Physiol Rev
84: 1097–1153, 2004; 10.1152/physrev.00043.2003.

Genetic Models of Mechanotransduction: The Nematode *Caenorhabditis elegans*

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Syntichaki, Popi, and Nektarios Tavernarakis. Genetic Models of Mechanotransduction: The Nematode *Caenorhabditis elegans*. *Physiol Rev* 84: 1097–1153, 2004; 10.1152/physrev.00043.2003.—Mechanotransduction, the conversion of a mechanical stimulus into a biological response, constitutes the basis for a plethora of fundamental biological processes such as the senses of touch, balance, and hearing and contributes critically to development and homeostasis in all organisms. Despite this profound importance in biology, we know remarkably little about how mechanical input forces delivered to a cell are interpreted to an extensive repertoire of output physiological responses. Recent, elegant genetic and electrophysiological studies have shown that specialized macromolecular complexes, encompassing mechanically gated ion channels, play a central role in the transformation of mechanical forces into a cellular signal, which takes place in mechanosensory organs of diverse organisms. These complexes are highly efficient sensors, closely entangled with their surrounding environment. Such association appears essential for proper channel gating and provides proximity of the mechanosensory apparatus to the source of triggering mechanical energy. Genetic and molecular evidence collected in model organisms such as the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the mouse highlight two distinct classes of mechanically gated ion channels: the degenerin (DEG)/epithelial Na^+ channel (ENaC) family and the transient receptor potential (TRP) family of ion channels. In addition to the core channel proteins, several other potentially

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interacting molecules have in some cases been identified, which are likely parts of the mechanotransducing apparatus. Based on cumulative data, a model of the sensory mechanotransducer has emerged that encompasses our current understanding of the process and fulfills the structural requirements dictated by its dedicated function. It remains to be seen how general this model is and whether it will withstand the impiteous test of time.

I. INTRODUCTION

Ubiquitous mechanical stimuli permeate the environment of every living cell and every organism. The process by which cells convert mechanical energy into electrical or chemical signals is called mechanotransduction and appears to be a universal property of all living organisms ranging from bacteria to humans (38, 130, 152, 353). The capacity to respond and adjust to mechanical inputs plays a pivotal role in numerous fundamental physiological phenomena such as the perception of sound and gravity, which underlie our senses of hearing and balance (137, 163, 221). Touch sensation and proprioception (the coordinated movement of our body parts) are additional manifestations of responsiveness to mechanical stimulation (137, 402, 409, 453). Somewhat less appreciated but by far not less important is the critical role of mechanotransduction in the stretch-activated reflexes of vascular epithelia and smooth muscle and in the regulation of systemic fluid homeostasis and blood pressure (137, 247, 404, 411, 453). Mechanotransduction is also critical for the prevention of polyspermy during fertilization, cell volume and shape regulation, cell locomotion, and tissue development and morphogenesis (199, 236, 347). In plants, mechanotransduction is the basis of gravitaxis and turgor control (265, 315). In protists (*Paramecium*, *Stentor*) mechanotransduction underlies gravikinesis, the swimming against the gravity vector to avoid sedimentation (36, 145, 184, 271).

All living organisms have developed highly specialized structures that are receptive to mechanical forces originating either from the surrounding environment or from within the organism itself. Among the most elaborate and greatly efficient, such structures are the mechanotransducers that are responsible for sensory awareness, for example, those facilitating touch, balance proprioception, and hearing (137, 152, 163, 404). In this article, we survey the current state of the art in the field of sensory mechanotransduction in *Caenorhabditis elegans* and highlight landmark discoveries in other model organisms, ranging from bacteria to mammals that have decisively shaped our understanding of the phenomenon of mechanotransduction. We further touch on the still missing pieces of the puzzle that impinge on the dominant concepts in the field and discuss potential experimental approaches that could help clarify sensitive open issues.

A. Emergence of Mechanotransduction

Perception of incident mechanical stimuli is critically important for interfacing with the physical world. Naturally, the mechanisms underlying the capability of living cells to receive and act in response to mechanical inputs are among the most ancient, implemented during evolution. Proteins with mechanosensitive properties are ubiquitously present in eubacteria, archaea, and eukarya and are postulated to have been an essential part of the physiology of the Last Universal Ancestor (137, 234, 237, 240, 276). The first mechanosensitive processes may have evolved as backup mechanisms for cell protection, e.g., to reduce intracellular pressure and membrane tension during osmotic swelling. Subsequent organismal diversification and specialization resulted in variable requirements for mechanotransduction in different organisms (304). Hence, evolutionary pressure has shaped a large repertoire of mechanotransducers, optimized for a great assortment of tasks that range from maintenance of intracellular osmotic balance and pressure to our impressive ability of hearing and discriminating sounds, and reading Braille code with our fingertips (152, 168).

Below, we briefly present mechanically gated ion channels from bacteria and archaea. Although these channels probably represent the simplest and more ancient forms of a mechanotransducer, they nevertheless comply with the same basic principles that dictate the function of mechanosensitive molecules and structures in all organisms.

1. Mechanically gated ion channels in bacteria

The best-characterized bacterial mechanosensitive channels are those of *Escherichia coli*, which are involved in cell turgor regulation. The primary role of this channel is osmoregulation or osmoprotection of bacterial cells (30, 249, 353). These mechanically gated channels can directly sense mechanical stretch on the membrane during severe osmotic challenges and can switch between open and closed conformations in response to bilayer tension (4, 25, 38, 40, 392). They have been studied in detail by patch-clamp methodologies in giant spheroplasts and in reconstituted membrane fractions (168, 278, 392). Based on their conductance and sensitivity to negative pressure applied to the patch-clamp pipette, three types of mechanosensitive channels (*Msc*) can be distinguished: *Msc*M (M for mini conductance), *Msc*S (S for small conductance), and *Msc*L (L for large conductance). Another bacterial channel, *Msc*K (previously called *Aef*A and *Kef*A), shares homology with *Msc*S but contains additional domains at its amino terminus, which may be required for the allosteric regulation of this channel by ionic concentration (251, 287). *Msc*L was the first identi-

fied and is the best-characterized of all mechanosensitive channels (39, 41, 277, 391).

Fractionation of *E. coli* membrane constituents by column chromatography and functional examination of the individual fractions for mechanically gated channel activity by patch-clamp electrophysiological recordings led to the identification of a membrane protein underlying the activity of *Msc*L and the cloning of the corresponding gene (391, 393). The *Msc*L secondary structure includes two α-helical transmembrane domains (TM1 and TM2) connected by a periplasmic loop, with the amino and carboxy termini located in the cytoplasm (41, 392). The *Msc*L ion channel is nonselective and exhibits very large conductivity, which is indicative of a large, water-filled pore, probably formed in the center of homomultimeric structures (91). The higher order structure of *Msc*L is somewhat controversial. Cross-linking experiments indicated that *Msc*L might form homohexamers (40, 41) or homopentamers (280, 394). Likewise, crystallographic studies have identified both homopentameric (*Tb-Msc*L; from *Mycobacterium tuberculosis*) and homoexameric (*Eco-Msc*L; from *E. coli*) structures (72, 356).

Molecular dissection of the *Msc*L channel through mutational analysis of the *mscL* gene identified structural domains critical for function (39, 41, 277, 391, 477). Several models of *Msc*L mechanosensitivity, based on structure-function relations, have been proposed (37, 168). The crystal structure of *Msc*L from *M. tuberculosis* (*Tb-Msc*L), at 3.5Å resolution, has provided a basis for molecular modeling of the channel gating mechanism (38, 72, 296, 305). At present, a combination of electrophysiological, biophysical, and structural studies in bacterial ion channels (25, 31, 33, 280, 311, 312, 378, 389, 390) support the following simplified model of the *Msc*L gating. The channel is composed of five identical subunits arranged around a central pore. The closed pore is formed by the hydrophobic constriction of the first transmembrane (TM1) helices at the cytoplasmic end of the channel. The mobile amino termini probably serve to stabilize the open-channel conformation and may interfere with the passage of ions, whereas the carboxy termini possibly play a role in stabilizing the closed configuration of the channel. The periplasmic loop domains most likely function as elastic springs, resisting the opening of the channel by membrane tension (168, 276). Membrane tension entails the channel opening through an intermediate step that involves small movements in the first transmembrane helices (TM1). Subsequent concerted, massive rearrangements in both TM1 and TM2 of the channel subunits result in irislike expansion and opening of the channel (31, 311). The mobile amino termini probably serve to stabilize the open-channel conformation and may interfere with the passage of ions. The carboxy termini possibly play a role in stabilizing the closed configuration of the channel (276).

*Msc*S and *Msc*M are two additional mechanically gated channels in *E. coli* that show different pressure sensitivity, with *Msc*M appearing less frequent in bacterial cells, compared with *Msc*L and *Msc*S, which are typically found in excess. *Msc*S is relatively nonselective, displaying a slight preference for anions over cations, whereas *Msc*M exhibits a slight preference for cations (30, 278, 393). Because all three channels can be activated by hyposmotic stress, this indicates that they are probably activated sequentially to provide a gradual efflux conduit (147, 168). Studies of the *E. coli Msc*S channel structure demonstrated that it folds as heptamer with three transmembrane helices (TM1–3) in each subunit (22).

2. Mechanically gated ion channels in archaea

Two types of mechanically gated channels have been identified in the cell membranes of the halophilic archaeon *Haloferax volcanii* using the patch-clamp technique, *Msc*A1 and *Msc*A2 (246). Both show large conductance, but they differ in their distinct rectification properties, and similarly to the bacterial mechanically gated channels, both respond to bilayer tension and are blocked by submillimolar concentrations of gadolinium (Gd^{3+}) (232, 246). In addition, mechanosensitive channels have been identified and cloned from two other archaeal species that occupy different environmental habitats: the thermophilic archaeon *Thermoplasma volcanium* and methanogenic archaeon *Methanococcus jannashii*. By reconstitution of detergent-solubilized membrane proteins onto liposomes and following their function in patch-clamp experiments, a 15-kDa membrane protein that forms a novel mechanosensitive channel, termed *Msc*TA, was identified in *T. volcanium* (232). The *T. volcanium* channel protein exhibited properties typical of a mechanosensitive ion channel when heterologously expressed in *E. coli* and used for channel reconstitution experiments onto liposomes (234). Secondary structure analysis indicates that the *Msc*TA channel has two α-helical transmembrane domains, similarly to the bacterial *Msc*L (232).

With the use of the TM1 transmembrane domain of *Eco-Msc*L as a genetic probe, the hypothetical protein MJ0170 was identified in the *M. jannashii* genome, which shares sequence similarity with both the TM1 of *Eco-Msc*L and the *Ygg*B protein underlying the activity of *Msc*S in *E. coli* (234, 249). This suggests that the hybrid *Msc*MJ protein might have evolved by gene duplication from an ancestral *Msc*L-like gene (233). These studies demonstrate that archaeal mechanosensitive channels share structural and functional similarity with bacterial mechanosensitive channels. A common gating mechanism is at operation where mechanical force transmitted via the lipid bilayer triggers channel opening (232, 234). Even though no sequence homologs for *Msc*L have been identified in eukaryotes, bacterial and archaeal mechanosen-

sitive channels share intriguing similarities in basic structural motifs and membrane topology with eukaryotic channels of different function [e.g., the degenerin (DEG)/epithelial Na^+ channel (ENaC) family of ion channels; see sect. IV*A*]. This indicates that common biophysical principles underlie the mechanosensitive properties of diverse classes of channels (168). Hence, mechanosensory transduction probably originated along with the appearance of the first life forms according to such biophysical principles. Phylogenetic analysis, which indicates that prokaryotic mechanosensitive channels derived from a common ancestral molecule resembling the bacterial *Msc*L channel protein, provides support for this hypothesis (232, 240, 295). Moreover, a recent study suggests that the *Msc*L channel shares a common evolutionary origin with the sensor module of eukaryotic mechano- and voltage-gated channels (243).

B. Regulatory Mechanotransduction

Mechanotransduction in living organisms can operationally be categorized as regulatory or sensory. Both cellular and organismal homeostasis often requires adjustment to mechanical forces generated by environmental sources or internal processes (168, 353). For example, osmotic balance, ion concentration homeostasis, cell volume and shape regulation, blood pressure, and turgor control all depend on appropriately responding to mechanical stretch or shearing forces (93, 112, 168, 353). Dedicated mechanotransducers in these paradigms serve as regulatory valves that initiate a cascade of events towards adjusting to or counteracting any substantial deviation from normal conditions. The requirement for regulatory mechanotransduction is probably as ancient as life itself. Cells constantly need to fight shearing and stretch forces they encounter, and the faculty of mechanotransduction was most likely decisive for the survival of the first cell. The universal occurrence of mechanotransduction capabilities in all living organisms argues for such early emergence of mechanotransducers (137, 233, 237). An alternative, plausible scenario is that mechanotransduction is the outcome of convergent evolution and evolved independently multiple times by the conversion of various types of ion channels into mechanotransducers (137).

C. Sensory Mechanotransduction

Sensory mechanotransduction or mechanosensation alerts the organism to mechanical inputs in the form of touch, pressure, stretch, sound, vibration, and acceleration (149, 152, 168, 353). Such stimuli provide vital awareness of the environment and information with regard to the organism's relative position and movement. This prowess is important in negotiating with the physical world and is based on highly adapted mechanotransducers that have evolved to optimally carry out the task. Sensory and regulatory mechanotransducers obey similar principles, and it is likely that the first derived from the second by refinement towards acquiring dedicated functions. In higher organism, specific neurons, the mechanoreceptors are equipped with a mechanotransducing apparatus and signal upon reception of a stimulus. Frequently these cells are implanted within accessory structures that serve to filter and amplify an incoming stimulus. For example, skin touch receptor neurons are occasionally associated with hair shafts, while hair cells of the inner ear are enclosed in elaborate anatomical structures that greatly facilitate capture and tunneling of sound wave energy (113, 134, 292, 317, 374, 375).

II. DECIPHERING MECHANOTRANSDUCTION

Mechanical forces are sensed by living cells in various ways and variably influence cellular biochemistry and physiology. For example, direct funneling of mechanical energy through the cytoskeleton can affect gene transcription and other intracellular events (235, 304, 313, 354, 355). However, such mechanisms are not suitable for rapid sensory transduction due to prohibitive latency characteristics. Rather, specialized ion channels are engaged, which open in response to mechanical stimuli (137, 152, 168). What is the molecular mechanism by which mechanical energy elicits ion channel opening? While the mechanism of mechanosensitive channel gating has been studied in great detail for prokaryotic *Msc*L-type channels (31, 311, 389), we only have circumstantial evidence about how eukaryotic mechanotransducers respond to mechanical stimuli (152, 168, 171, 404; reviewed in Ref. 402).

A. Theoretical Concepts for Mechanotransduction

The nature of the triggering stimulus puts specific constraints on the gating properties of a mechanotransducer. Specifically, two physical parameters are of paramount importance: sensitivity and speed (88, 151, 152, 317). An efficient mechanotransducer is capable of detecting minute forces and responding within microseconds upon stimulation (195, 197). These requirements exclude slow mechanisms involving second messengers and ligands from gating sensory mechanotransducers (152, 196, 236, 354). Rather, mechanical force needs to be directly applied to the channel, reducing immediate response. An additional essential feature of a mechanotransducer is adaptation to background, constitutive mechanical forces. Adaptation bolsters the capacity to differentiate

between weak signals and relatively large constant mechanical forces (196, 197, 211, 317).

Two general models have been proposed for gating mechanosensitive ion channels: the "lipid bilayer" and the "tethered channel" models (Fig. 1). In the lipid bilayer model, mechanosensitivity of ion channels inserted in lipid bilayers arises from the intimate association of the channel with the bilayer, which allows dilation, thinning, or changes in local membrane curvature, to directly shift the equilibrium between open and closed channel conformations (168, 273, 276, 322). Bilayer reconstitution experiments have provided unequivocal evidence for a bilayer model of mechanical gating for bacterial channels, and there is growing evidence that at least some eukaryotic channels are gated by tension developed in the bilayer (e.g., the SAT-CAT channel in *Xenopus* oocytes; Ref. 479). Another eukaryotic channel that retains mechanosensitivity when reconstituted into lipid bilayers is the epithelial Na^+ channel (ENaC; Refs. 12, 200, 201). However, it should be noted that, when expressed in *Xenopus* oocytes, ENaC proteins do not form mechanosensitive channels, which casts doubts as to the physiological relevance of lipid-bilayer observations (13, 346).

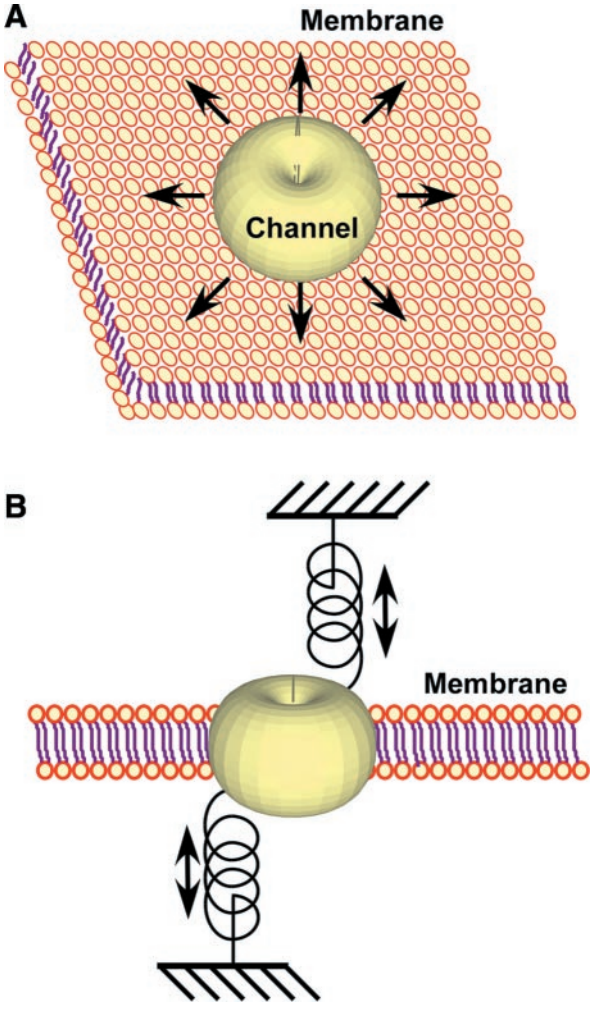

FIG. 1. Two general models for mechanotransduction involving plasma membrane ion channels. *A*: in the "lipid bilayer" model, surface tension that develops on the membrane drives the channel open. *B*: in the "tethered channel" model, the ion channel is linked to both inside and outside anchor points. Force is applied to the channel via these links and modulates its conductance.

The lipid bilayer model is based on one critical assumption: despite its fluid properties, the cellular membrane is rigid enough to develop tension capable of influencing the conformation of proteins or complexes inserted to it, such as the mechanosensitive ion channels (168, 273). Although this may be the case with prokaryotic cells that possess a rigid cell wall, it appears unlikely in animal cells with excess membrane area that can effectively buffer developing tension before it becomes capable of triggering the opening of ion channels (168, 479). Under such conditions, tension needs to be focally applied to the mechanotransducer for maximal effect. In the tethered channel model, the mechanically gated ion channels are embedded in the cell membrane and are also closely linked to firm points of reference both intracellularly (the cytoskeleton) and extracellularly (the extracellular matrix). These anchors serve as a "gating spring" that provides the tension required to open the channel (5, 152). Mechanical force is transmitted directly to the channels through the cytoskeleton and extracellular matrix tethers, without increasing tension in the lipid bilayer in a manner which is reminiscent of integrin-mediated mechanotransduction (353, 442). The tethered channel model is more relevant to mechanically gated channels in animal sensory cells (152, 272). Although this model is compatible with an extensive body of genetic findings from nematodes to mammals, there is no experimental evidence that directly supports it (i.e., analogous to liposome reconstitution, in the case of the lipid bilayer model).

B. Molecular Characterization of Eukaryotic Mechanotransducers: Methodological Limitations

For years, mechanically gated ion channels tenaciously eluded cloning and molecular characterization efforts for reasons pertinent to their highly specific and efficient function. For example, owing to their high sensitivity, the relative density of skin touch receptors is exceedingly low; there are only ~17,000 such receptors in the finger and palm skin pad (105, 127, 152, 239). Also, in the specialized hair cells of the vertebrate inner ear, only a few hundred mechanosensitive ion channels may exist (47, 88, 195, 317, 379). These are prohibitive densities for biochemical isolation of the molecules involved. Moreover, there are no known reagents that will interact with these channels with high specificity and high affinity, thus further thwarting efforts at biochemical purification.

Second, mechanosensory channels are embedded and intertwined with materials that attach to the surrounding environment (152, 168). These contacts, while probably critical for function, make physical dissociation of the channel a challenge, and hinder studies in heterologous expression systems where such components and intricate associations are either lacking or are difficult to reproduce faithfully (140, 147, 152, 404). Apart from raising concerns about heterologous expression studies, the delicate surroundings of eukaryotic mechanosensitive channels also encumber in situ electrophysiological recordings. In sharp contrast to voltage- or ligand-gated ion channels, the very act of measurement using electrophysiological probes is likely to inadvertently disturb the structure and the properties of the mechanotransducing apparatus. Together with the subunits of the mechanically gated, core ion channel, the interacting molecules that provide the necessary gating tension need to be identified and included in potential mechanotransducer reconstitution attempts. Without these, it is highly likely that the core ion channel would not retain its native mechanosensitive characteristics.

C. Genetic Models

The intrinsic difficulties of directly isolating and studying mechanosensitive macromolecular complexes and ion channels necessitate the development and utilization of genetic models for mechanotransduction. Indeed, genetic approaches have been exceptionally successful in identifying candidate mechanically gated ion channels and other components of mechanotransducing complexes. Pioneering genetic screens conducted by Sydney Brenner, Martin Chalfie, Marilyn Dew and John Sulston, using the nematode *Caenorhabditis elegans*, have allowed the detailed dissection of touch sensation in this simple organism (55, 58, 59, 64, 65, 395). Extensive follow-up studies have culminated in the identification of a plethora of proteins implicated in mechanotransduction and the formulation of an elegant model for a mechanosensory apparatus (15, 18, 58, 138, 402). Similarly motivated genetic approaches in the fly *Drosophila melanogaster* have resulted in the dissection of a different type of mechanotransducer (113–115, 205, 223, 438). Two vertebrate organisms have also contributed to our understanding of mechanotransduction, the fish *Danio rerio* (zebrafish) and the mouse (9, 106, 158, 300, 302, 326, 327). Remarkably, investigations in such distant organisms have converged to a limited number of mechanisms and many common components for the metazoan mechanotransducer (134, 152, 168, 171, 404). This conservation suggests that, while individual implementations of mechanosensitive structures may somewhat differ, a similar basic principle underlies mechanotransduction from nematodes to mammals (113, 115, 152, 404). Below, we elaborate on findings in *C. elegans* and describe work in *Drosophila* and vertebrates, aiming to portray the commonalities that epitomize this general principle.

III. MECHANOTRANSDUCTION IN *CAENORHABDITIS ELEGANS*

Sydney Brenner introduced *Caenorhabditis elegans* as a model organism particularly suitable for the study of neuronal functions in 1974 (46). The simplicity of this animal coupled with many methodological advantages have allowed its remarkably rapid and detailed characterization (187, 210, 413, 444). The nematode has thus emerged as the organism of choice in which to study numerous biological processes (18, 58, 63, 100, 290, 334). *C. elegans* has facilitated many landmark discoveries in the fields of embryogenesis, development, ageing, cell death, and neurobiology and was the first metazoan with a completely sequenced genome (54, 126, 132, 191, 396, 397, 413, 467). Many behaviors of this animal are direct manifestations of mechanosensitivity, making it exceptionally attractive for investigating mechanotransduction (18, 26, 55, 64, 65, 104, 186, 215, 267, 332, 458, 469; recently reviewed in Ref. 118). The best characterized of such behaviors is the response to a gentle mechanical stimulus delivered transversely along the body of the animal, typically by means of an eyelash hair attached onto a toothpick (the "gentle body touch response"; Refs. 64, 65, 186). Other mechanosensory responses are the generation and maintenance of the characteristic coordinated sinusoidal pattern of locomotion (analogous to proprioception; Refs. 402, 409); the nose touch response, which can be further categorized into the head-on collision response and the head withdrawal response (103, 215); the response to harsh mechanical stimuli (70, 103, 141); and the tap withdrawal reflex, where animals retreat in response to a tap on the culture plate (76, 335, 460). Less obvious and not well understood is the role of mechanotransduction in matting, in egg laying, in feeding, and in defecation (10, 108, 257, 259, 414, 469).

A. Introducing *C. elegans*

C. elegans is a small (~1 mm) soil-dwelling, free-living nematode worm (46). In the laboratory, animals feed on an *E. coli* diet and complete a reproductive life cycle in 2.5 days at 25°C, progressing from fertilized embryos through four larval stages to become egg-laying adults, which then live for ~2 wk (see Fig. 2; Refs. 46, 231, 470). Because *C. elegans* can reproduce by self-fertilization, it is possible to raise genetically identical populations that do not undergo inbreeding depression. While the dominant sexual form is the hermaphrodite (geno-

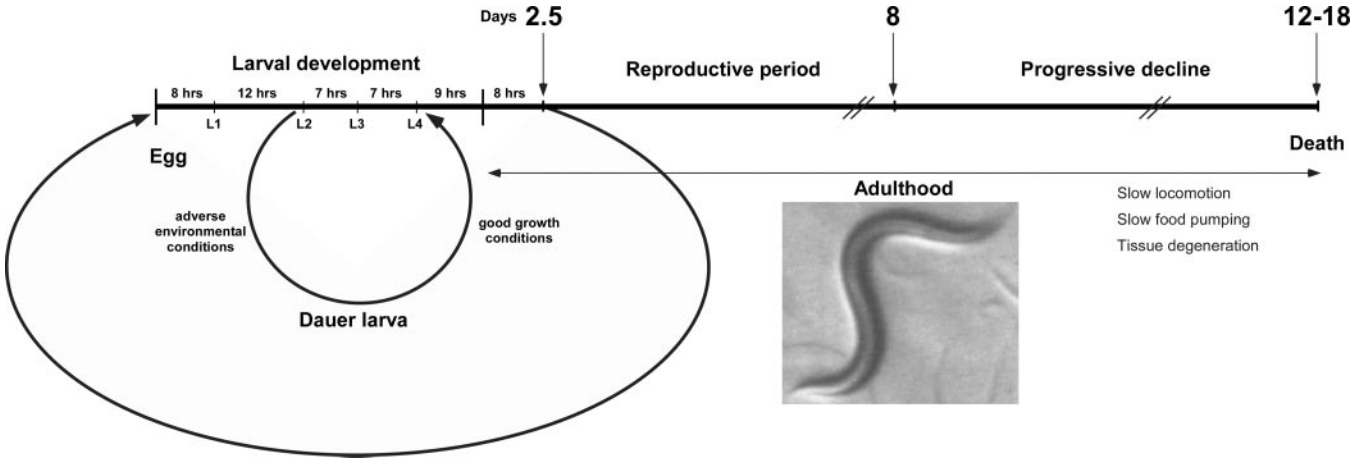

FIG. 2. The *C. elegans* life cycle. After hatching, worms progress through four larval stages before reaching adulthood (46). The duration of each stage at 25°C is shown in hours. Adult nematodes lay eggs for ~5 days. The average lifespan of animals is ~15 days.

type: XX), males (genotype X0) can also be propagated and used to construct strains carrying multiple mutations (46). Under adverse conditions such as starvation, overcrowding, or high temperature, larvae can enter an alternative life stage called the dauer (enduring) larva, during which animals move but do not feed (53, 119). The dauer larva is a "nonaging" developmental form that survives for weeks or even months (153, 222). When a dauer larva encounters favorable environmental conditions, it reenters the life cycle at the fourth larval stage, progresses into adulthood to reproduce, and then completes the final week or so of its life span.

The simple body plan, the transparent egg and cuticle, and the nearly invariant developmental plan of this nematode have facilitated exceptionally detailed developmental and anatomical characterization of the animal (367, 443, 455; http://www.wormatlas.org). The complete sequence of cell divisions and the normal pattern of programmed cell deaths that occur as the fertilized egg develops into the 959-celled adult have been elaborated (396, 397; Fig. 3). In addition, the pattern of synaptic connections made by each of the 302 neurons of the animal has been described so that the full "wiring diagram" of the animal is known (18, 63, 443, 455, 456). Microsurgery with a laser beam can be used to specifically ablate individual cells, and whole classes of cells can be rendered nonfunctional or killed by cell-specific expression of toxic genes (17, 172). *C. elegans* is a well-established, powerful genetic system. When a hermaphrodite parent is subjected to a mutagenizing agent, the F1 progeny self-fertilize to produce F2 animals that are homozygous for recessive mutations (46, 116, 146, 261, 476). In this way, thousands of mutations that disrupt development or various behaviors have been identified and, after crossing with males and standard gene mapping, positioned on a detailed genetic map (46, 187). Rapid and precise genetic mapping can be achieved by taking advantage of a dense single nucleotide polymorphism map (203, 238, 461). Primary culture methodologies are available for the analysis of specific groups of cells and neurons ex vivo (79). Recently, electrophysiological study of cultured nematode neurons and muscles has also become possible (80; recently reviewed in Ref. 386).

C. elegans molecular biology enables a considerable amount of information on in vivo activities of genes of interest to be determined rapidly. A physical map of the *C. elegans* genome, consisting of overlapping cosmid and YAC clones covering most of the six chromosomes, has been constructed to facilitate cloning of genes that have been positioned on the genetic map (62, 90, 187, 444). Sequencing and high-quality annotation of the complete *C. elegans* genome organized in six chromosomes (5 autosomes and the sex chromosome X) has been accomplished (413, 465; http://www.wormbase.org). In addition, ongoing efforts to obtain expressed sequence tags (ESTs) and open reading frame sequence tags (OSTs) for all *C. elegans* genes have provided an extensive collection of nematode cDNAs (266, 337). All ~20,000 predicted open reading frames (ORFs) have been subjected to expression profiling under numerous conditions using microarray technology (42, 208, 228, 229, 339). Detailed gene expression and protein-protein interaction maps have been developed and are publicly available (44, 95, 279, 435; http://www.wormbase.org).

C. elegans is also particularly amenable to reverse genetics studies. Investigators can take advantage of the wealth of genome data available to perform "reverse genetics," directly knocking out genes (116, 261, 476). A novel method of generating mutant phenocopies, called double-stranded RNA-mediated interference (dsRNAi), enables probable loss-of-function phenotypes to be rapidly evaluated (126, 198, 213, 410, 420, 421). A comprehen-

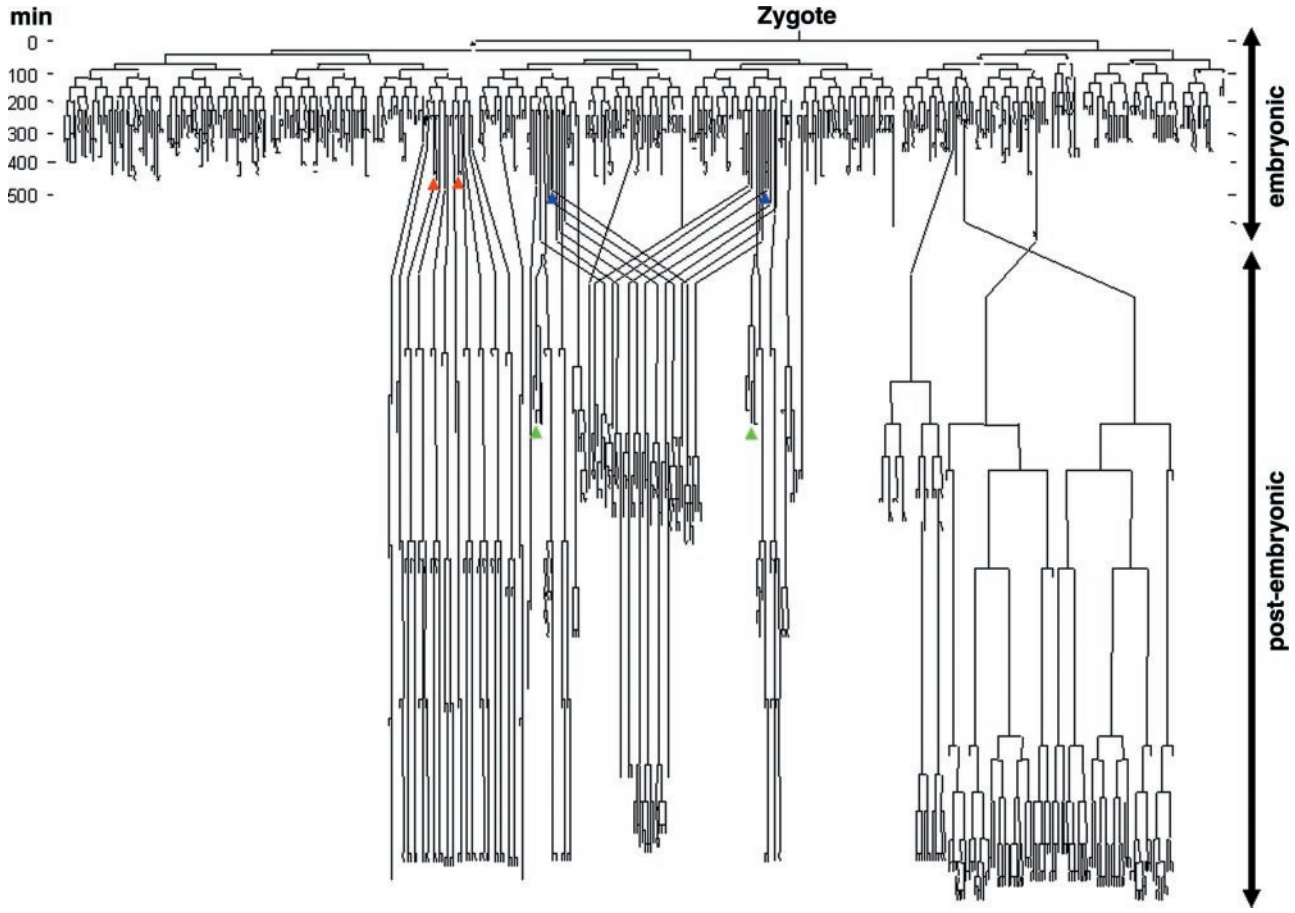

FIG. 3. The *C. elegans* cell lineage. A remarkable feature of the *C. elegans* model system is the availability of the complete cell lineage description from the fertilized oocyte to the 959-celled adult animal (396, 397). The positions of the six touch receptors are shown. Four are born embryonicaly (ALML/R, red; PLML/R, blue), and two are born after hatching (AVM, PVM; green) More specific information on individual cells and sublineages of *C. elegans* is available at the following web address: http://www.wormbase.org/db/searches/pedigree. [Drawing adapted with permission from Nick Rhind (http://elegans.swmed.edu/parts/lineage.gif).]

sive RNAi approach to knock down expression of each of the 20,000 ORFs was recently published (212a), and examination of all genes on chromosomes I and III as well as of those expressed in the ovary has already been published (16, 129, 154, 266, 314). DNA manipulated in vitro can be microinjected or bombarded back into animals for functional assays (125, 288, 324). Vectors are available for identification of transformants, cell-specific expression, and generation of fusions to marker genes such as *E. coli* β-galactosidase and the jellyfish green fluorescent protein (GFP) so that individual cells can be visualized in stained or living animals (57, 69, 124, 291).

B. Gentle Body Touch Response

The laboratory assay for the gentle body touch response involves a mild stroke of the animal with an eyelash hair attached to a toothpick, transversely to the anterior-posterior body axis (64, 65, 395). Forces exerted during the stimulation, which can be sensed by wild-type animals, are in the range of 10–20 μN (137, 157). When no response is observed, animals are prodded with a thin platinum wire to confirm that they are touch insensitive rather than paralyzed (gentle-touch insensitive animals typically still respond to a strong stimulus, the harsh touch response, with forces in excess of 100 μN) (65, 70, 103, 157, 469). Depending on the part of the body touched, animals will either accelerate or initiate forward movement (when stimulated at the posterior or the tail), or reverse and move backwards (when stimulated at the anterior part of the body). Hermaphrodite, male, juvenile (except L1), and dauer animals respond identically to touch. The response is adaptive: repetitive stimulation leads to short periods of insensitivity (267, 335, 458).

1. *Neuroanatomy: the neuronal circuit for touch response*

The neuronal morphologies, chemical synapses, and gap junctions of all *C. elegans* neurons have been de-

scribed by means of reconstruction from serial section electron micrographs (443, 455, 456; http://www.wormatlas.org). The significance of identified synaptic relationships has been tested using laser microsurgery. The touch reflex of the mature animal involves 6 touch receptor neurons, 5 pairs of interneurons, and 69 motorneurons (55, 65). The 6 touch receptor neurons were originally designated as the microtubule cells because of distinctive bundles of 15-protofilament (tubulin dimmer filaments) microtubules that fill their processes (ALML/R, anterior lateral microtubule cell, left/right; AVM, anterior ventral microtubule cell; PLML/R, posterior lateral microtubule cell, left/right; PVM, posterior ventral microtubule cell; Refs. 55, 60, 65–67). All six cells are dispensable for the viability of the organism. Apart from insensitivity to gentle body touch, laser ablation of all six neurons does not result in any additional adverse effects (56, 65).

Two fields, anterior and posterior, of touch sensitivity are defined by the arrangement of the six touch receptor neurons along the body axis (Fig. 4; Ref. 65). All the touch receptor neuron cell bodies have anteriorly directed processes. ALM processes are embedded in the hypodermis, extend along the cuticle, and form a short branch into the circumpharyngeal nerve ring (Fig. 4; Refs. 59, 68). Most of the synapses of the ALM cells, including coupling via gap junctions to the AVM cell, are formed on this branch. In addition to a long anteriorly directed process, the PLM cells have a short posteriorly directed process (65, 456). Similarly to ALM, the anteriorly directed process is embedded in the hypodermis and runs close to the cuticle. The PLM process turns and enters the ventral cord near the vulva. PLML does not make it around the hypodermal ridge and has no synapses. PLMR runs along the neuropile and makes direct interneuron synapses (65, 456). The two postembryonic touch cells have single processes that enter the ventral nerve cord and run anteriorly at its extreme ventral edge. AVM branches at its anterior end. The branch enters the nerve ring where it forms synapses with the ALM cells and other neurons (Fig. 4). PVM does not branch or enter the nerve ring (68, 455, 456). Touch receptors also are uniquely surrounded by an osmiophillic extracellular material referred to as the mantle. The amount of mantle varies along the length of the process. Periodic darkly staining patches are often seen in the cuticle (65, 456). These patches resemble those seen under muscle cells, and it has been speculated that they may represent touch cell attachment sites (Fig. 5; Refs. 103, 402). Several lines of evidence support that touch cell processes are mechanosensory organs (103). First, the touch cell processes

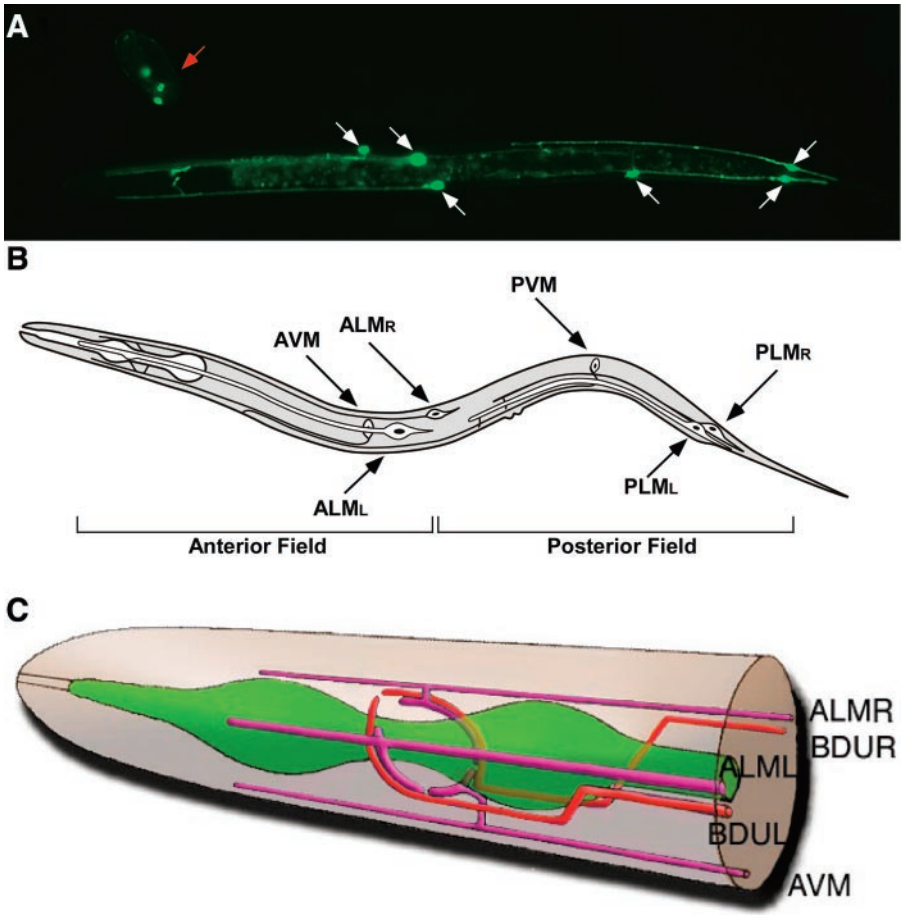

FIG. 4. The *C. elegans* touch receptor neurons. *A*: expression of green fluorescent protein (GFP) under the control of the *mec-4* promoter, which is active only in the six touch receptor neurons (55, 57, 69, 101). White arrows indicate touch receptor cell bodies. Some touch receptor axons are visible. A developing embryo inside an egg with embryonically born touch receptors, just starting to express *mec-4*, is also shown (red arrow). *B*: the two fields of touch sensitivity are defined by the arrangement of touch receptor neurons along the body axis. The anterior lateral microtubule cells (ALMs; R, right; L, left) and anterior ventral microtubule cell (AVM) mediate the response to touch over the anterior field, whereas posterior lateral microtubule cells (PLMs; R, right; L, left) mediate the response to touch over the posterior field. The posterior ventral microtubule cell (PVM) does not mediate touch response by itself (56, 58, 65). *C*: synaptic partners of anterior touch receptor neurons. The three touch neurons (ALMs and AVM) have branches that enter the nerve ring and make synapses to interneurons (BDUs; Refs. 65, 103, 456). The feeding organ of the animal, the pharynx, is shown in green. [Adapted from original drawing by W. W. Walthall and M. Chalfie; courtesy of WormAtlas (http://www.wormatlas.org).]

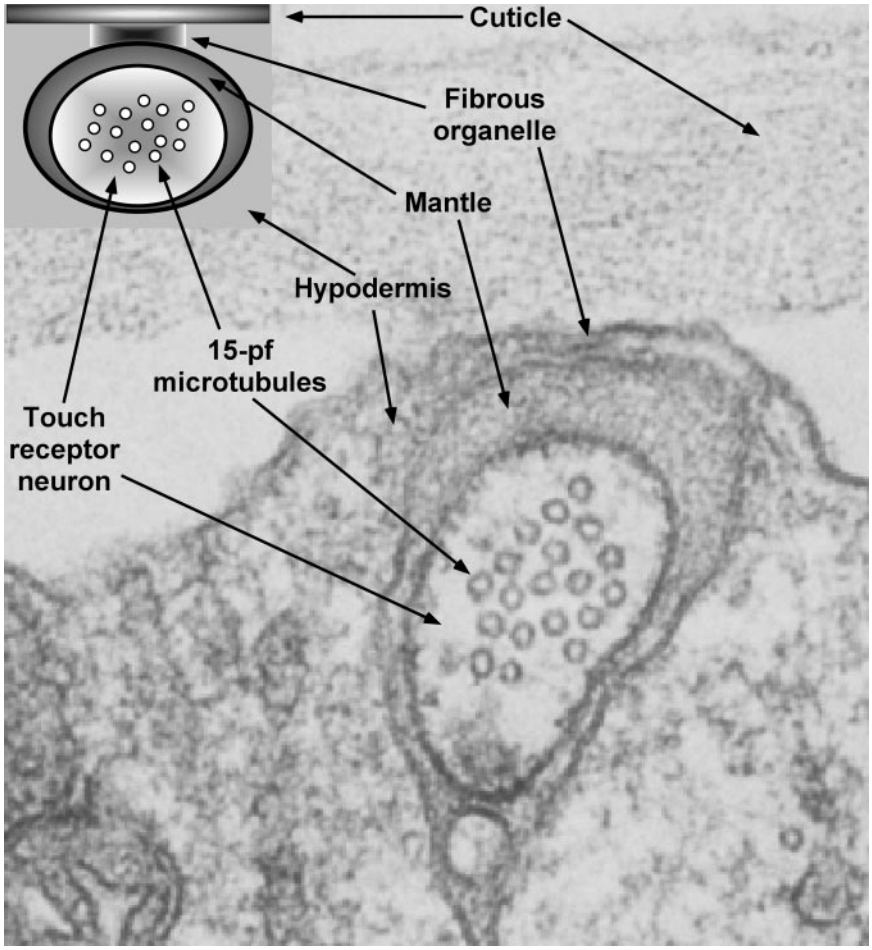

FIG. 5. Ultrastructural features of the touch receptor neurons. An electron micrograph of a cross-section of a touch receptor neuron process is shown. The touch cell process is filled with 15-pf microtubules (55, 56, 64, 66, 67). The process is embedded in the hypodermis and surrounded by the mantle. A schematic representation of a touch receptor neuron cross-section is also shown for clarity. A darkly staining region, labeled fibrous organelle, is depicted here as a bar-shaded rectangle connecting the mantle and the cuticle. Such structural specializations appear periodically along the length of the touch receptor process and may serve to attach the process to the cuticle.

lack synaptic specializations, and hence are likely to be dendritic (68, 456). Second, the touch cell processes are embedded in the hypodermis adjacent to the cuticle, positioning expected to facilitate detection of mechanical stimuli (55, 65). Third, the position of the processes along the body axis correlates with the sensory field of the touch cell (65, 103).

Laser ablation microsurgery established that PLML and PLMR are required for response to a touch to the tail. If either is present, tail touch sensitivity is observed. When both are ablated, animals are completely insensitive to gentle touch stimuli administered to the posterior (65, 68, 230). Either ALML or ALMR can mediate a response to a mechanical stimulus delivered to the anterior part of the body. AVM, which is added into the touch circuitry postembryonically, can mediate a weak response to some touches but not all, by itself. In animals in which both ALM cells are killed, partial touch sensitivity returns 35–40 h after hatching, which is attributable to AVM being added. PVM cannot mediate a touch response by itself. Other cells or neurons cannot differentiate and take the place of missing touch receptor neurons (55, 59, 65).

In newly hatched larvae, the processes of touch cells lie between the lateral hypodermis and the adjacent muscle quadrant, while at ~12 h they are engulfed by the hypodermis (55, 65). All touch receptors except PVM have a short synaptic branch, which is the site of most synapses to other neurons. The ALMs, AVM, and PVM also make synaptic connections to other neurons on their receptor processes. Interestingly, the induction of synaptic branching appears to depend on the position of the cell body (68). If the PVM cell is situated more anteriorly as in *mab-5* mutant animals, a branch forms (64, 174, 358). *mab-5* encodes a homeodomain protein, related to *Drosophila antennapedia*, that functions at the posterior part of the body to determine cell fate and migration (discussed in section III*B2*). The displaced cell can mediate a weak touch response. This indicates that PVM has the potential to branch if situated in the appropriate environment. However, it is not clear if in the *mab-5* genetic background, PVM adopts an anterior touch cell fate (358).

Bundles of darkly staining large-diameter mictotubules distinguish the touch receptor neurons (66, 456). Cross-bridges between microtubules of a bundle are observed in micrographs obtained with electron microscopy and may increase the structural integrity of the bundle. These microtubules are unique to the nematode touch receptor neurons and contain 15-protofilament microtu-

bules, a unique feature of these six cells (67). In most eukaryotic cells, α- and β-tubulin coassemble into 13-protofilament microtubules, whereas the vast majority of microtubules in *C. elegans* cells have 11 protofilaments (67, 456). In normal touch receptors, 11-protofilament microtubules typical of most other cells in this nematode are occasionally observed. If the 15-protofilament microtubules are eliminated by mutation, the number of 11-protofilament microtubules in the touch cell processes increases (55, 60, 66, 135, 360). Normally there are 450 15-protofilament microtubules in a touch cell and none or very few 11-protofilament microtubules; when 15-protofilament microtubules are absent, ~100 11-protofilament microtubules fill the cell process. Fifteen-protofilament microtubules are differentially sensitive to colchicine. At low concentrations, 15-protofilament microtubules and touch sensitivity are disrupted, but other behaviors and microtubules in other cells are unaffected (66). Colchicine also has the potential to block the production of the 11-protofilament microtubules to a lesser extent (67). At hatching, lateral microtubule cells contain very few microtubules, which are short (2.5 μm). The array is not yet continuous as it is in adults. Still, these larvae are touch sensitive. By 12 h, microtubules have increased in number and length. At 12–36 h posthatching, numbers increase but length remains constant, whereas after 36–48 h, they increase in number and length to adult level. After 48 h, the microtubule number still increases, but the length is constant (67). Overall, process length increases 2.4-fold, while total length of microtubules increases ~5 times (66).

Microtubules may provide a rigid intracellular "point of reference," against which a touch stimulus could exert mechanical force to the mechanotransducing apparatus (103, 402). This function of the microtubule network is further discussed in section IV*B*. Microtubules also appear to play a role in process outgrowth, since processes are lacking in cells that have been treated with colchicine and benomyl (benomyl interferes with the 11-protofilament microtubules that take over in the absence of 15-protofilament microtubules; Ref. 102). Continuity of the microtubules does not appear necessary for axonal outgrowth (60, 371). Examination of serial section electron micrographs revealed that the 15-protofilament microtubules do not span the entire length of the touch receptor process. The processes are 400–500 μm long, whereas the microtubules are 10–20 μm long (443, 456). Mature processes are filled with overlapping bundles of 15-protofilament microtubules. The average microtubule length varies with cell type, with lateral cell processes containing more microtubules than the ventral cell processes (66, 456). Such short microtubules may facilitate sliding relative to each other, which would be required to accommodate changes in cell length that is likely to accompany the sinusoidal motion of the animal. Microtubules have a distinct polarity: the distal end is found on the outside of the microtubule bundle and the proximal end is preferentially found within the bundle (66, 456). The distal end is distinguished by its diffuse ending, which is a diffuse patch of stained material with a diameter up to twice that of the microtubules. Proximal ends often have a filled appearance. Intriguingly, the diffusely stained structure of the distal end often appears to associate with the plasma membrane (66, 456).

There is no significant synaptic input to the touch receptor neurons. The branches of the ALM cells make gap junctions with AVM in the nerve ring. PVM is connected to AVM by a gap junction in the ventral cord. PLML and PLMR are not coupled to each other or to other microtubule cells (65, 268). The major synaptic output of touch receptor neurons is channeled to interneurons that connect to motorneurons controlling body wall muscle contractions (Fig. 6). Four pairs of interneurons synapse onto both motorneurons and touch receptor neurons, as well as onto each other, and are thus candidates for function in the touch circuit: AVA, AVB, PVC, and AVD (65, 455, 456). Initially ALM cells work independently via AVD. When AVM is added to the circuit, it couples the anterior cells into a circuit. AVM makes gap junctions to AVD and chemical synapses to AVB (65, 268). There is a reciprocal pattern of chemical synapses formed between interneurons and sensory neurons. Such an arrangement may serve to reinforce the potency of the signal while quenching an inappropriate response (103). For example, PVC makes a gap junction to the posterior touch receptors, and at the same time, this interneuron forms a chemical synapse to anterior touch receptors (Fig. 6). The chemical synapse might negatively regulate other neuronal input, thus preventing inadvertent forward locomotion upon stimulation at the anterior part of the body. When PVC is ablated, posterior touch response is eliminated, while ablation of AVD abolishes anterior touch response (65). In both cases, gap junctions to the touch receptor neurons are involved in relaying the signal (65, 268). These neurons modify movement but are not essential for normal locomotion. Hence, they are principally engaged in mediating the touch response. Contrary to PVC and AVD, ablation of AVA and AVB results in touch sensitive but uncoordinated animals (65). Therefore, these two interneurons are primarily involved in regulating normal locomotion. The posterior touch system is more complex. Although PLMR makes direct synapses to interneurons AVA and AVD, PLML does not. However, both tail touch receptors form gap junctions with a pair of cells, LUAR and LUAL. LUAL/R in turn form chemical synapses with AVM and AVD and gap junctions with PVR (65, 103). Because of the complexity of these synaptic relationships, it has not been possible to confirm experimentally the role of the LUA cells in the touch response

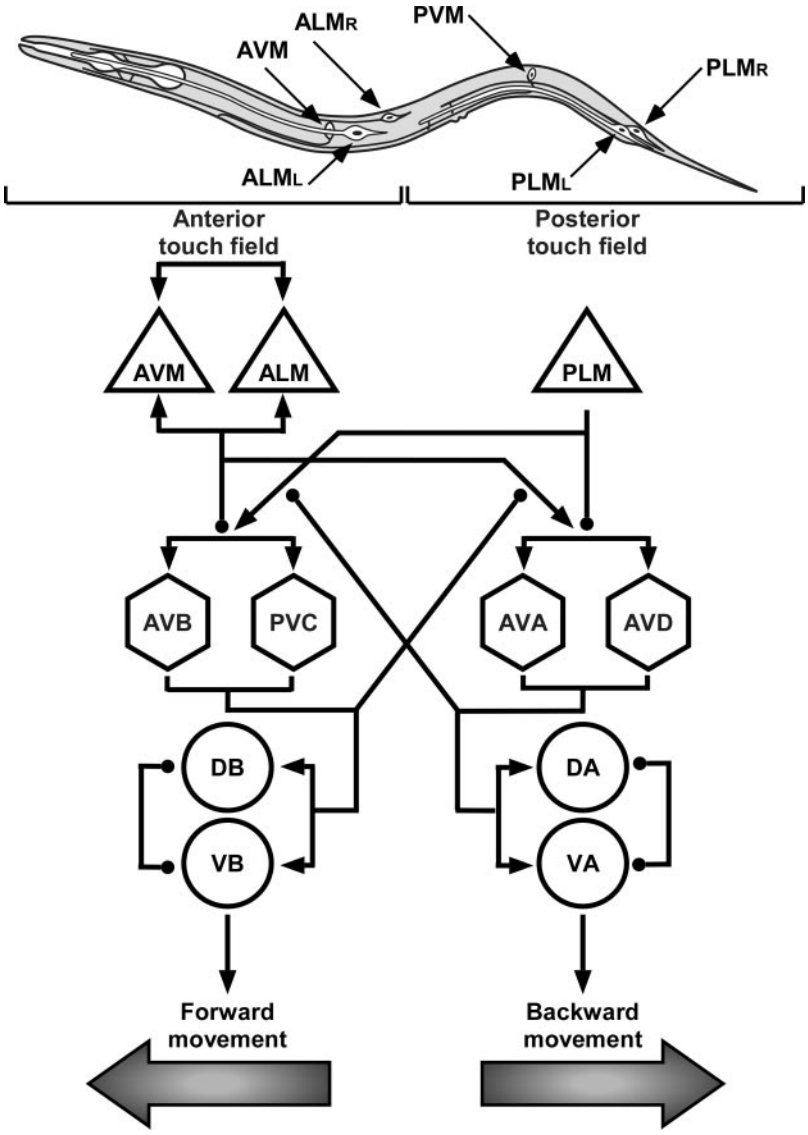

FIG. 6. Neuronal circuitry for locomotion in response to gentle body touch. Interconnections between sensory neurons (triangles; ALMs, AVM, PLMs), interneurons (hexagons; AVB, PVC, AVA, AVD) and motorneurons (circles; DB, VB, DA, VA) are shown (65, 103). Arrowheads represent stimulatory connections, and dark circles represent inhibitory connections. Sensory input from the anterior touch field inhibits forward movement and stimulates backward movement. Sensory input from the posterior field produces the opposite effect.

circuit. Nevertheless, this connectivity plan adds to the asymmetry of the touch circuit; the anterior touch neurons form chemical synapses anterior to the ventral cord, while the LUA interneurons make synapses posterior to the ventral cord.

Interestingly, the touch cells synapse onto many neurons that do not appear to be involved in locomotion (65, 456). These include the cephalic neurons (CEP), the derids (ADE) and the postderids (PDE), which are presumed sensory neurons, and in addition, the RIP interneurons that connect the 20 neurons of the pharynx, the feeding organ of the animal, with the rest of the nervous system, and the HSN motorneurons, which control the egg laying muscles of the vulva (55, 456). Although the significance of these synapses has not been elaborated in detail, such connectivity suggests that touch sensation interfaces with other behaviors of the animal that are either modified by gentle body touch or modify the response to it. Indeed, body touch has been shown to regulate pharyngeal pumping, egg laying, and defecation (103, 469). However, the circuitry underlying these effects has not been characterized. Possibly PVM, which apparently plays no role in touch mediated control of locomotion, might mediate mechanosensory control of some of these other behaviors (469).

2. Development and differentiation of touch receptors

Four of the six touch receptors (ALML/R and PLML/R) are born during embryogenesis. Two additional cells, the AVM and the PVM, arise from identical lineages postembryonically (55, 396; Fig. 3). These touch receptors are situated sublaterally toward ventral on the anterior right and posterior left, respectively. The positions of postembryonic touch receptors AVM and PVM are determined by the migrations of their precursor cells QR and

QL, correspondingly (55, 64, 396). The Q neuroblasts are born at symmetrical positions (left and right) in lateral epidermis, ~1 h before hatching. While they start from the same position, shortly after hatching the precursor to AVM (QR) moves anteriorly and the precursor to PVM (QL) moves slightly posterior (64, 180). AVM arises ~10 h after hatching and forms functional connections via its branch ~20 h later. ALM neurons reach midbody positions by extended migration in the embryo. They migrate posteriorly, and migrations are completed before the general elongation of the embryo (55, 64, 397). Defective ALM migrations would leave the region between ALM and the ends of the PLM axons without mechanosensory innervations. Touch neurons situated in different positions differ in branching, connectivity, and function. Neuronal branching patterns and process trajectories appear dependent in correct cell positioning, but the length of neuron process outgrowth and ultrastructural features are not affected (55, 68).

Even though ALM, PLM, and AVM/PVM cells are derived from three distinct lineages via different patterns of cell divisions, the six touch receptor neurons express nearly identical terminally differentiated features (55, 456). The common touch receptor fate is specified by the concerted action of several groups of genes. For example, genes that function in the execution of cell lineages that generate the touch receptors, the specification of touch cell fate, the restricted expression of touch cell structural genes, the precursor cell migration, and touch cell process outgrowth have been identified in various genetic screens (59, 60, 64, 104, 108). Here, we briefly describe the key players.

The activities of at least three regulatory proteins are required in the final steps of the cell lineages that generate some or all of the touch receptor neurons. Lin-14 acts as a genetic switch that controls the choice to execute the sublineage that generates the postembryonic touch cells AVM/PVM (191, 293). Lin-32, a transcription factor related to *Drosophila aschete-scute* of the basic helix-loop helix family, is needed for the generation of precursors for the PLM and the AVM/PVM cells (108, 293, 323, 367, 481). The unc-86 POU homeodomain protein and the MEC-3 LIM homeodomain protein are essential for directing appropriate differentiation of precursors that generate all six touch receptor neurons (discussed in detail in section III*B3*; Refs. 27, 122, 123, 252). LIN-14, LIN-32, MEC-3, and UNC-86 also appear to be required for the function of differentiated touch receptor neurons (108, 111, 293). In addition, EGL-5 and MAB-5, which are homeodomain proteins, related to the *Drosophila Abdominal-B* and *antennapedia*, respectively, determine posterior cell fate and migration and are needed for touch sensitivity in the tail (77, 367). *mab-5* expression is necessary and sufficient to make the QL (the progenitor of PVM) neuroblast distinct from QR (the progenitor of AVM; Ref. 174). *mab-5* affects migration of the QL cell, but not the ability of descendents to differentiate to mechanosensory neurons. In the *mab-5(e1239)* mutant, QL is situated more anteriorly than in wild type (174, 358). Consequently, the resulting PVM cell adopts an anterior touch cell fate similar to AVM; it branches and becomes capable of mediating a weak touch response akin to AVM (103). Mutations in *mig-1*, which encodes a *frizzled*-related, G protein-coupled *wnt* receptor, also affect QL migration (174, 448).

Another important aspect of specification of touch cell fate is the action of regulatory genes that restrict expression of touch cell-specific genes (103, 293). *egl-44* and *egl-46* turn off expression of touch receptor neuron-specific genes in the FLP neurons, *pag-3* turns off their expression in the lineage sisters of the ALM cells, the BDUs, and *sem-4* prevents *mec-3* expression in the tail PHC neurons (293). Moreover, two genes involved in the regulation and execution of programmed cell death, *ced-3* and *ced-4*, help restrict the number of cells expressing touch cell-specific features. The activity of *ced-3* and *ced-4* ensures the elimination of cells destined to die within the lineages that give rise to the six touch receptors (191, 293). In their absence, extra cells with touch neuron-specific characteristics emerge, located near the PLM and AVM and PVM cells. Additional spurious touch cells are also occasionally seen in *unc-40* (extra ALM and PLM), *unc-73* (duplicated ALML, AVM, PVM and PLM), and *vab-8* mutants (382, 448, 462).

An assortment of >25 genes affects axonal guidance and/or positioning of the touch receptors (59, 371). Here, we epigrammatically catalogue the genes involved and defects observed in mutant animals. Mutations in *unc-13*, *unc-14*, *unc-33*, *unc-44*, *unc-51*, *unc-53*, *unc-59*, *unc-69*, *unc-71*, *unc-73*, *unc-75*, and *unc-85* cause the most pronounced defects in touch cell morphology, axonal outgrowth, and positioning (abnormal varicosities, arborizations, and growth in abnormal directions). Mutations in 13 others, *unc-1*, *unc-3*, *unc-5*, *unc-6*, *unc-27*, *unc-30*, *unc-34*, *unc-40*, *unc-55*, *unc-61*, *unc-62*, *unc-98*, and *unc-104*, result in less severe defects. Specifically, *unc-34*, *unc-76*, and *unc-71* are considered necessary for normal longitudinal axon elongation and fasciculation (180). *unc-14*, *unc-33*, *unc-44*, and *unc-73* affect multiple aspects of axonogenesis, namely, circumferential growth, elongation fasciculation, and axonal ultrastructure (181). *unc-53* and *unc-73* are particularly involved in the proper guidance of PLMs; PLM outgrowth is affected in mutant animals, and neurons veer abnormally into the ventral nerve cord (183, 448). *unc-59* and *unc-85* affect cytokinesis, and mutant animals show more than one axonal process (191). Ventral growth of AVM is affected by mutations in *unc-1*, *unc-5*, *unc-6*, *unc-27*, *unc-34*, *unc-51*, *unc-55*, *unc-59*, and *unc-61* (181, 289). *unc-6*, *unc-13*, *unc-33*, *unc-44*, *unc-51*, *unc-61*, *unc-71*, *unc-73*, and *unc-98* mutants

show abnormal PLM processes among other defects (181, 306, 382).

The picture that emerges is that the concerted activity of genes forming a complex pattern of regulatory relationships, specifies touch cell fate, restricts this fate to the appropriate cells, and ensures their proper development, anatomic integrity, and fine structure. We further elaborate on the genes that function within the touch receptors themselves to provide mechanosensory characteristics in the following section.

3. Genetics and molecular biology of the gentle body touch response: the mec genes

To identify molecules dedicated to touch transduction, Brenner, Chalfie, Dew, Sulston, and colleagues (59, 60, 64, 108, 162, 193, 395) mounted a tour-de-force exercise of forward genetics, to isolate gentle body touch-insensitive nematode mutants. Briefly, populations of wild-type, touch-sensitive animals were mutagenized, and touch-insensitive individuals were sought among their descendants by stroking with an eyelash hair and prodding with a platinum wire (58). During the course of this very tedious screening process, over 417 mutations in 17 different genes, randomly distributed in all six chromosomes of *C. elegans*, were isolated (103, 402). By design, the screen yields mutations in genes that are fairly specific for normal gentle body touch perception. For example, gene mutations with pleotropic effects that result in lethality or uncoordinated and paralyzed phenotypes would have been missed. In addition to being touch insensitive, *mec* mutants tend to be lethargic when grown normally in the presence of ample food (103). Reduced spontaneous movement is probably due to their inability to sense microvibrations in their environment, interaction with external objects, or stretch produced by the locomotory movements themselves. However, when starved or during mating they move as well as wild type. The 17 genes isolated are designated as the *mec* genes for their "mechanosensory abnormal" phenotype (see Table 1). It is likely that the screen has reached statistical saturation, since multiple alleles have been isolated for each gene (103, 190). Corroborating the high specificity of the screen, while most of the alleles generated cause complete touch insensitivity, only a few other abnormalities accompany the mutants (103). As expected, certain types of mutations are missing from the *mec* collection. For example, mutations that specifically disrupt cell migrations and process outgrowth were not isolated. Thus genes that dictate aspects of process extension and precursor cell positioning, which affect the patterning of the nervous system in general rather than being touch cell specific, have not been identified with the screen. Furthermore, genes that are required specifically for the function of the anterior or posterior touch cells also have not been identified (59, 103). Hence, despite the fact that various touch neurons have distinct morphologies and positions, they appear to operate under the same set of instructions.

Depending on their role and point of action, *mec* genes can be loosely classified into three main categories: *1*) the regulatory/specification genes that control the expression touch receptor neuron specific genes or modify the activity of the mechanotransducer complex; *2*) the *mec* genes encoding core structural components of the mechanosensitive ion channel; and *3*) the genes encoding peripheral, associated proteins.

A) REGULATORY/CELL-SPECIFICATION GENES. *mec* genes that control the expression of other *mec* genes or genes specific to the touch receptor neurons belong to this category. We also discuss here *mec* genes encoding putative regulators or modifiers of the activity of the touch receptor mechanotransducer. The key member of this group is *mec-3*, which encodes a LIM homeodomain transcription factor, 42% similar to the *paired* protein, with a strongly acidic region at the carboxy terminus of the protein (54, 59, 446–449). In *mec-3* loss-of-function mutants, the pattern of cell divisions that give rise to touch receptor neurons is typical. However, cells that would normally differentiate to touch cells express none of their distinguishing features, such as the specialized mantle and 15-protofilament microtubules (59, 111). Affected cells do not simply lack the ability to differentiate into touch cells; they also appear to adopt different neuron fates (446). ALMs in *mec-3* background are positioned more anteriorly and more laterally, have smaller cell bodies, and have additional posteriorly directed processes (55, 59). In these respects they resemble their lineage sister the BDU cells. Interestingly, the positions of PLMs, or other cells are not affected. In contrast, increase in *mec-3* dosage can induce additional cells expressing touch receptor neuron-specific genes (111).

mec-3 is expressed in 10 cells, which are all anterior daughters of terminal asymmetric cell divisions, the six touch receptor neurons and the PVDs and FLPs (446, 448). The posterior sister cell is either an interneuron or undergoes programmed cell death (108, 191, 396, 397). *lin-17*, a gene involved in maintaining asymmetry of cell divisions, and *unc-73* and *unc-40*, genes that influence polarity, contribute to the asymmetric expression of *mec-3* (27, 448, 449). This demonstrates that *mec-3* expression alone is not sufficient to determine touch cell fate. PVD mediates a response to harsh mechanical stimuli, and FLP has features that suggest it could be a mechanoreceptor (anterior process that has a ciliated ending characteristic of other mechanosensory cells; Refs. 215, 447). PVD has long anterior and posterior processes that make essentially no synapses and are likely to be sensory. Both PVD and FLP have ventral processes that synapse onto interneurons that control forward and backward movement (443, 455, 456). Loss of *mec-3* does not cause

TABLE 1. *The 17 Caenorhabditis elegans mec genes*

Name	ORF	Chromosome	Similarity	Function	Associated Phenotype	Reference Nos.
mec-1	T07H8.4	V	EGF/Kunitz repeat protein	Mantle component	Touch insensitive; lethargic; microtubule cells lack extracellular mantle often displaced; some amphidial neurons also displaced, defective fasciculation	L. Emptage, G. Gu, and M. Chalfie, unpublished data
mec-2	F14D12.4	X	Stomatin-like	Channel-associated protein	Touch insensitive; lethargic	194
mec-3	F01D4.6	IV	LIM homeodomain transcription factor	Specification of touch cell differentiation	Touch insensitive; lethargic; microtubule cells small and lacking processes, ALM and PLM cells displaced	446
mec-4	T01C8.7	X	Amiloride-sensitive Na^+ channel protein (degenerin)	Mechanosensitive channel	Touch insensitive; lethargic	101
mec-5	E03G2.3	X	Collagen	Extracellular matrix component	Touch insensitive; lethargic; mantle of microtubule cells not stained by peanut lectin	109
mec-6	W02D3.3	I	Paraoxonase/arylesterase	Regulator of channel stability/activity	Touch insensitive; lethargic	73
mec-7	ZK154.3	X	β-Tubulin	15-Protofilament microtubule component	Touch insensitive; microtubule cells lack microtubules	360
mec-8	F46A9.6	I	RNA-binding domain	RNA splicing	Touch insensitive lethargic; disrupted fasciculation of amphid and phasmid channel cilia	263
mec-9	C50H2.3	V	EGF/Kunitz repeat protein	Extracellular matrix component	Touch insensitive; lethargic	109
mec-10	F16F9.5	X	Amiloride-sensitive Na^+ channel protein (degenerin)	Mechanosensitive channel	Touch insensitive; lethargic	193
mec-12	C44B11.3	III	α-Tubulin	15-Protofilament microtubule component	Touch insensitive; lethargic, few microtubules in touch cell processes, no 15-protofilament microtubules	135
mec-14	F37C12.12?	III	Aldo-keto reductases; *Shaker*-type K^+ channel β-subunit	Regulator of channel activity	Touch insensitive; lethargic	M. Chalfie and G. Caldwell, unpublished data
mec-15	?	II	?	May interact with microtubules	Touch insensitive; lethargic	G. Gu and M. Chalfie, unpublished data
mec-16	F31E8.3	II	Homeobox transcription factor; *bsh* (brain-specific homeobox)	?	Fails to respond to prodding with a platinum wire; also known as *tab-1* (touch abnormal) and *ceh-29* (*C. elegans* homeobox)	L. Carnell and M. Chalfie, unpublished data
mec-17	F57H12.7	IV	?	Maintenance of touch cell differentiation	Larvae touch sensitive, older animals touch insensitive	480
mec-18	C52B9.9	X	AMP-binding motif	Negative channel regulator	Touch insensitive; lethargic	G. Gu and M. Chalfie, unpublished data

mec-13 is not listed since it has been shown to correspond to the *mec-4* locus (64, 101).

PVD to adopt the fate of its sister, which dies by programmed cell death (59, 293, 446). In this case *mec-3* is acting on a single cell at the end of the lineage. Furthermore, unlike in touch receptor neurons, *mec-3* mutations do not change the ultrastructural features of PVD and FLP (448).

The *unc-86* gene product is needed to initiate expression of *mec-3*. *unc-86* is expressed exclusively in 57 neurons, only 10 of which express *mec-3* (123). As such, *unc-86* is always expressed in the parent of the *mec-3* expressing cell (122, 123). Therefore, *unc-86* is not sufficient to activate *mec-3* expression, since another 47 neurons express *unc-86* but not *mec-3*. *unc-86* is expressed throughout the touch cell life, suggesting that it may function in maintenance as well as in lineage specification (27, 122, 191). MEC-3 and UNC-86 associate to form heterodimers that bind to well-defined sites in the *mec-3* and other touch cell-specific gene promoters (such as *mec-4* and *mec-7*) to stimulate gene expression (252, 344, 345, 447, 449, 474, 475). The MEC-3 LIM homeodomain and the UNC-86 POU homeodomain are sufficient to mediate interaction in vitro, but more of each protein is needed for heterodimer formation on DNA in vivo (474, 475). Therefore, once *mec-3* expression is triggered in touch receptors, a positive-feedback loop stimulates its own synthesis (447). *unc-86* continues to be expressed in differentiated touch receptors and is continuously needed for their function (123). Genetic analysis suggests that later in development, *mec-17* also contributes to the maintenance of *mec-3* expression (see below; Refs. 447, 480). The biochemical properties of MEC-17 have not been determined, and the protein does not bear significant similarity to proteins of known function (480). Interestingly, *mec-17* was among the 71 genes identified by microarray analysis to be dependent on MEC-3 for their expression in touch receptor neurons (480). Seventeen of these newly identified *mec-3*-dependent genes contain the consensus sequence for the binding of the MEC-3::UNC-86 heterodimer in their 5′-region, indicating that they are direct targets of *mec-3* regulation (449, 480). Apart from *mec-17*, eight more *mec* genes were picked by microarray analysis as being *mec-3* dependent: *mec-1*, *mec-3*, *mec-4*, *mec-7*, *mec-9*, *mec-12*, *mec-14*, and *mec-18* (480). Likewise, an additional gene, *unc-24*, which shares similarity with *mec-2* and was previously known to be expressed in touch receptor neurons, also appears to be under *mec-3* control (19, 480). *unc-24* has been implicated in regulation of locomotion and may play a role in mechanotransduction, which we discuss more extensively in section III*C*. Although many of the remaining identified genes encode proteins of unknown function, some others may have specific and intriguing function in the touch receptors. Thus the *mec-3*-dependent expression of genes encoding components of the T-complex chaperonin (*cct-1*, *cct-2*, and *cct-4*) in touch receptors is in concert with the role of this chaperonin in the correct folding of α- and β-tubulin and the assembly of microtubules that are packed in *C. elegans* touch receptor neurons. Additional *mec-3*-dependent genes encode a TASK potassium channel protein, a GABA receptor subunit; four putative enzymes (metallo-β-lactamase, acid phosphatase, tyrosine kinase, and esterase); and two proteins that are similar to antigens from the parasitic nematode *Onchocerca volvulus* (480).

Two other genes needed for body touch sensitivity, *mec-8* and *mec-14*, appear to play regulatory roles. *mec-8* alleles disrupt touch sensitivity (92, 264), but they also affect other sensory structures, attachment of body wall muscle to the hypodermis and cuticle, and embryonic and larval development (263, 310). Molecular analysis has shown that the MEC-8 protein, which includes two RNA binding motifs, is required for splicing of several messages including that of the MEC-2 protein (194, 264). *mec-14* mutations do not perturb touch receptor ultrastructure but can partially suppress *mec-10*(A673V)-induced death (see below and sect. IV*C*), suggesting that MEC-14 could influence channel function (162). *mec-14* encodes a member of the aldo-keto reductase superfamily (M. Chalfie and G. Caldwell, unpublished data). Interestingly, the β-subunit of the *Shaker*-type K^+ channels, which modifies channel properties (161, 182), is also a member of this family (283).

Three more *mec* genes have been hypothesized to serve various regulatory roles in touch receptor neurons: *mec-15*, *mec-16*, and *mec-18* (103, 104, 162). Genetic evidence suggests that MEC-15 may interact with microtubules (G. Gu and M. Chalfie, unpublished data). *mec-16* encodes a brain-specific homeobox (*bsh*) transcription factor (L. Carnell and M. Chalfie, unpublished data). Finally, MEC-18 contains an AMP binding motif and may negatively regulate the channel formed by MEC-4 and MEC-10, since mutations in *mec-18* enhance *mec-10*(A673V)-induced cell death (G. Gu and M. Chalfie, unpublished data; Ref. 162).

B) MECHANOSENSOR CORE STRUCTURAL COMPONENTS. Four *mec* genes can be classified in the category of core structural components of the putative mechanosensory ion channel in touch receptor neurons: *mec-2*, *mec-4*, *mec-6*, and *mec-10*. The standard model for mechanotransduction in *C. elegans* dictates that MEC-4 and MEC-10 form the core ion channel while MEC-2 and MEC-6 physically interact with the channel subunits to shape and modulate their gating properties.

Animals bearing loss-of-function mutations in *mec-4* or *mec-10* are touch insensitive despite the fact that in these mutant backgrounds the touch receptor neurons develop normally and exhibit no apparent defects in ultrastructure (56, 59). *mec-4* and *mec-10* encode homologous proteins related to subunits of the multimeric amiloride-sensitive Na^+ channel which mediates Na^+ reabsorption in vertebrate kidney, intestine, and lung epithelia (the ENaC channel; Refs. 50–52, reviewed in Refs. 221, 348). By analogy, MEC-4 and MEC-10 are likely ion channel subunits. Indeed, channel activity has recently been directly demonstrated for MEC-4 and MEC-10 (155) and for UNC-105, a related degenerin that is expressed in muscle

cells (140; see below). In addition, chimeric nematode/rat proteins function in *C. elegans* and in *Xenopus* oocytes, implying that the nematode and rat proteins are functionally similar (189; reviewed in Ref. 221). *mec-4* is expressed only in the six touch receptor neurons, and *mec-10* is expressed in the six touch receptor neurons and in two other neuron pairs that may mediate stretch-sensitive responses (FLPs and PVDs; Refs. 101, 104, 193). Interestingly, a MEC-4::GFP fusion localizes in distinct puncta along the processes of the touch receptor neurons (Fig. 7). Such punctuate localization may reflect the distribution of mechanotransducing complexes on the axon of the touch receptor neuron. Because the MEC-4 and MEC-10 subunits are expressed exclusively in mechanosensitive neurons and are essential for the function of these neurons, it has been proposed that MEC-4 and MEC-10 coassemble into a mechanically gated ion channel that plays a central role in touch transduction. These proteins have been shown to assemble into ion channels, which are amiloride sensitive in heterologous systems (140, 155). Such pharmacological properties are intriguing because amiloride is a general inhibitor of mechanosensitive ion channels (7, 167, 170, 188, 245, 347, 428). Experimental verification that the MEC-4/MEC-10 channel is mechanically gated remains a challenge for the future.

In addition to being involved in mechanotransduction, MEC-4, MEC-10, and several other related nematode proteins have a second, unusual property: specific amino acid substitutions result in aberrant channels that induce the swelling and subsequent necrotic death of the cells in which they are expressed (100, 101, 165, 172, 369). This pathological property is the reason that this family of proteins was originally called degenerins (61, 70, 101, 403, 404). For example, unusual gain-of-function (dominant; d) mutations in the *mec-4* gene induce degeneration of the six touch receptor neurons required for the sensation of gentle touch to the body (101, 172). *C. elegans* degenerins, together with their relatives, in other species ranging from snails to *Drosophila* and mammals, comprise the large DEG/ENaC family of ion channels (see Fig. 13; Ref. 87). We discuss the intriguing capacity of mutant degenerins to inflict neuron degeneration in section IV*C*.

mec-2 encodes a 481-amino acid protein and is expressed in the touch receptor neurons and in a few additional neurons in the nerve ring region (108, 162, 194). The MEC-2 protein appears to be localized all along the length of the touch receptor process as well as in the cell body (194). MEC-2 features three candidate protein-protein interaction domains (Fig. 8). First, part of the amino-terminal domain (situated in part between amino acids 42–118) is needed for the proper localization of a MEC-9::LacZ fusion protein to the touch receptor process. Second, the carboxy-terminal domain includes a proline-rich region that is similar to SH3-binding domains. Third, the central region (amino acids 114–363) encompasses an SPFH domain with a membrane-associated hydrophobic part (amino acids 114–141) and a cytoplasmic hydrophilic part that together exhibit 65% identity to the human red blood cell protein stomatin (194, 407). The SPFH domain is the common denominator of stomatins, prohibitins, flotilins, and bacterial *Hfl*K/C proteins, all of which are membrane-associated regulators (Fig. 9; Ref. 407). Stomatin, also known as band 7.2b protein, is a membrane-associated protein originally identified as a component of human red blood cells (97, 364, 376, 383, 384, 484). In humans, stomatin is missing from erythrocyte membranes in autosomal dominant hemolytic disease overhydrated hereditary stomatocytosis, despite an apparent normal stomatin gene. In addition, knocked-out mice for the stomatin gene did not have any hemolytic phenotype, and thus the precise function of mammalian stomatin remains unknown (484). In both *C. elegans* and mammals, the stomatins have been shown to exist as oligomers and appear to interact with other gene products. In mammals, stomatin was found to interact with a G protein-coupled receptor and to colocalize with glycophosphoinositol (GPI)-anchored proteins and lipid rafts (364, 377, 407). Lipid rafts are microdomains in the cell membrane that are thought to facilitate the localization of multiple membrane proteins into complexes, thus ensuring proper relative posi-

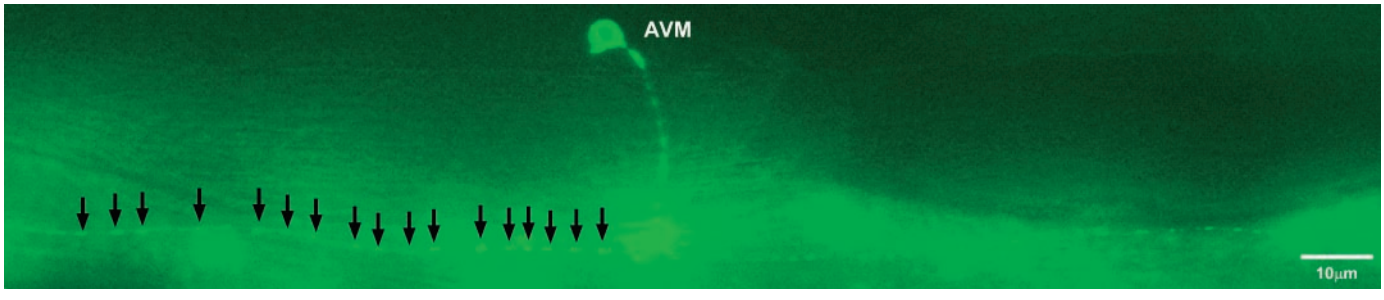

FIG. 7. Punctate localization of a putative mechanosensitive ion channel subunit. Image of an AVM touch receptor neuron expressing a GFP-tagged MEC-4 protein. Fluorescence is unevenly distributed along the process of the neuron in distinct puncta, which may represent the location of the mechanotransducing apparatus.

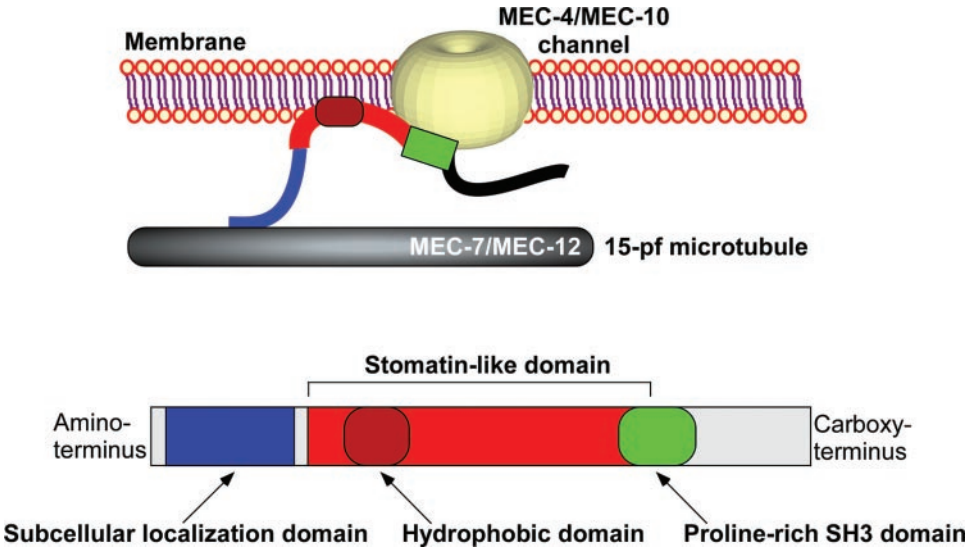

FIG. 8. Schematic representation and topology of the MEC-2 protein. Conserved domains as well as hydrophobic regions are highlighted. Putative interactions with the degenerin channel and the cytoskeleton are indicated (155, 193, 194, 407).

tioning required for physical interactions (364). Interestingly, stomatin also shows limited similarity to caveolin, which is a protein crucial to the formation of caveolae (structures analogous to lipid rafts; Refs. 343, 425). These intriguing similarities have prompted us to suggest that one possible function for stomatins is the stabilization or the regulation of the activity of proteins localized in lipid rafts and other membrane microdomains as well as the trafficking and turnover of specific plasma membrane proteins (407). In addition to MEC-2 the *C. elegans* genome encodes eight stomatin-related proteins, two of which, UNC-1 and UNC-24, have been genetically characterized. UNC-1 and UNC-24 are required for normal locomotion. In addition, UNC-1 is required for normal responsiveness to volatile anesthetics, which suggests that it is a molecular target for volatile anesthetics (discussed further in sect. III*C2*; Refs. 329, 330, 364).

Many of the 54 mutant *mec-2* alleles have dominant effects and exhibit a complex pattern of interallelic complementation (64, 162), indicating that MEC-2 protein

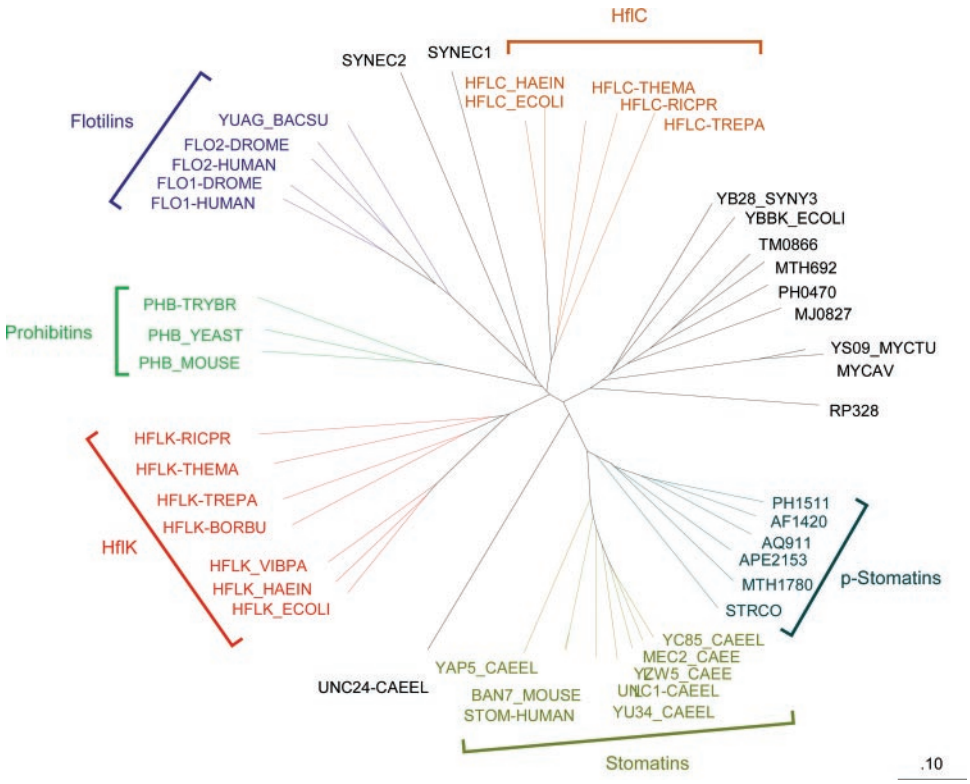

FIG. 9. Phylogenetic relations among SPFH domain proteins. The dendrogram shows distance relationships among most of the stomatin protein superfamily members. (The complete ClustalW generated alignment on which the dendrogram was based is available at http://www.imbb.forth.gr/worms/worms/alignment.gif.) The dendrogram was constructed with the neighbor-joining method (357) based on pairwise distance estimates of the expected number of amino acid replacements per site (0.10 in the scale bar) and visualized by TreeTool (http://www.hgmp.mrc.ac.uk/Registered/Option/treetool.html). Protein subfamilies are denoted in different colors (407).

molecules form higher order complexes. However, there is also genetic data that suggest MEC-2 interacts with the specialized touch cell microtubules encoded by *mec-7* and *mec-12* (β-tubulin and α-tubulin, respectively; see below and Refs. 162, 194). Normally, a MEC-2::LacZ fusion protein is distributed along the touch receptor axon (194). The axonal distribution of a MEC-2::LacZ fusion protein is mildly disrupted in a *mec-7* null or *mec-12* strong loss-of-function background, implying that the 15-protofilament microtubules are not essential for the localization of MEC-2 to the neuronal process. However, two specific *mec-12* missense alleles interfere dramatically with localization of MEC-2 fusion proteins, restricting the fusion proteins to the cell body (194). One of the *mec-12* alleles encodes a single amino acid substitution close to a microtubule-associated protein (MAP)-binding region in other α-tubulins; the other affects a residue in the carboxy-terminal domain (194). These analyses of the MEC-2::LacZ fusion protein suggest that residues in the MEC-2 amino terminus and the MEC-12 α-tubulin carboxy terminus could interact (162, 194). There is also genetic and biochemical evidence that the MEC-2 protein functionally interacts with the touch receptor channel. Certain *mec-2* alleles partially suppress *mec-10*(A673V)-induced death (193). In addition, some recessive *mec-2* alleles act as dominant enhancers of a weak *mec-4(ts)* allele (162, 194). In other words, when a temperature-sensitive *mec-4* mutant is reared at the maximum temperature at which the touch receptors still function, adding a single mutant copy of the *mec-2* gene to the strain background can push the touch receptor neuron over the threshold into a nonfunctional state (402). These genetic studies, which do not by themselves prove a direct interaction, have recently been complemented by elegant heterologous expression experiments in *Xenopus* oocytes that support physical interaction between MEC-2 and the channel subunits MEC-4 and MEC-10 (155). Reconstitution of channel activity in *Xenopus* oocytes revealed that MEC-2 regulates the activity of the MEC-4/MEC-10 channel, providing the first direct support for the hypothesis that stomatin-like proteins interact with and regulate ion channels (155, 384). This interaction appears to dramatically potentiate the conductivity of the channel in oocytes. Coexpression of MEC-2 with the hyperactive MEC-4(d) and MEC-10(d) derivatives in *Xenopus* oocytes resulted in an ~40-fold increase in the amplitude of amiloride-sensitive ionic currents, and this amplification allowed currents to be detected even with wild-type MEC-4 and MEC-10 proteins (155). Visualization of tagged MEC-4(d) and MEC-10(d) in live oocytes demonstrated that MEC-2 does not increase the number of MEC-4(d)/MEC-10(d) channels that reach the plasma membrane, and probably acts by regulating their activity.

All three domains of MEC-2 are needed for full activity of the protein in *Xenopus* oocytes. Interestingly, human stomatin can partially substitute for MEC-2 in the generation of amiloride-sensitive currents, suggesting a common function of stomatin-like proteins (155). In addition, both the stomatin-like domain of MEC-2 and human stomatin produced a strong negative effect when coexpressed with full-length MEC-2 protein, supporting the hypothesis that MEC-2 forms multimers via the central domain (155). Taken together, these results are consistent with the simple hypothesis that MEC-2 tethers the 15-protofilament microtubules to the degenerin channel and largely determines its physiological properties.

Recessive *mec-6* mutations disrupt touch sensitivity but do not cause detectable changes in touch cell ultrastructure (64). *mec-6* alleles have the interesting property that they completely block *mec-4(d)* and *mec-4*(A673V)-induced touch cell degeneration, i.e., in *mec-6(lf); mec-4(d)* and *mec-6(lf); mec-10(A673V)* double mutant strains, cell death is suppressed (*lf*, loss-of-function allele; Refs. 172, 193). *mec-6* encodes a protein with limited similarity to paraoxonases/arylesterases that physically interacts with MEC-4 and MEC-10 (73). How exactly MEC-6 acts to influence MEC-4/MEC-10 channel activity is unknown. Nevertheless, it appears that *mec-6* mutations do not affect *mec-4* transcription, although they do cause full-length MEC-4::LacZ or MEC-4::GFP reporter fusion chimeras to be rapidly degraded (N. Tavernarakis, unpublished observations; Ref. 73). Thus working hypotheses concerning the function of MEC-6 focus on two possibilities. First, MEC-6 is another subunit needed for channel function or assembly, or second, it mediates localization or posttranslational modification essential for MEC-4 and MEC-10 activity/stability. It should be noted that MEC-6 function is not exclusively related to the MEC-4/MEC-10 touch receptor channel. *mec-6* mutations also suppress the deleterious consequences of neurodegeneration-inducing mutations in other *C. elegans* degenerins including *deg-1*, *unc-8*, and partly *unc-105* (70, 258, 369, 409) (N. Tavernarakis, unpublished observations).

C) PERIPHERAL ASSOCIATED PROTEINS. Two subgroups of *mec* genes encoding peripheral components required for mechanotransduction in the touch receptor neurons can be defined: those encoding intracellular protein (*mec-7*, *mec-12*) and those encoding extracellular protein (*mec-1*, *mec-5*, *mec-9*) (103, 104, 402).

As described previously, the touch receptor processes are filled with bundled 15-protofilament microtubules. Mutations in two genes, *mec-7* and *mec-12*, disrupt the formation of these microtubules (59, 64, 135, 166, 360, 361). Interestingly, even in the absence of the 15-protofilament microtubules, the touch receptor processes grow out seemingly normally and become filled with 11-protofilament microtubules (60, 66, 67). Such touch receptors do not function, however, suggesting that the extensively

cross-linked 15-protofilament microtubules contribute a specific role in touch transduction.

mec-7 encodes a β-tubulin expressed at high levels in the touch receptor neurons (166, 360, 361). MEC-7 is highly conserved, apart from the carboxy-terminal domain that is characteristically highly variable; only 7 amino acids differ from other β-tubulins. It is not known whether any of the unique residues are instructive for the formation of 15-protofilament microtubules, although it is interesting that one of the amino acid differences affects a strictly conserved cysteine residue (Cys-293) that has been implicated in protofilament assembly by analysis of *Drosophila* mutants (349, 361). *mec-7* mutations isolated in the screen for touch-insensitive mutants range in severity from recessive to strongly dominant, and most of the amino acid changes that disrupt MEC-7 function are known (360, 361). Domains affected by mutations include sites for GTP binding and hydrolysis, sites for heterodimerization with α-tubulin, and sites for higher order microtubule assembly (361).

mec-12 encodes an α-tubulin expressed at high levels in the touch receptor neurons but also expressed in several other neurons that do not assemble 15-protofilament microtubules (135). Thus the presence of the MEC-12 tubulin is not sufficient to nucleate assembly of the touch-cell specific microtubules. As is the case for *mec-7*, many *mec-12* mutations are semi-dominant or dominant and are likely to disrupt subunit interactions or protofilament assembly (162). MEC-12 is the only *C. elegans* tubulin that is acetylated (135). This posttranslational modification has been implicated in the regulation of microtubule stability and function. However, a *mec-12* point mutant lacking the acetylation site can also mediate touch sensitivity, indicating that acetylation is not essential for this function (135). The totality of the studies on *mec-7* and *mec-12* strongly support that unique α- and β-tubulins assemble to form the 15-protofilament microtubules required for touch receptor function. Whether these specialized microtubules play a direct role in the function of the mechanotransducing complex remains to be determined. Perhaps these microtubules form a rigid intracellular grid that connects to the mechanotransducing ion channel, providing a necessary anchoring point (see discussion below).

In *mec-1* mutants, touch cells generally lack the mantle and associated periodic specializations of the overlying cuticle (64, 162, 361). The ALM processes are somewhat displaced and run along body wall musculature rather than within the hypodermis (55). Where portions of the touch processes are embedded within the hypodermis in *mec-1* mutants, however, mantle is present. Whether the mantle acts to position the touch cell processes or, alternatively, whether incorrect positioning of the process leads to the failure to produce the mantle remains to be determined. *mec-1* is expressed in touch receptor neurons, other lateral neurons, and intestinal muscles (L. Emptage, G. Gu, and M. Chalfie, unpublished data). It encodes a likely secreted protein with multiple Kunitz-type serine protease inhibitor and EGF domains (L. Emptage, G. Gu, and M. Chalfie, unpublished data). The Kunitz and EGF domains are likely to be protein interaction domains.

mec-5 mutations disrupt the extracellular matrix in a subtle manner; the mantle in a wild-type animal can be stained with peanut lectin, whereas the mantle in *mec-5* mutants cannot (64, 109, 162). *mec-5* encodes a novel collagen type that is secreted by hypodermal cells (109). The central portion of the MEC-5 protein is made up of Pro-rich Gly-X-Y repeats. *mec-5* mutations, many of which are temperature sensitive, cluster toward the carboxy terminus of the protein and affect these repeats. What role the unique sequences in the amino and carboxy termini contribute to MEC-5 function is not clear, since there are no *mec-5* mutations that map to these regions. Genetic interactions suggest that *mec-5* influences MEC-4/MEC-10 channel function [for example, *mec-4* and *mec-10* mutations can enhance the *mec-5(ts)* mutant phenotype; Ref. 162]. Thus a specialized collagen could interact with the touch receptor channel, perhaps acting to provide gating tension. The potential importance of collagen::degenerin interactions is underscored by studies of another degenerin family that is expressed in muscle, *unc-105* (258). Dominant, gain-of-function mutations in *unc-105* cause severe muscle hypercontraction (307). Specific alleles of *let-2*, which encodes a type IV basement membrane collagen, suppress the *unc-105(d)* phenotype (see sect. III*C*; Refs. 258, 307). Thus, although direct interactions of collagens and degenerin channels remain to be proven, such associations may emerge as a common theme in the function of this channel class.

mec-9 mutations do not alter mantle ultrastructure in a detectable manner, despite the fact that *mec-9* encodes a protein that appears to be secreted from the touch receptor neurons (64, 109). The *mec-9* gene generates two transcripts, the larger of which encodes an 834-amino acid protein (MEC-9L) that is expressed only by the touch receptors (109). Akin to MEC-1, the predicted MEC-9L protein contains several domains related to the Kunitz-type serine protease inhibitor domain, the Ca^{2+}-binding EGF repeat, the non-Ca^{2+}-binding EGF repeat, and a glutamic acid-rich domain (109). Single amino acid substitutions that disrupt MEC-9 function affect the two Ca^{2+}-binding EGF repeats, the sixth EGF repeat, and the third Kunitz-type domain, thus implicating these regions as important in MEC-9 function (109). How MEC-9 is needed for touch cell activity is not clear, but it is interesting that MEC-9 appears specialized for protein interactions and that agrin, a protein that acts to localize acetylcholine receptors, has a domain structure that appears similarly specialized (agrin features multiple EGF and Kazal-type serine protease inhibitor repeats; Refs. 350–352). *mec-9*

mutations are dominant enhancers of a *mec-5(ts)* allele, suggesting that these proteins might interact in the unique mantle extracellular matrix outside the touch receptor neuron (109, 162).

C. Proprioception: Regulation of Locomotion

C. elegans locomotion ensues from alternate contraction and relaxation of dorsal and ventral body wall muscles, which generates a canonical sinusoidal pattern of movement (456, 469). The arrangement of the body wall muscles and their synaptic inputs restricts locomotion to dorsal and ventral turns of the body. The body wall muscles are organized into two dorsal and two ventral rows. Each row consists of 23 or 24 diploid mononucleate muscle cells arranged in an interleaved pattern (128, 297, 445, 463). Distinct classes of motorneurons control dorsal and ventral body muscles. To generate the sinusoidal pattern of movement, the contraction of the dorsal and ventral body muscles must be out of phase. For example, to turn the body dorsally, the dorsal muscles contract, while the opposing ventral muscles relax. Interactions between excitatory and inhibitory motorneurons produce a pattern of alternating dorsal and ventral contractions (128, 192). Relatively little is known about how the sinusoidal wave is propagated along the body axis. Adjacent muscle cells are electrically coupled via gap junctions, which could couple excitation of adjacent body muscles. Alternatively, ventral cord motorneurons could promote wave propagation, since gap junctions connect adjacent motorneurons of a given class (65, 455, 456). A third possibility is that motorneurons could themselves act as stretch receptors so that contraction of body muscles could regulate adjacent motorneuron activities, thereby propagating the wave (405, 409). We discuss this model further in the following section.

1. Neuroanatomy: the neuronal circuit for locomotion

The adult motor system involves five major types of ventral nerve cord motorneurons, defined by axon morphologies and patterns of synaptic connectivity. A motorneurons (12 VA and 9 DA), B motorneurons (11 VB and 7DB), D motorneurons (13 VD, 6 DD), AS motorneurons, and VC motorneurons command body wall muscles arranged in four quadrants along the body axis (128, 192, 441). At hatching, the locomotory circuit is simpler with only DA, DB, and DD classes of motorneurons present. The rest of the motorneurons are born postembryonically (396). Consequently, early L1 larvae are somewhat uncoordinated (65, 103). Motorneuron processes have presynaptic regions, which form neuromuscular junctions and provide input to other neurons, and postsynaptic regions, which receive input from other neurons. Some classes of neurons form neuromuscular junctions with the ventral body muscles (VA, VB, VC, and VD), while others innervate the dorsal muscles (AS, DA, DB, and DD). Each motorneuron class is composed of multiple members, which form an array along the length of the ventral cord in repeating units (e.g., VA1–VA6). Equivalent motorneuron classes are found in the nervous system of *Ascaris suum*. Ablation studies establish that elimination of DA disrupts backward movement, elimination of DB disrupts forward locomotion, and elimination of DDs disrupts locomotion in either direction (94, 103, 338). Depolarization of cells that correspond to A and B motorneurons of *C. elegans* in the nervous system of the larger nematode *Ascaris lumbridoides* established that these are excitatory. Stimulation of the D motorneurons established that these are inhibitory (209, 387). These observations suggest that A motorneurons stimulate contraction of muscles that mediate backward movement, B motorneurons stimulate contraction of those muscles that mediate forward movement, and D cells mediate contralateral inhibition.

A pattern of alternating dorsal and ventral contractions is produced by interactions between excitatory and inhibitory motorneurons. The A, B, and AS motorneurons utilize the neurotransmitter acetylcholine and are likely to be excitatory (439, 440). Consistent with this are physiological measurements showing that the analogous *Ascaris* motorneurons are both cholinergic and excitatory (365, 387). The D-type neurons are inhibitory and utilize GABA as their transmitter (209, 218, 285, 286, 320). The A- and B-type neuromuscular junctions are organized into characteristic dyadic complexes in which an A or B synaptic terminus is apposed to two distinct postsynaptic elements, a body wall muscle, and a D neuron dendrite (128, 192). The pattern of these dyadic synapses is highly asymmetric. The VD neurons receive input at the dorsal A and B type neuromuscular junctions, and they form neuromuscular junctions ventrally that appear to relax the ventral muscles (320). Thus they are likely to be active during dorsal muscle contractions. The converse set of connections is established by the DD neurons (456). This pattern of connectivity led to the proposal that the D neurons act as cross-inhibitors that prevent the simultaneous contraction of the dorsal and ventral muscles (103, 209).

Forward and backward locomotion are antagonistic behaviors, controlled by distinct neural circuits (see Fig. 6). Four bilaterally symmetric interneuron pairs (AVA, AVB, AVD, and PVC) have large-diameter axons that run the entire length of the ventral nerve cord and provide input to the ventral cord motorneurons (65, 455). These interneurons have distinct patterns of connectivity. AVA and AVD provide input to the A type motorneurons whereas AVB and PVC provide input to the B type motorneurons (76, 103, 455, 459). Laser killing experiments have shown that the ventral cord interneurons and mo-

torneurons can be subdivided according to their function in forward or backward movement (65, 103, 186). The circuit comprising AVB, PVC, and the B motorneurons drives forward locomotion while that comprising AVA, AVD, and the A motorneurons drives backward movement (458, 466). Although to a first approximation forward and backward locomotion reflect the activities of competing circuits, there are several indications that these circuits functionally interact. First, disabling either circuit with a laser also causes mild defects in the opposing behavior (65, 103). Second, mosaic analysis of GLR-1 glutamate receptors, which are expressed in the locomotory interneurons, suggests that both the forward and backward interneurons play a role in backward movement (18, 176, 216, 270, 482). Third, simultaneous activation of the forward and backward circuits with a diffuse mechanical stimulus reveals that these circuits functionally inhibit each other (332, 458). Interaction between these opposing circuits may be mediated by the unusual connectivity of the ventral cord interneurons. The forward and backward interneurons have multiple reciprocal connections, which could mediate coordination of opposing circuits.

How does this circuit create and sustain the rhythmic pattern of sinusoidal locomotory movement? Two general models have been proposed for the generation of sinusoidal locomotion: the central oscillator model and the chain-reflex mechanism of movement pattern generation (303, 380, 381). In several cases in other organisms, networks of neurons have been shown to control rhythmic behaviors, and specific cells in these networks have intrinsic oscillating activity that engenders the observed rhythm (133, 368, 373, 464). These oscillating cells have been termed pattern generators (373, 380). In the case of *C. elegans* locomotion, relatively little is known about how the rhythmicity is engendered. In the related *Ascaris*, the GABA-containing ventral cord motorneurons (equivalent to the D-type motorneurons of *C. elegans*) have an oscillating pattern of electrical activity, leading to the speculation that the GABA motorneurons act as the pattern generator for locomotion (286, 320). A similar pattern-generating mechanism seems unlikely in the case of *C. elegans* because *unc-25* and *unc-30* mutants that lack functional D neurons still generate rhythmic sinusoidal movement, albeit with a reduced amplitude (209). An alternative model has been proposed that bears similarity to the chain-reflex mechanism of locomotory pattern generation, inspired by the subtle, peculiar anatomical features of some ventral nerve cord nematode motorneurons (455, 456). Both A and B motorneurons have long undifferentiated processes distal to the regions containing their neuromuscular junctions. The fine structure of these processes is reminiscent of touch receptor neuron processes (59, 65, 455, 456). Based on such telling characteristics, these terminally undifferentiated processes have been proposed to be stretch sensitive (409). Interestingly, the anterior/posterior polarity of motorneuron processes correlates with function in either forward or backward movement. A-type neurons, required for backward movement (i.e., anteriorly propagated waves), have anteriorly directed processes, whereas B neurons drive forward movement (i.e., posteriorly propagated waves) and have posteriorly directed processes. This model was originally proposed by R. L. Russell and L. Byerly and is described in References 402 and 456.

2. Genes involved in the regulation of locomotion

Numerous mutations disrupt normal sinusoidal locomotion in *C. elegans*, resulting in animals with movement defects ranging from total paralysis, to severe uncoordination, to subtle and almost imperceptible irregularities in movement (308, 409). In this section, we present an overview of those genes implicated in mechanotransduction phenomena pertinent to regulation of locomotion.

A) UNC-8 AND DEL-1. Unusual, semi-dominant (*sd*), gain-of-function mutations in the *unc-8* gene induce transient neuronal swelling of embryonically derived motorneurons as well as some neurons in the head and tail ganglia and severe uncoordination (46, 308, 369, 409). Swelling is absent at hatching and peaks in severity late in L1 and L2. The exact pattern of swelling varies from animal to animal, but all 15 midbody motorneurons have been observed to swell in some animals (369, 409). Interestingly, not all swollen cells die. Swelling is transient rather than lethal in most cells. DAPI staining of ventral cord nuclei does not largely change. Swelling is not as pronounced as with the PVCs in *deg-1(d)* or the touch receptors in *mec-4(d)* animals. Regression of swelling has also been observed in L1 animals over an 8- to 10-h period (369). However, some cells do appear to die (by criteria of lack of GFP reporter fusion expression; N. Tavernarakis, unpublished observations).

unc-8 encodes a degenerin expressed in several motorneuron classes and in some interneurons and nose touch sensory neurons. Interestingly, semi-dominant *unc-8* alleles alter an amino acid in the region hypothesized to be an extracellular channel-closing domain defined in studies of *deg-1* and *mec-4* degenerins (see sect. IV*A* for a description of the structural features and topology of degenerins). The genetics of *unc-8* are further similar to those of *mec-4* and *mec-10*; specific *unc-8* alleles can suppress or enhance *unc-8(sd)* mutations in *trans*, suggesting that UNC-8::UNC-8 interactions occur (369, 409). Another degenerin family member, *del-1* (degenerin-like), is coexpressed in a subset of neurons that express *unc-8* (the VA and VB motorneurons) and is likely to assemble into a channel complex with UNC-8 in these cells (409). The UNC-8 and DEL-1 proteins include all domains characteristic of degenerin family members and

are likely to adopt similar transmembrane topologies (amino and carboxy termini situated inside the cell and a large extracellular domain that includes three cysteine-rich regions; see sect. IV*A*). Neither degenerin has any primary sequence features that are markedly different from other *C. elegans* family members, although one somewhat atypical feature of UNC-8 is that it has a relatively long carboxy-terminal domain that shares some primary sequence homology with the extended carboxy terminus of another degenerin implicated in locomotion, UNC-105 (258, 307).

What function does the UNC-8 degenerin channel serve in motorneurons? *unc-8* null mutants have a subtle locomotion defect; they inscribe a path in an *E. coli* lawn that is markedly reduced in both wavelength and amplitude compared with wild type (Fig. 10; Ref. 409). This phenotype indicates that the UNC-8 degenerin channel functions to modulate the locomotory trajectory of the animal. How does the UNC-8 motorneuron channel influence locomotion? As mentioned earlier, one highly interesting morphological feature of some motorneurons (in particular, the VA and VB motorneurons that coexpress *unc-8* and *del-1*) is that their processes include extended regions that do not participate in neuromuscular junctions or neuronal synapses (456). These "undifferentiated" process regions have been hypothesized to be stretch sensitive (456). Given the morphological features of certain motorneurons and the sequence similarity of UNC-8 and DEL-1 to the candidate mechanically gated channels MEC-4 and MEC-10, we have proposed that these subunits coassemble into a stretch-sensitive channel that might be localized to the undifferentiated regions of the motorneuron process (409). When activated by the localized body stretch that occurs during locomotion, this motorneuron channel potentiates signaling at the neuromuscular junction, which is situated at a distance from the site of stretch stimulus (Fig. 11). The stretch signal enhances motorneuron excitation of muscle, increasing the strength and duration of the pending muscle contraction and directing a full size body turn. In the absence of the stretch activation, the body wave and locomotion still occur, but with significantly reduced amplitude because the potentiating stretch signal is not transmitted. This model bears similarity to the chain reflex mechanism of movement pattern generation. However, it does not exclude a central oscillator that would be responsible for the rhythmic locomotion. Instead, we suggest that the output of such an oscillator is further enhanced and modulated by stretch-sensitive motorneurons (409). One important corollary of the *unc-8* mutant studies is that the UNC-8 channel does not appear to be essential for motorneuron function. If this were the case, animals lacking the *unc-8* gene would be severely paralyzed. This observation strengthens the argument that degenerin channels function directly in mechanotransduction rather than merely serving to maintain the osmotic environment so that other channels can function. As is true for the MEC-4 and MEC-10 touch receptor channel, the model of UNC-8 and DEL-1 function that is based on mutant phenotypes, cell morphologies, and molecular properties of degenerins remains to be tested by determining subcellular channel localization, subunit associations, and, most importantly, channel-gating properties.

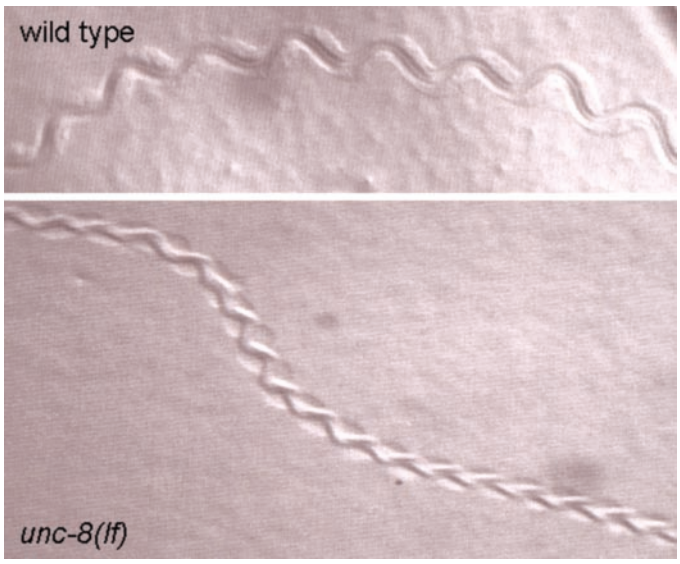

FIG. 10. Proprioception in the nematode. *Top*: wild-type animals inscribe a sinusoidal track as they move on an agar plate evenly covered with an *E. coli* bacterial lawn. *Bottom*: the characteristic properties (amplitude and wavelength) of tracks inscribed by *unc-8(lf)* mutants are drastically reduced.

The striking coiled phenotype of *unc-8(sd)* alleles facilitated exhaustive genetic screens for rare extragenic suppressor mutations that restore normal locomotion. Screens of ~600,000 mutagenized chromosomes identified extragenic mutations in 5 genes that could suppress the severely Unc phenotype (369, 370, 409). Mutations in four of these genes, *sup-40*, *sup-41*, *sup-42*, and *sup-43*, were isolated at a frequency considerably lower than that expected for a simple knockout of gene activity (369, 370). This suggests that the alleles generated are not loss-of-function mutations but rather, either reduce the activities of their encoded proteins or alter the properties of the UNC-8 channel in a highly specific manner. However, none of the *sup* genes has been cloned at the present time. One additional gene revealed by this genetic screen was *mec-6*, known previously to suppress *unc-8(sd)* mutations (172, 369, 409). Because MEC-6 associates with and may regulate the MEC-4/MEC-10 mechanotransducing complex, it is likely that the proteins encoded by the *sup* genes will also be involved in modifying the function of mechanotransducers.

B) UNC-1 AND UNC-24. In *C. elegans*, the UNC-1 protein has an important role in determining volatile anesthetic sen-

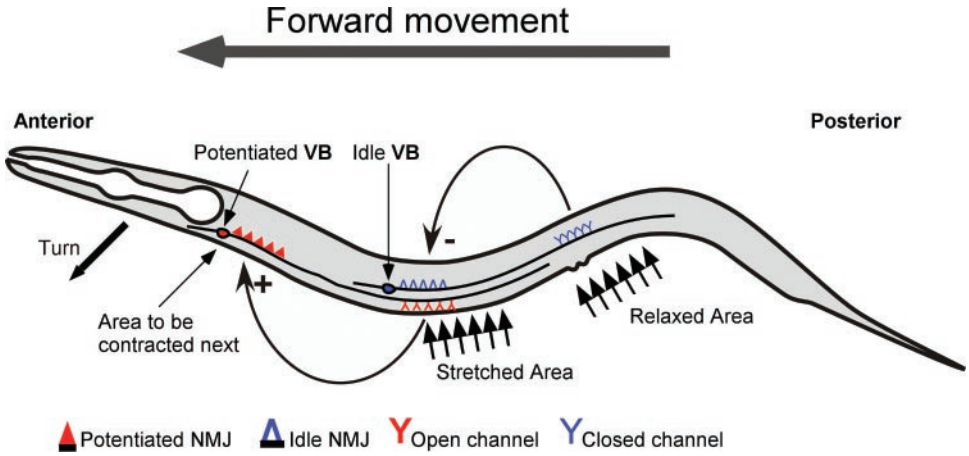

FIG. 11. A model for UNC-8 involvement in stretch-regulated control of locomotion. Schematic diagram of potentiated and inactive VB class motorneurons. Neuromuscular junctions (NMJ; signified by triangles) are made near the cell body (456). Mechanically activated channels (signified by Y figures), which are postulated to include UNC-8 subunits, and possibly DEL-1 subunits in VB motorneurons, are hypothesized to be concentrated at the synapse-free, undifferentiated ends of the VB neuron. Body stretch is postulated to activate these mechanically gated channels that augment the motorneuron signal that excites a specific muscle field. For example, mechanically gated channels may function to increase motorneuron excitability or enhance local muscle excitation at the other, cell body-proximal end of the neuron. A strong muscle contraction results in a sustained body turn. In *unc-8(lf)* mutants, VB motorneurons lack the stretch-sensitive component that potentiates their signaling and consequently elicit a muscle contraction that is shortened in intensity or duration so that the body turns less deeply. Note that although we depict VB as an example of one motorneuron class that affects locomotion, other motorneuron classes may also be involved in the modification of locomotion in response to body stretch (402, 409). Sequential activation of motorneurons that are distributed along the ventral nerve cord and signal nonoverlapping groups of muscles amplifies and propagates the sinusoidal body wave (+/− symbols indicate positive/negative feedback on motorneuron activity).

sitivity and is similar to MEC-2 and mammalian stomatin (194, 329). UNC-1 interacts genetically with UNC-8 (330). Staining of wild-type *C. elegans* with anti-UNC-1 antibody showed a punctuate distribution primarily in cells of nervous system, most notably in the nerve ring, retrovesicular ganglion, and the ventral and dorsal nerve cord (364). This is consistent with the expression of an UNC-1::GFP reporter chimera (330). Examination of the UNC-1 subcellular localization demonstrated that it is distributed along the axons of neurons and is most likely not found at synapses (364). Interestingly, experimental evidence suggests that UNC-1 interacts with an additional stomatin-like protein, UNC-24 (364). It is intriguing that UNC-24, apart from the domain with similarity to stomatin, also features a sterol carrier protein (SCP) domain. This domain is found in proteins that mediate the transfer of phospholipids between membranes and facilitate the organization of microdomains such as lipid rafts and caveolae (19). The potential colocalization of UNC-1 to lipid rafts supports the theory that volatile anesthetics partition into lipid rafts and exert their effects by interfering with the lipid-protein complexes that are present in these structures (364).

unc-24 is expressed in a variety of motorneurons, interneurons, and sensory neurons (including the touch receptor neurons; Refs. 19, 480). *unc-24* mutants display severe inability to move forward, indicating a major defect in the forward locomotion circuit. UNC-1 antibody staining is dramatically reduced in *unc-24* mutants, compared with wild-type animals. Furthermore, *unc-24* mutations are epistatic to *unc-1* (364). UNC-24 does not affect the expression of *unc-1* gene, but rather the stability of the UNC-1 protein, which in the absence of UNC-24 is localized close to nucleus instead of being distributed to the neuronal axons. This suggests that UNC-24 affects the trafficking of UNC-1 to the cell periphery and its proper localization to lipid rafts. Failure of UNC-1 to be transported to the periphery could in turn affect the stability or, via a feedback mechanism, the synthesis of the protein (364). It is possible that UNC-24 could serve a similar function in touch receptor neurons, where its partner would be MEC-2 instead of UNC-1. However, *unc-24* mutants show normal touch responses and no interference with the function of MEC-4 and MEC-10, as assessed by the capacity of toxic dominant alleles of these degenerins to inflict necrotic cell death in *unc-24* mutant background (N. Tavernarakis, unpublished observations). It is tempting to suggest that defects in the human homolog of UNC-24 are responsible for the absence of stomatin from erythrocytes, in patients with stomatocytosis, despite them having an apparently normal stomatin gene (364, 366).

C) UNC-105. The *unc-105* gene encodes a member of the DEG/ENaC family of ion channels and is mainly expressed in body wall muscles of *C. elegans*, where it is believed to mediate stretch sensitivity (258, 307). UNC-

105 contains ~150 amino acids at the carboxy terminus that are not represented in other degenerin proteins. Although loss-of-function mutations in *unc-105* do not result in any readily observable phenotype, gain-of-function mutations cause muscle hypercontraction and result in severe paralysis of the animal (307). These mutations in *unc-105* disrupt extracellular residues situated near the predicted transmembrane domain, where degeneration-causing mutations are found in MEC-4, MEC-10, and DEG-1 and result in constitutive channel activation producing the hypercontraction phenotype (140, 258). The muscle hypercontraction phenotype of dominant *unc-105* mutations can be suppressed by certain mutations near the carboxy terminus of *let-2*, a gene that encodes the α_2-chain of type IV collagen found in the basement membrane between muscle cells and the hypodermis (258). The nature of the functional link, implied by the suppression effect, between UNC-105 and LET-2 collagen is unknown. A possible interpretation is that LET-2 normally carries gating tension to the UNC-105 channel when the muscle is stretched, thus providing regulatory feedback for muscle contraction (258). Suppressor mutations in LET-2 may relieve conformational alterations to the UNC-105 channel induced by dominant mutations, allowing the channel to close. This putative connection between a collagen and a degenerin is reminiscent of a similar relationship between the MEC-5 collagen and MEC-4 in touch receptor neurons (402). Similarly, mechanosensory transduction in the auditory system requires extracellular tip links that physically deliver mechanical energy to the mechanosensitive channels in the hair cell stereocilia of the inner ear (see sect. IV*B*; Refs. 316, 317).

Expression of the wild-type *unc-105* gene in two heterologous systems [*Xenopus* oocytes and human embryonic kidney (HEK) cells] resulted in no detectable currents, suggesting that the channel requires a stimulus for gating (140). In contrast, expression of two mutant forms of *unc-105*, carrying gain-of-function mutations predicted to cause constitutive activation, resulted in constitutive currents in both heterologous systems (140). These currents occurred without additional exogenous proteins, indicating that UNC-105 channels can assemble as homomultimers, at least in oocytes and human embryonic kidney cells. In addition, UNC-105 is only partially dependent on MEC-6 for its function, unlike other degenerins, which are fully reliant on it (N. Tavernarakis, unpublished observations; Ref. 172). Phylogenetic analysis suggests that UNC-105 is one of the most ancient degenerins, and thus may have not developed dependencies on other subunits (87). The two mutations that constitutively activate the heterologously expressed UNC-105 channel are the one causing muscular hypercontraction (P134S) and the mutation (A692V) that corresponds to degeneration inducing substitutions in *deg-1* and *mec-4*. In cultured human embryonic kidney cells, both mutants triggered nonapoptotic cell death accompanied by accumulation of whorls and vacuoles that resembles neurodegeneration inflicted in nematode cells expressing other mutant degenerins (140, 165). Although there are differences in cell swelling and mitochondrial damage between nematode neurons and mammalian cultured epithelial cells, the ultrastructural features of dying cells appear similar in both cases (140, 165; see sect. IV*B*).

D. The Nose Touch Responses

The nose of *C. elegans* is highly sensitive to mechanical stimuli. This region of the body is innervated by many sensory neurons that mediate mechanosensitivity. Responses to touch in the nose can be classified into two categories: the head-on collision response and the foraging and head withdrawal response.

1. Head-on collision response

When animals collide with an obstacle in a nose-on fashion during the course of normal locomotion, they respond by reversing the direction of movement (18, 84, 175, 458). Three classes of mechanosensory neurons, ASH, FLP, and OLQ, mediate this avoidance response (18, 186, 215, 458). Each of these sensory neurons accounts for a part of the normal response, which is quantitative with normal animals responding ~90% of the time. Laser ablation and genetic studies have demonstrated that each sensory neuron contributes to the overall responsiveness as follows: ASH, 45%; FLP, 29%; and OLQ, 5%. The remaining 10% of the responses are mediated by the ALM and AVM neurons, which sense anterior body touch (103, 215). It is unclear what distinguishes the function of the three nose touch neurons. One attractive possibility is that these cells differ in their sensitivities and that the intensities of nose touch stimuli vary according to the violence of the collision. If this were the case, it would be expected that the most sensitive neuron (ASH) would account for the majority of responses while less sensitive neurons (FLP and OLQ) would account for the remainder. In addition to their mechanosensory properties, the ASH neurons are part of a chemosensory organ, the amphid sensilla, with their sensory endings exposed to the external environment (310, 443). The ASH neurons serve chemosensory and osmosensory functions, mediating avoidance of osmotic repellents (175, 176, 215). Several classes of chemosensory neurons respond to multiple chemical stimuli in *C. elegans*. However, ASH is unique among them in responding to such divergent stimuli. In this respect, ASH neurons are similar to vertebrate neurons that sense painful stimuli, which are called nociceptors. For their multisensory capabilities, the ASH neurons have been categorized as polymodal sensory neurons (Fig. 12; Refs. 103, 215).

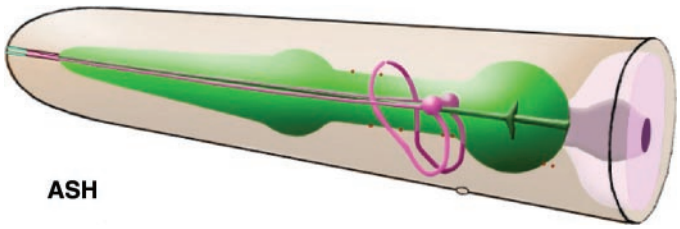

FIG. 12. The set of the ASH polymodal amphid neurons. The ciliated endings of these neurons are exposed to the environment and can be filled with dyes (181). The pharynx is shown in green. [Courtesy of WormAtlas (http://www.wormatlas.org).]

On the basis of anatomical data, nose mechanosensory neurons signal to the AVA, AVB, and AVD interneurons (ASH and FLP connect directly to these interneurons, while OLQ provides synaptic input indirectly via the RIC interneuron; Refs. 103, 175, 215). Such synaptic connectivity is further supported by genetic and molecular studies of mutants with defective synaptic function such as *glr-1* and *osm-10*. *glr-1* mutants are defective for nose touch avoidance but are normal for ASH-mediated osmotic sensitivity, while *osm-10* mutants are defective for ASH-mediated osmotic avoidance but are normal for ASH-mediated nose touch sensitivity (175, 215; reviewed in Ref. 103). These phenotypes indicate that separate signaling pathways relay the two ASH sensory modalities. The *glr-1* gene encodes an AMPA-type glutamate receptor subunit and *osm-10* a novel cytosolic protein with a major sperm protein (MSP) domain. *glr-1* is expressed in both the forward (AVB and PVC) and backing (AVA and AVD) interneurons, while *osm-10* is expressed in four classes of chemosensory neurons in all larval and adult animals (ASH, ASI, PHA, and PHB). Expression of both *glr-1* and *osm*-10 commences during late embryogenesis (175, 176, 270). The expression of *glr-1* in postsynaptic interneurons coupled with analysis of genetic mosaics suggests that GLR-1 glutamate receptor subunits function in both the forward and backing interneurons (186). These neurons derive from the cell lineages AB.p and AB.a, respectively. Taken together, these observations indicate that the sensory transmitter for the head-on collision response is glutamate and that GLR-1 participates in the reception of the nose touch receptor signal from the interneuron side of the synapse (270).

Even though GLR-1 is only required for ASH-mediated nose touch sensitivity and not for osmotic avoidance, the *glr-1* gene is expressed in all interneurons that are synaptic targets of ASH. Interestingly, ASH presynaptic termini contain two distinct kinds of synaptic vesicles, clear and dense core vesicles, which indicates that ASH neurons utilize a repertoire of distinct neurotransmitters (175, 176). The totality of the evidence suggests that the ASH neurons produce different synaptic signals in response to mechanosensory and osmotic stimuli and that GLR-1 may be involved in distinguishing between different ASH sensory modalities. Such differential signaling at the ASH-to-interneuron synapses may allow animals to distinguish between mechanosensory, chemosensory, and osmotic inputs to a single neuron (175).

2. Foraging and the head withdrawal response

While the body of *C. elegans* is restricted to moving in a sinusoidal manner on a single two-dimensional plane, the nose has more degrees of freedom and is capable of moving in a three-dimensional space. This complex pattern of continuous, and apparently exploratory, head movements is called foraging. The reason for the higher flexibility of the nose is due to the motor anatomy of the nematode head. Unlike body wall muscles that are arranged in four quadrants and generate only dorsoventral contractions, head muscles are divided into eight radial symmetric sectors (456). Muscles in these 8 sectors are independently innervated by 10 classes of motorneurons (443, 456). This elaborate muscle and motorneuron arrangement allows animals to move their head through 360°. Touch also regulates/modifies foraging. If worms are touched on either the dorsal or ventral side of their nose with an eyelash hair, they interrupt the typical pattern of foraging and execute a sharp head-withdrawal pirouette. This reflex is mediated by the OLQ and IL1 mechanosensory neurons, which connect to the RMD motorneurons (103, 176). All three classes of neurons are required for normal head withdrawal upon mechanical stimulation, since laser ablation of any or combinations of these cells reduces the reflex. Similarly to the nose touch response, the sensory neurons involved in head-withdrawal contribute differently, with OLQ mediating the majority of normal responses. Apart from their role in head withdrawal upon stimulation, IL1, OLQ, and RMD appear to also control spontaneous foraging movements. Animals with laser-ablated IL1 and OLQ neurons forage abnormally slowly and make exaggerated dorsal and ventral nose turns (176). Furthermore, the RMD neurons express *glr-1* and *glr-1* mutants and are defective for the head withdrawal reflex (176; reviewed in Ref. 103). Therefore, mechanosensory stimuli such as touch/collisions or stretch modulate overall spontaneous foraging activity.

Intriguingly, all the neurons involved in head-on collision and head withdrawal responses have ciliated sensory endings at the tip of the nose (443). These specialized structures that contain a single cilium are essential for the physiological function of these neurons. Mutations in *che-3*, *daf-11*, *eat*-4, and *osm-1*, which are otherwise unrelated genes, disrupt the ultrastructure of these ciliated sensory endings and precipitate the common phenotypes of defective nose touch responses and abnormally slow foraging behavior (175, 214, 215, 250, 310, 336, 429, 430).

CHE-1 is a C2H2-type zinc finger-containing transcription factor (426), and *osm-1* encodes a G-β, WD domain protein (241). DAF-11 is a membrane-bound guanylate cyclase that is required in ciliated chemosensory neurons for normal chemotaxis and dauer formation (35). EAT-4 shares extensive sequence similarity (46% identity) with the brain and neural specific sodium-dependent inorganic phosphate cotransporter BNPI (248, 331) and is expressed in >24 neurons, including the pharyngeal M3 neuron and AVM neurons. Apart from defects in nose touch responses and irregular foraging movements, *eat-4* mutants are also generally defective for glutamatergic neurotransmission.

E. Other Mechanosensitive Behaviors

C. elegans displays several additional behaviors that are based on sensory mechanotransduction that have been characterized to a lesser extent. These include the response to harsh mechanical stimuli, the tap withdrawal reflex, where animals retreat in response to a tap on the culture plate (267, 333, 458). Furthermore, mechanotransduction appears to also play a regulatory role in processes such as mating, egg laying, feeding, defecation, and maintenance of the pseudocoelomic body cavity pressure (18, 405). These behaviors add to the large repertoire of mechanosensitive phenomena, amenable to genetic and molecular dissection in the nematode.

1. The harsh touch response

Animals, which are insensitive to gentle body touch with an eyelash hair because they lack functional touch cells or genes essential for mechanotransduction (for example, *mec-4*), are still sensitive to prodding with a stiff platinum wire, and typically respond by initiating backward movement (103, 405, 402). The capacity to respond to mechanical stimuli in the absence of elements required for gentle body touch suggests that a separate mechanosensory circuit mediates sensitivity to harsh touch stimuli. The PVD neurons with features characteristic of gentle body touch receptors are thought to be harsh touch sensory neurons. Similarly to the touch receptor neurons and motorneurons, the PVD neurons have long undifferentiated processes that run along the lateral body wall, which could be mechanosensory (456, 458). In addition, the PVD neurons express genes involved in touch cell differentiation such as *mec-3*, implying that they may also be mechanosensory (109, 447, 448). Laser ablation of the PVD neurons in animals that lack touch cell function eliminates harsh touch sensitivity (65, 103). The interneurons AVA and PVC are direct synaptic targets of PVD. Mutants lacking GLR-1 glutamate receptors, which are expressed by these interneurons, are insensitive to harsh touch, which suggests that glutamate is the neurotransmitter released by PVD in response to harsh touch at its synapses with locomotory interneurons (270). The involvement of PVC in relaying harsh touch stimuli is exemplified by the phenotype of specific mutations in the *deg-1* degenerin gene. Animals carrying dominant, gain-of-function mutations in *deg-1* are touch abnormal (Tab; Refs. 70, 141, 369). They do not respond to gentle touch in the tail or prodding with a wire. *deg-1(d)* mutants exhibit late-onset degeneration of several types of neuron expression the mutant gene, including the PVC interneuron. These animals are touch-sensitive at hatching and become touch abnormal later in L2 and L3 larval stages. PVC death can occur in different times depending on temperature, gene dosage, and nature of the mutation in *deg-1*, after synapse formation occurs and neurons are functional (70, 369).

2. The tap withdrawal reflex

A diffuse mechanical stimulus such as a tap to the side of the Petri dish that worms are resting on elicits either forward acceleration or initiation of backward movement (76, 335; reviewed in Ref. 103). The stochastic nature of the response coupled with the stimulus, which is not spatially coherent, suggests that the tap response reflects the simultaneous activation of both the anterior and posterior touch cells. The final behavioral outcome is determined by the integration of these two antagonistic circuits. As such, the tap reflex may represent a manifestation of competition between the anterior and posterior touch circuits.

The cellular basis of the reaction to tap has been examined extensively (459, 458). As described in section III*B*, the anterior and posterior touch circuits are interconnected in two ways. First, touch cells make reciprocal connections to the opposing classes of interneurons, and second, the forward and backward interneurons form reciprocal connections (332, 459). These interconnections suggest that the anterior and posterior touch circuits functionally interact, perhaps allowing integration of opposing mechanosensory inputs. The capacity of these circuits for integration is beginning to be understood through the analysis of the response to tap. Both the anterior (ALM and AVM) and posterior (PLM) touch cells, and their interneuron targets (AVD and PVC), contribute to the tap response. Anterior cells promote reversals, and the posterior cells promote accelerations. Tampering with either the anterior or posterior touch circuit tips the balance in favor of the opposite circuit and results in amplification of the opposing response (reviewed in Ref. 103). As predicted, animals with laser-ablated PLM neurons respond to tap solely with reversals, and these reversals are of greater magnitude than those of unoperated controls. Likewise, animals lacking the PVC interneurons, which receive synaptic input from PLM neurons and me-

diate accelerations, always respond to tap by reversing. These observations are in agreement with the notion that the anterior and posterior touch circuits functionally inhibit each other, exemplified in Figure 6. However, elimination of PLM and PVC produces distinct phenotypes. Unlike in the case of PLM ablation, the magnitude of reversals in the case of PVC removal is indistinguishable from unoperated controls. This lack of equivalence suggests that PLM neurons make additional functional connections, other than the gap junctions with PVC. Perhaps, PLM attenuate the extent of reversals via inhibitory chemical synapses with the backing interneurons AVA and AVD.

The propensity of animals to respond to tap with accelerations versus reversals varies over the course of development. Accelerations outweigh reversals in larvae, while the opposite behavior is observed in adults (76, 335). This developmental switch occurs in young adults, at around 46–50 h after hatching, and it may reflect the formation of novel functional connections at that stage by the AVM neuron. However, laser-ablation of AVM does not phenocopy larva behavior; operated adults respond to tap by reversing much more often than accelerating (76, 460). This discrepancy indicates that in addition to AVM neurons, other neurons, such as the PVD, enter and modify the touch circuit during development. Animals with laser-ablated PVD and DVA neurons, which synapse to both forward and backing interneurons, respond to tap with diminished accelerations and reversals. Given that PVD neurons are mechanosensory, and that they promote the activities of both circuits, it is possible that the excitability of the touch circuit is modulated by mechanical stimuli relayed by PVD and DVA (332, 459; reviewed in Ref. 103).

3. Regulatory mechanotransduction in C. elegans

Although no direct supporting evidence has become available yet, mechanotransduction is likely to play a role or regulate several nematode behaviors, in addition to the ones described above.

One example is the mating of male with hermaphrodite animals. During mating the male needs to carry out the task of locating the vulva opening on the body of its hermaphrodite partner, through which sperm will be injected (136, 259). In trying to do so, the male scans the body of the hermaphrodite with its specialized tail, which is equipped with an elaborate network of neurons and auxiliary structures (256, 259). Once the vulva on the hermaphrodite is located, scanning stops, the male tail becomes stably hooked on the opening, and sperm is injected (21, 259). It is likely that mechanosensory neurons in the male tail facilitate the recognition of the vulva anatomy and aid the secure attachment to the hermaphrodite by sensing relative movement (see the discussion on LOV-1 and PDK-2 TRP proteins below).

Opening of the vulva is controlled by a set of muscles, innervated by the HSN neurons (98, 99, 143, 415). Vulva muscles are responsible for egg laying in adult hermaphrodite nematodes. We hypothesize that mechanotransducers sense stretch generated within the uterus by continuous egg accumulation to trigger vulva opening and egg release. It is also possible that specialized mechanosensory ion channels function in the feeding organ of *C. elegans*, the pharynx, which continuously pumps bacterial food through to the intestine. Once nutrients are absorbed in the intestine, bacterial remains and debris are released to the environment. The process of defecation is highly rhythmic, occurring about once every 50 s. The necessary feedback to generate this rhythmicity may be provided by a mechanotransducer that senses the peristaltic movements of the intestine and signals to the anal sphincter muscle of the animal. It is intriguing that several uncharacterized members of the DEG/ENaC family are expressed in tissues and cells involved in all these processes (N. Tavernarakis, unpublished observations).

C. elegans possesses a hydraulic skeleton. As in other nematodes, the liquid-filled body cavity, the pseudocoelom, functions as a hydroskeleton (120, 321). Loss of body cavity pressure, when for example an animal is punctured, results in acute hydroskeleton collapse and inevitable death. Therefore, monitoring and maintenance of the pseudocoelomic body cavity pressure is essential. The stretch applied on various body structures due to internal pressure may be sensed by mechanosensitive ion channels similar to the mammalian baroreceptor. Again, gene expression studies reveal that members of the DEG/ENaC family may serve as baroreceptors in the nematode (N. Tavernarakis, unpublished observations).

IV. DEGENERINS: FROM NEURODEGENERATION TO MECHANOTRANSDUCTION

The DEG/ENaC family of ion channels is a large group of proteins sharing a high degree of sequence and overall structure similarity. Intense genetic, molecular, and electrophysiological studies have implicated DEG/ENaC ion channels in mechanotransduction in nematodes, flies, and mammals (221, 405). Therefore, these proteins are strong candidates for a metazoan mechanosensitive ion channel.

A. Features of the DEG/ENaC Ion Channels

Members of the DEG/ENaC family of ion channel subunits have been identified in organisms ranging from nematodes, snails, flies, and many vertebrates including

humans and are expressed in tissues as diverse as kidney epithelia, muscle, and neurons (7, 269, 404; recently reviewed in Ref. 221). With the sequence analysis of the *C. elegans* genome now complete, it is possible to survey the entire gene family within this organism. At present, 30 genes encoding members of the DEG/ENaC family have been identified in the *C. elegans* genome, 7 of which have been genetically and molecularly characterized (*deg-1*, *del-1*, *flr-1*, *mec-4*, *mec-10*, *unc-8*, and *unc-105*; Table 2). While DEG/ENaC proteins are involved in many diverse biological functions in different organisms, they share a highly conserved overall structure (28, 269). This strong conservation across species suggests that DEG/ENaC family members shared a common ancestor relatively early in evolution. The basic subunit structure may have been adapted to fit a range of biological needs by the addition or modification of functional domains. This conjecture can be tested by identifying and isolating such structural modules within DEG/ENaC ion channels. *C. elegans* degenerins exhibit ~25–30% sequence identity to subunits of the vertebrate amiloride-sensitive ENaCs (Fig. 13; Ref. 61), which are required for ion transport across epithelia (see Ref. 221 for a recent comprehensive review). DEG/ENaC proteins range from ~550 to 950 amino acids in length and share several distinguishing blocks of sequence similarity (Fig. 14*A*). Subunit topology is invariable: all DEG/ENaC family members have two membrane-spanning domains with cysteine-rich domains (CRDs, the most conserved is designated CRD3) situated between these two transmembrane segments (406, 404). DEG/ENaCs are situated in the membrane such that amino and carboxy termini project into the intracellular cytoplasm while most of the protein, including the CRDs, is extracellular (Fig. 14*B*; Refs. 137, 405). Highly conserved regions include the two membrane-spanning domains (MSD I and II), a short amino acid stretch before the first membrane-spanning domain, extracellular CRDs, an extracellular regulatory domain and a neurotoxin-related domain (NTD) before predicted transmembrane domain II (Fig. 15; Ref. 408). The high degree of conservation of cysteine residues in these extracellular domains suggests that the tertiary structure of this region is critical to the function of most channel subunits and may mediate interactions with extracellular structures. Interestingly, the NTD is also distantly related to domains in several other proteins including the *Drosophila* protein *crumbs*, required for epithelial organization (412); *agrin*, a basal lamina protein that mediates aggregation of acetylcholine channels (351); and the selectins, which participate in cell adhesion (such as ELAM-1; Ref. 32). The presence of related domains in proteins such as *crumbs* and *agrin* implies that such domains might act as interaction modules that mediate analogous interactions needed for tissue organization or protein clustering. We hypothesize that the appearance of neurotoxin-related domains in a specific class of ion channels might be the result of convergent evolution, driven by the requirement for high-affinity interaction modules in these proteins. That the extracellular domain of degenerins may participate in interactions with the extracellular matrix is further supported by the presence of the muscin repeats, which are selectin binding sites, close to the extracellular regulatory domain in DEG-1 (141).

Amino and carboxy termini are intracellular, and a single large domain is positioned outside the cell (Fig. 14*B*; Refs. 221, 244, 408). The more amino-terminal of the two membrane-spanning domains (MSDI) is generally hydrophobic, whereas the more carboxy-terminal of these (MSDII) is amphipathic (189, 190). In general, MSDI is not distinguished by any striking sequence feature except for the strict conservation of a tryptophan residue (corresponding to position W111 in MEC-4), and the strong conservation of a Gln/Asn residue (corresponding to po-

TABLE 2. *The current list of Caenorhabditis elegans DEG/ENaC family members and their chromosomal distribution*

Gene Name	ORF	Chromosome	Behavior/Phenotype	Reference Nos.
deg-1	C47C12.6	X	Touch abnormality	70
del-1	E02H4.1	X	Locomotory defects	409
mec-4	T01C8.7	X	Touch insensitivity	101
mec-10	F16F9.5	X	Touch insensitivity	193
flr-1	F02D10.5	X	Fluoride resistance	217
unc-8	R13A1.4	IV	Locomotory defects	409
unc-105	C41C4.5	II	Muscle function defects?	258
	C11E4.3	V	ND	413
	C11E4.4	X	ND	413
	C18B2.6	X	ND	413
	C24G7.1	I	ND	413
	C24G7.2	I	ND	413
	C24G7.4	I	ND	413
	C27C12.5	X	ND	413
	C46A5.2	X	ND	413
	F23B2.3	IV	ND	413
	F25D1.4	V	ND	413
	F26A3.6	I	ND	413
	F28A12.1	V	ND	413
	F55G1.12	IV	ND	413
	F59F3.4	IV	ND	413
	T21C9.3	V	ND	413
	T28B8.5	I	ND	413
	T28D9.7	II	ND	413
	T28F2.7	I	ND	413
	T28F4.2	I	ND	413
	Y69H2.2	V	ND	413
	Y69H2.11	V	ND	413
	Y69H2.13	V	ND	413
	ZK770.1	I	ND	413

Genes have been listed alphabetically with the 7 genetically characterized ones on top. Phenotypes are those of loss-of-function alleles. All 23 uncharacterized putative degenerin genes encode proteins with the sequence signature of amiloride-sensitive channels. However, some lack certain domains of typical degenerin (DEG)/epithelial Na^+ channel (ENaC) ion channels. These incomplete proteins could associate with channels to regulate their function. Alternatively, the corresponding genes may be defective pseudogenes. ND, not determined.

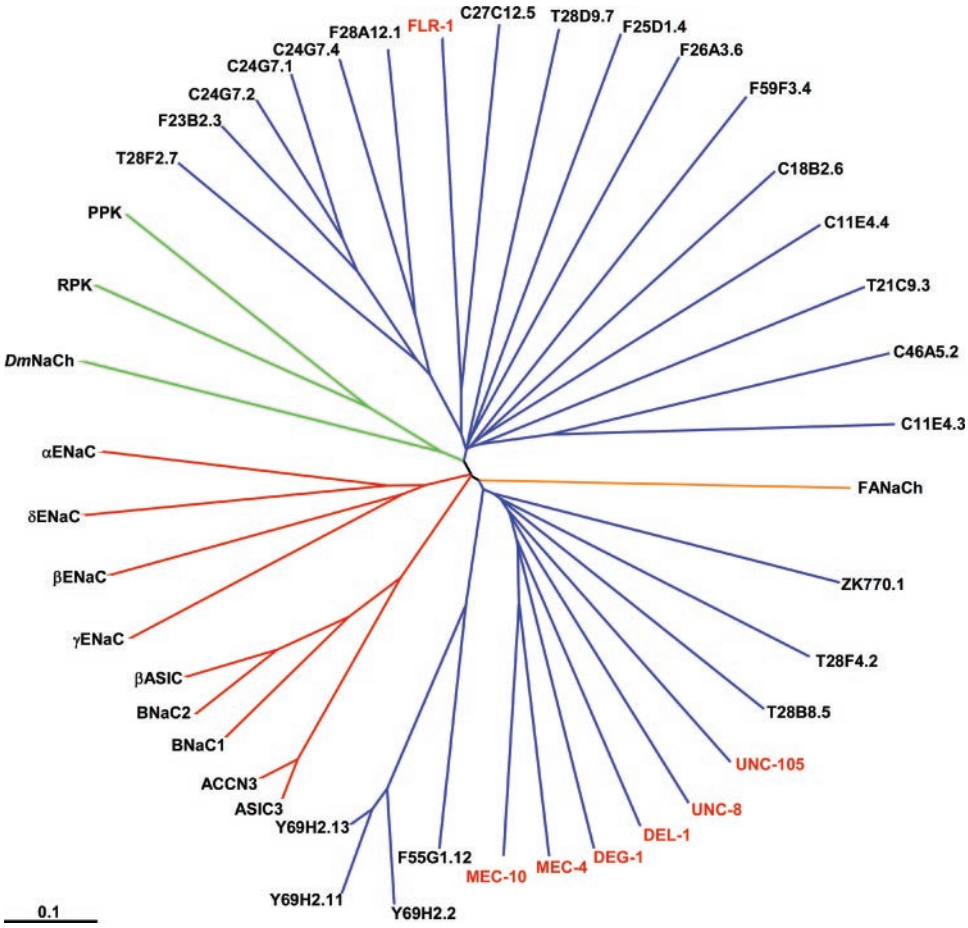

FIG. 13. Phylogenetic relationships among degenerin (DEG)/epithelial Na^+ channel (ENaC) proteins. Nematode degenerins are shown with blue lines. The current degenerin content of the complete nematode genome is included. The seven genetically characterized (DEG-1, DEL-1, FLR-1, MEC-4, MEC-10, UNC-8, and UNC-105) are shown in red. Representative DEG/ENaC proteins from a variety of organisms, ranging from snails to humans, are also included (mammalian, red lines; fly, green lines; snail, orange line). The scale bar denotes relative evolutionary distance equal to 0.1 nucleotide substitutions per site (357).

sition N125 in MEC-4). MSDII is more distinctive, exhibiting strong conservation of hydrophilic residues (consensus GLWxGxSxxTxxE) that has been implicated in pore function (189). The short highly conserved region before the minimal transmembrane domain is thought to loop back into the membrane to contribute to the channel pore (28, 144, 220). The extended MSDII homology region (loop + transmembrane part) can be considered a defining characteristic of DEG/ENaC family members.

Amino acids on the polar face of amphipathic transmembrane MSDII are highly conserved and are essential for mec-4 function (189). Consistent with the idea that these residues project into the channel lumen to influence ion conductance, amino acid substitutions in the candidate pore domain (predicted to disrupt ion influx) block or delay degeneration when the channel-opening A713V substitution is also present in MEC-4 (141, 189, 220, 221). Electrophysiological characterization of rat and rat/nematode chimeras supports the hypothesis that MSDII constitutes a pore-lining domain and that highly conserved hydrophilic residues in MSDII face into the channel lumen to influence ion flow (362, 363). In the region immediately preceding MSDII is a key residue that influences channel activation. Amino acid substitutions for a conserved small residue situated close to MSDII favor an open channel conformation (alanine-713 for MEC-4; Refs. 101, 140). In *C. elegans*, this genetically induced channel hyperactivation can induce degenerative cell death of cells expressing the mutant genes (Fig. 14*B*; see below for a discussion on neurodegeneration). There is a correlation between the size of the amino acid side-chain at position 713 and toxicity: tests of *mec-4* mutant alleles engineered to include all possible amino acid substitutions established that a large side-chain amino acid at this site is toxic to touch neurons, whereas a small side-chain amino acid (alanine, serine, cysteine) is not lethal (101). Interestingly, small side-chain amino acids are present at the position corresponding to MEC-4 (A713) in all characterized DEG/ENaC family members (61, 101). Other *C. elegans* family members (e.g., *deg-1* and *mec-10*) can be altered by analogous amino acid substitutions to induce neurodegeneration (70, 193). Noticeably, a mutant variant of neuronally expressed mammalian family member (BNC1; also known as ASIC2, MDEG, BNaC1; Refs. 139, 328, 433), engineered to encode valine or phenylalanine at the corresponding position, induces swelling and death when introduced into *Xenopus* oocytes and hamster embryonic kidney cells (221, 433; see below).

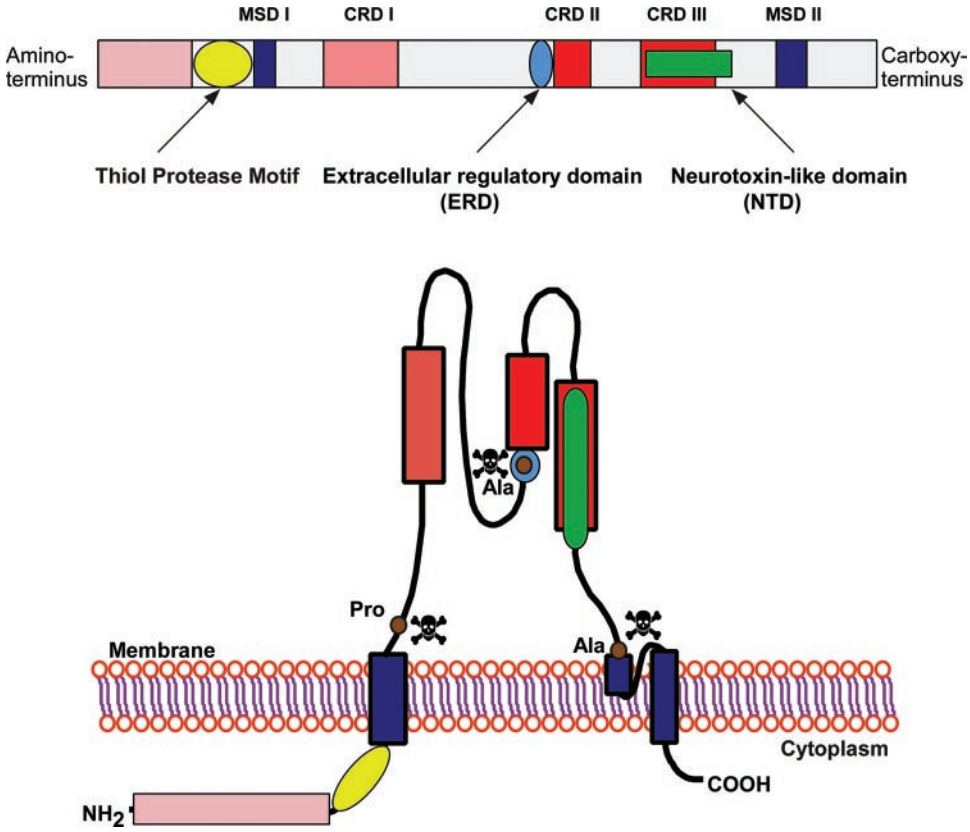

FIG. 14. Schematic representation of DEG/ENaC ion channel subunit structure and topology. *Top*: functional/structural domains. Colored boxes indicate defined channel modules. These include the two membrane-spanning domains (MSDs; dark-blue shading) and the three cysteine-rich domains (CRDs; red shading; the first CRD is absent in mammalian channels and is depicted by light red shading). The small light-blue oval depicts the putative extracellular regulatory domain (ERD) identified by J. García-Añoveros and co-workers in *C. elegans* degenerins (141). The green box overlapping with CRDIII denotes the neurotoxin-related domain (NTD). The conserved intracellular region with similarity to thiol-protease histidine active sites is shown in yellow. Shown in pink is the amino-terminal domain model based on protease Pro-domains (see Fig. 17; Refs. 404, 406, 408). *Bottom*: transmembrane topology. Both termini are intracellular with the largest part of the protein situated outside the cell (244, 340). The brown dots (after MSDI, within ERD and near MSDII) represent the amino acid positions (proline-134 in UNC-105, alanine-393 in DEG-1, glycine-387 in UNC-8, and alanine-713 in MEC-4) affected in dominant, toxic degenerin mutants.

In the saturation genetic screen for touch-insensitive mutants, more than 50 *mec-4* loss-of-function alleles were isolated. Sequence analysis of recessive loss-of-function *mec-4* alleles has highlighted two other regions of MEC-4 in addition to MSDII, which appear especially important in channel function. Amino acid substitutions that disrupt MEC-4 function cluster within a conserved region that is situated on the intracellular side, close to MSDI (Fig. 16; Ref. 190). This region of the channel could interact with cytoskeletal proteins. A three-dimensional model of this region, based on weak but significant similarity to a protease of known structure, suggests a configuration with a hydrophobic stretch, which is exposed and has potential for protein-protein interactions (408; Fig. 17). Interestingly, the effects of semidominant alleles of *unc-8* can be completely blocked by mutations in this conserved re-

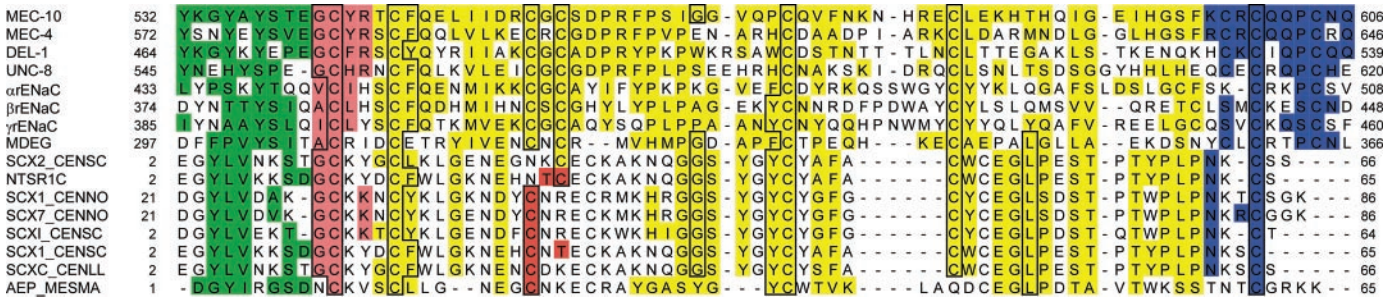

FIG. 15. Similarity of neurotoxins to a DEG/ENaC ion channel domain. Amino acid alignment of 8 DEG/ENaC family members and 8 venom neurotoxins (406). Identical residues that occur in more than 60% of the sequences are boxed. Gray-shaded residues represent sequence similarity (>85%; for homology analysis the first sequence is used as primary, i.e., homology is displayed with respect to MEC-10 sequence). Residue positions are noted on both sides of the alignment for each of the designated sequences. Representative members of the DEG/ENaC family characterized to date were included in the alignment (without orthologs in different species). Antiepilepsy peptide (AEP_MESMA) belongs to the α-subfamily of neurotoxins, whereas the remaining 7 toxin sequences are typical of the β-class. Multiple sequence alignments were generated with the ClustalW algorithm (416) and displayed with SeqVu (The Garvan Institute of Medical Research, Sydney, Australia).

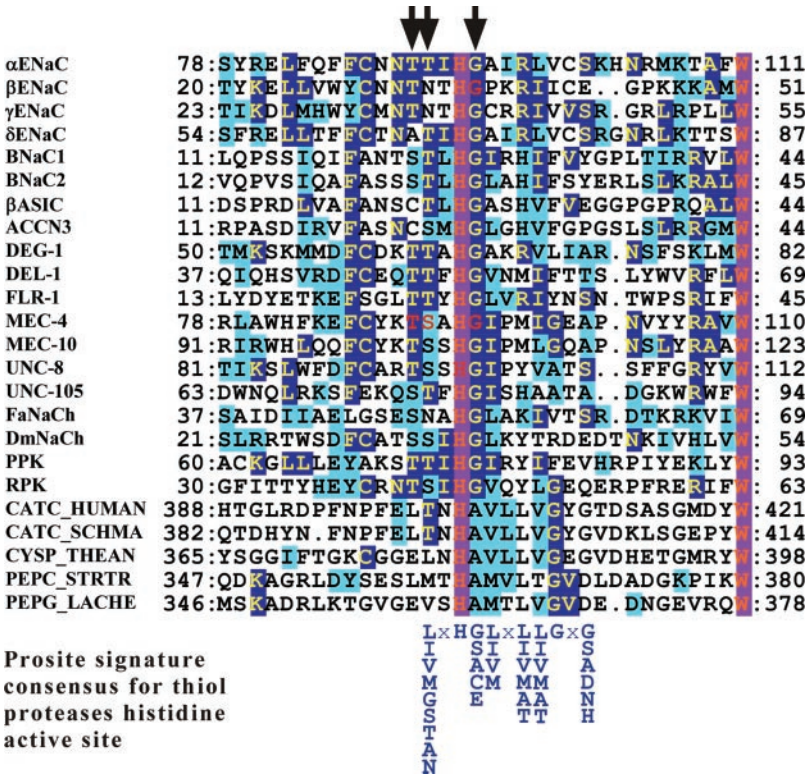

FIG. 16. Protease-like, structural features of the conserved amino-terminal region of DEG/ENaC family members. Alignment of the conserved intracellular region of DEG/ENaC proteins, adjacent to MSD I, with representative cathepsin B proteases is shown. The protease signature is shown at the *bottom* of the alignment (408). The significance of the conserved residues is highlighted by mutations at these sites in MEC-4, which disrupt normal touch sensitivity (indicated by arrows; Ref. 190).

gion, highlighting its functional importance (369, 370, 408, 409). This suppression is observed both when such mutations reside in *cis*, on the same protein molecule as the semi-dominant mutations, or in *trans*, on different coexpressed genes, as observed in heterozygote animals carrying a semidominant allele on one chromosome and a mutation in the conserved intracellular amino-terminal region on the other (369, 408). Such a pattern of genetic suppression suggests that UNC-8 proteins interact to form a dimeric or multimeric complex where more than one molecule associates to form a channel. The conserved intracellular amino-terminal region could play a role in facilitating such interac-

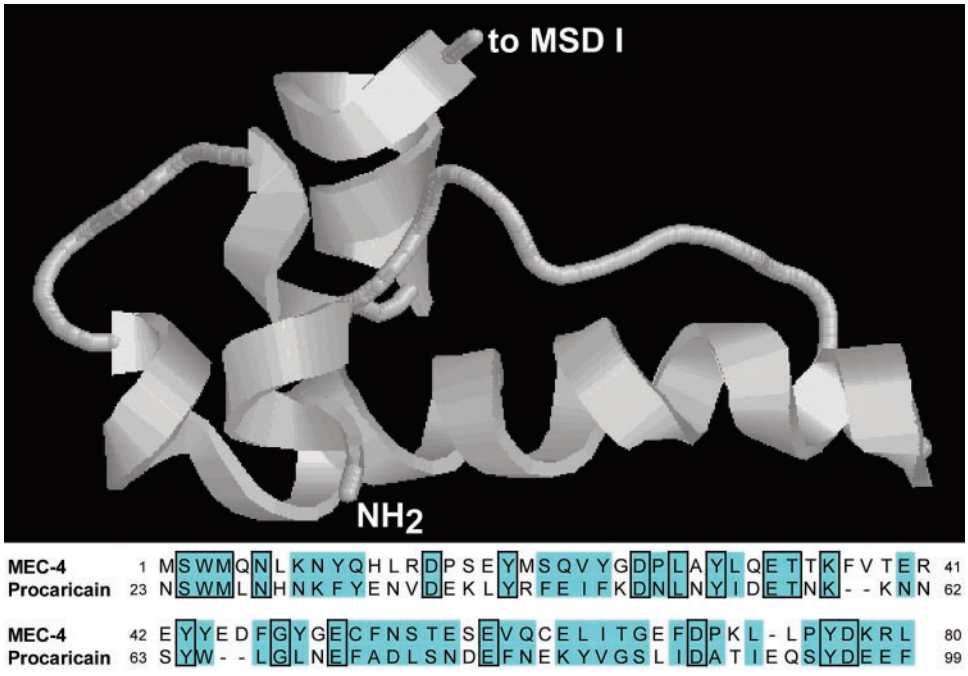

FIG. 17. A three-dimensional model of the extreme, intracellular amino terminus of MEC-4. The domain has been modeled by homology to the protease procaricain (the relevant alignment is shown at the *bottom*). The resulting structure appears to have the capacity for protein-protein interactions via a potential hydrophobic surface (408).

tions. A second hot-spot area for channel-inactivating substitutions overlaps with NTD and CRDII (406). It is proposed that this region participates in interactions of the channel with the extracellular matrix (162, 190).

The subunit compositions and stoichiometries for DEG/ENaC channels have not yet been unequivocally determined (7, 221). Electrophysiological assays of the rat ENaC channel reconstituted in oocytes established that at least three homologous subunits (α-, β-, and γ-ENaC) must be coexpressed to assemble an active channel with the pharmacological properties similar to the in vivo channel (7, 29, 144, 200, 221, 363, 427). The touch receptor channel also appears to be multimeric. Evidence that MEC-4 and MEC-10 coassemble into the same channel complex include that *1*) MEC-4 and MEC-10 subunits are coexpressed in the touch receptor neurons (101, 193), *2*) MEC-4 and MEC-10 proteins can coimmunoprecipitate (155), and *3*) genetic interactions between *mec-4* and *mec-10* have been observed (162). For example, *mec-10* can be engineered to encode a death-inducing amino acid substitution [*mec-10*(A673V); Ref.193]. However, if *mec-10*(A673V) is introduced into a *mec-4* loss-of-function background, neurodegeneration does not occur. This result is consistent with the hypothesis that MEC-10 cannot form a functional channel in the absence of MEC-4. In support of this functional interaction, the coexpression of the degenerin mutants MEC-4(d) and MEC-10(d) in *Xenopus* oocytes gives rise to Na^+ currents, which increase further by coexpression of MEC-2 and MEC-6 (73, 155). Genetic experiments also suggest that MEC-4 subunits interact with each other. The toxic protein MEC-4(A713V) can kill cells even if it is coexpressed with wild-type MEC-4(+) [as occurs in a *trans* heterozygote of genotype *mec-4(d)/mec-4(+)*]. However, if toxic MEC-4(A713V) is coexpressed with a specific *mec-4* allele that encodes a single amino acid substitution in MSDII [for example, *mec-4(d)/mec-4*(E732K)], neurodegeneration is partially suppressed (162, 189). Because one MEC-4 subunit can interfere with the activity of another, it can be inferred that there may be more than one MEC-4 subunit in the channel complex. Genetic analysis of intragenic suppressor mutations, both in *cis* or in *trans* to dominant alleles, suggests that *deg-1* and *unc-8* are each likely to form multimeric complexes (70, 141, 308, 369).

An increasing amount of evidence suggests that specific DEG/ENaC ion channels may be mechanically gated (see Table 3 for a list of all DEG/ENaC proteins implicated in mechanotransduction). Because it has not yet been possible to directly demonstrate mechanical gating of the MEC-4/MEC-10 touch receptor channel or the UNC-8 channels using electrophysiological approaches, two models for the biological activities of degenerin channels have been considered (404, 405). In the simplest model, the degenerin channel mediates mechanotransduction directly. The alternative model is that the degenerin channel acts indirectly to maintain a required osmotic balance within a neuron so that a mechanosensitive channel, yet to be identified, can function. In the case of the touch

TABLE 3. *DEG/ENaC proteins implicated in mechanotransduction in Caenorhabditis elegans, Drosophila, and mammals*

Protein	Expression Pattern	Postulated Function	Organism	Reference Nos.
DEL-1	Motorneurons Sensory neurons	Stretch sensitivity Proprioception	*Caenorhabditis elegans*	409
DEG-1	Interneurons Sensory neurons Muscle Hypodermis	Harsh touch sensitivity Muscle stretch	*Caenorhabditis elegans*	70, 141
MEC-4	Touch receptor neurons	Touch sensitivity	*Caenorhabditis elegans*	101
MEC-10	Touch receptor neurons Other sensory neurons	Touch sensitivity	*Caenorhabditis elegans*	193
UNC-8	Motorneurons Interneurons Sensory neurons	Stretch sensitivity Proprioception	*Caenorhabditis elegans*	409
UNC-105	Muscle	Stretch sensitivity	*Caenorhabditis elegans*	258
PPK (DmdNaC1)	Sensory dendrites of peripheral neurons	Touch sensitivity Proprioception	*Drosophila melanogaster*	1
BNC1 (ASIC2, MDEG, BNaC1)	Lanceolate nerve endings that surround the hair follicle	Touch sensitivity	*Mus musculus*	142, 326
β-ENaC	Skin nerve terminals	Touch sensitivity		105, 131
γ-ENaC	Baroreceptor nerve terminals innervating the aortic arch and carotid sinus Skin nerve terminals	Touch sensitivity Pressure sensitivity	*Rattus norvegicus*	106, 131
ASIC3 (DRASIC)	Dorsal root ganglia neurons Large-diameter mechanoreceptors Small-diameter peptidergic nociceptors	Mechanosensation Acid-evoked nociception	*Mus musculus*	327

receptor channel, the absence of either MEC-4 or MEC-10 renders the mechanosensory neuron nonfunctional, making it impossible to distinguish between the two alternative hypotheses (101, 193). The situation with the UNC-8 channel is different. It is clear from the phenotype of *unc-8* null mutants that the majority of neurons that express *unc-8* must remain functional in the absence of UNC-8 activity (409). Our understanding of neuronal circuitry and characterized behavioral mutants argues that if these neurons were not functional, *unc-8* null mutants would exhibit severely defective locomotion. Given that *unc-8* null mutants move in a manner only marginally different from wild-type animals, the case that the UNC-8 channel maintains an osmotic milieu required for the function of other neuronal channels is weakened. One caveat to this discussion is that we cannot rule out the possibility that a functionally redundant and as yet unidentified degenerin family member might be coexpressed with *unc-8* and could nearly compensate for its absence. A key question that remains to be resolved is how broadly DEG/ENaC family members will prove to be involved in mechanotransduction. Analyses of the mammalian ENaC channel in lipid bilayers suggests that its gating can be influenced by membrane stretch (12, 201), although interpretation of these studies requires attention to experimental caveats (346).

Some DEG/ENaC family members are gated by nonmechanical stimuli. For example, one distant member, the snail *Helix aspersa*, FaNaC, is gated by FMRF-amide (254). In *C. elegans*, the FLR-1 member of the family is thought to be gated by fluoride ions via a signal transduction mechanism (217, 401). *flr-1* loss-of-function mutations confer resistance to fluoride toxicity, show slow growth, and increase the frequency of ultradian rhythms such as the defecation cycle (217). Unfortunately, the mechanism of fluoride poisoning is unknown, so it is difficult to build models of FLR-1 activity. Members of the mammalian ASIC (acid sensing ion channel) subfamily are gated by protons (14, 23, 24, 29, 431, 432, 454). Expression of these subunits individually in heterologous cells generates transient H^+-gated, amiloride-sensitive, Na^+-selective currents (221, 431, 471). Moreover, when coexpressed, they associate and produce currents with unique functional properties, suggesting that they have the capacity to form heteromultimers (14, 29). It has been suggested that they might be involved in the perception of pain during tissue acidosis (75, 432), as well as in mechanosensation (see sect. VI*B*; Refs. 221, 404). For example, loss of ASIC3 (also known as DRASIC) reduced sensitivity of some skin mechanoreceptors (Aδ fibers) to a noxious pinch and the responsiveness of acid- and noxious heat-sensitive (C-fiber) nociceptors (327, 471).

B. Degenerin-Induced Cell Death

As noted earlier, apart from forming a candidate mechanosensitive channel, MEC-4, MEC-10, and other nematode degenerins can be mutated to harbor specific amino acid substitutions that result in hyperactivated channels, which induce swelling and necrosis of the cells expressing them (Fig. 18; Refs. 70, 101, 141, 193, 369). The capacity to inflict neurodegeneration when mutated appropriately inspired the term *degenerin* for DEG-1, the

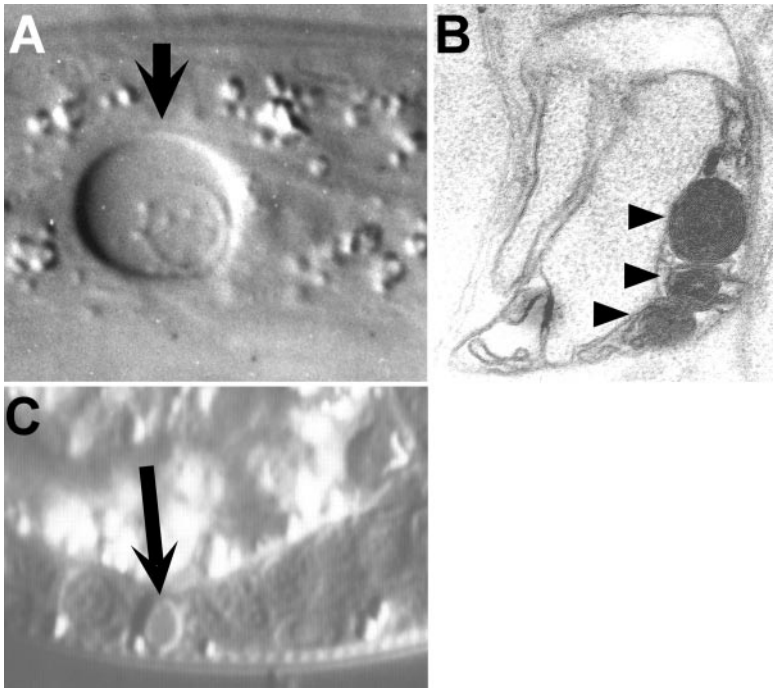

FIG. 18. Neurodegeneration in *C. elegans*. *A*: a dying PVM, expressing the toxic mec-4(d) allele is shown (arrow). The cell has swollen dramatically, and the nucleus appears highly distorted (165, 399, 403). *B*: under the electron microscope, the radical membrane invaginations effected within the failing cell take the form of electron-dense whorls that coalesce and become endocytosed (arrowheads; Ref. 165). *C*: the hollow look of the degenerating neuron is easily contrasted to that of an apoptotic cell shown in *C*, which has the characteristic buttonlike compact appearance (arrow).

first member of the family identified, and is now collectively used for related proteins in *C. elegans*. We note here that not all genes, which can mutate to trigger necrotic cell death in *C. elegans*, encode for degenerins. The term was initially coined to describe a terminal phenotype caused by a mutation rather than a class of proteins related in sequence. For example, toxic *deg-3* alleles cause neurodegeneration similarly to toxic *deg-1* or *mec-4* alleles, yet *deg-3* encodes an acetylcholine receptor Ca^{2+} channel, not a degenerin (424).

The unusual gain-of-function (dominant; *d*) mutations in the *mec-4* gene induce degeneration of the six touch receptor neurons required for the sensation of gentle touch to the body (101). In contrast, most *mec-4* mutations are recessive loss-of-function mutations that disrupt body touch sensitivity without affecting touch receptor ultrastructure or viability (64). *mec-4(d)* alleles encode substitutions for a conserved alanine that is positioned extracellularly, adjacent to pore-lining membrane-spanning domain II (see Fig. 14*B*). Similarly, dominant mutations at the same amino acid site in *deg-1*, the founding member of the degenerin family, induce death of a group of neurons that includes the PVC interneurons of the posterior touch sensory circuit (70, 141). Loss-of-function mutations in *deg-1* appear wild type in behavior (70). The capacity to mutate to toxic forms appears to be a general property of degenerins, since engineering the corresponding mutation in MEC-10 [*mec-10(A673V)*] generates a dominant allele that inflicts neurodegeneration, similarly to *mec-4(d)* (193).

The size of the amino acid side chain at this position is correlated with toxicity. Substitution of a small side-chain amino acid does not induce degeneration, whereas replacement of the alanine with a large side chain amino acid is toxic (101). This suggests that steric hindrance plays a role in the degeneration mechanism and supports the following working model for *mec-4(d)*-induced degeneration: MEC-4 channels, like other channels, can assume alternative open and closed conformations. In adopting the closed conformation, the side chain of the amino acid at MEC-4 position 713 is proposed to come into close proximity to another part of the channel (189). Steric interference conferred by a bulky amino acid side chain prevents such an approach, causing the channel to close less effectively. Increased cation influx ensues, which initiates neurodegeneration (Fig. 19). The proposal that ion influx is critical for degeneration is supported by electrophysiological studies of heterologously expressed, mutant *C. elegans* proteins carrying hyperactivating amino acid substitutions (140, 155). Also, large side-chain substitutions in some neuronally expressed mammalian family members at the position analogous to that of toxic mutations in nematode degenerins markedly increase channel conductance (433). In addition, amino acid substitutions that disrupt the channel-conducting pore can prevent neurodegeneration when present in *cis* to the A713 substitution (189, 190).

Degenerins can mutate in a second way to cause toxicity. Studies of recessive *deg-1(u506)* allele, which causes neurodegeneration, established that it encodes a

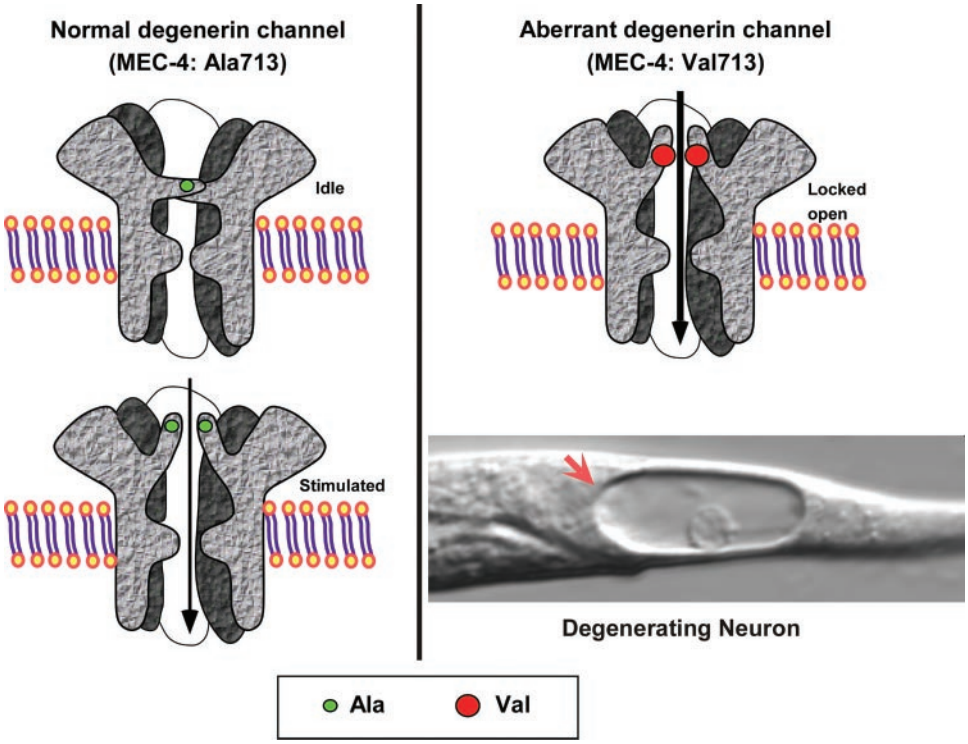

FIG. 19. Degenerin-induced neurodegeneration. MEC-4 is used as a paradigm. Gain-of-function mutations in the degenerin gene *mec-4* encode substitutions for a conserved alanine adjacent to MSDII and result in neuronal degeneration. Amino acids with bulkier side chains at this position are thought to favor an open-channel conformation by causing steric hindrance, resulting in Na^+ influx that triggers the necrotic-like cell death shown at the *bottom right* (red arrow) (101, 140, 399, 403).

substitution of alanine-393 for threonine, situated within a 22-amino acid stretch in the predicted extracellular regulatory domain (ERD) that is conserved among the *C. elegans* degenerin subfamily, but is missing from mammalian family members (Fig. 14; Ref. 141). Introduction of the same amino acid change in MEC-4 (A404T) or a small deletion (Δ399–407) in this region also creates a toxic allele (141). This observation is consistent with the idea that these mutations disrupt a channel closing domain that is situated on the extracellular side. Alternatively, death-inducing substitutions in the extracellular domain could change the MEC-4 three-dimensional structure so as to favor the open channel conformation. Interestingly, dominant *unc-8* alleles that induce neuronal swelling also map to this domain (glycine-387 for glutamic acid; Ref. 409). Elevated ion influx has been implicated in toxicity, suggesting that this region may be required for conformational changes that close, or stabilize, a closed state of the channel (141).

In addition, a mutation in the conserved, extracellular domain of UNC-105, after the first transmembrane domain, hyperactivates the channel. A substitution of proline-134 for serine results in muscle hypercontraction and can cause degeneration when expressed in nonmuscle cells (140, 258).

The touch receptor neurons in *mec-4(d)* mutants express terminally differentiated properties before they die, and the PVC neurons in *deg-1(d)* mutants differentiate and function before they degenerate (59, 64). *mec-4(d)*- and *deg-1(d)*-induced cell deaths have therefore sometimes been referred to as the nematode version of "late-onset" neurodegeneration. *mec-4(d)*- and *deg-1(d)*-induced neurodegeneration occurs autonomously and independently of programmed cell death executors (81). Genetic mosaic analyses first indicated that *mec-4(d)* kills because of toxic activity within the cells that die (185). Ectopic expression of *mec-4(d)* can induce swelling and death of cells other than the touch receptor neurons, confirming the cell autonomy of *mec-4(d)* action. The execution of degenerative cell death occurs by a mechanism that appears distinct from that in programmed cell death (apoptosis). Degenerative cell death triggered by *mec-4(d)*, *deg-1(d)*, and *mec-10*(A673V) mutations differs from programmed cell death and exhibits morphological features of necrotic cell death (165). There are several macroscopic distinctive features of degenerin-induced cell death. First, cells undergoing programmed cell death appear compacted and "buttonlike," whereas cells undergoing degenerative cell death appear swollen and enlarged (Fig. 18; Refs. 399, 436). Second, discrete ultrastructural changes accompany the two types of death (see below). Third, programmed cell deaths transpire within a 1-h time frame, whereas execution of degenerative death can span several hours (165). Fourth, at the genetic level, it has been demonstrated that *ced-3* and *ced-4* mutations, which suppress programmed cell death, do not block *mec-4(d)*- and *deg-1(d)*-induced cell degeneration (81). Finally, *mec-4(d)* and *deg-1(d)* alleles do not disrupt programmed cell deaths (70, 81, 101).

Although *mec-4(d)* and *deg-1(d)* mutations kill different groups of neurons, the morphological features of cell deaths they induce are the same. Careful studies of the timing of *mec-4* expression relative to the onset of degeneration support that the time of onset of degenerative death correlates with initiation of degenerin gene expression (70, 165). In general, vacuolated degenerating cells appear ~4 h before hatching and in some cases persist >10 h after hatching (165). For PVM, vacuoles appear at a variable time after cells are produced, ~10–12 h after hatching. The rapidity of death occurrence correlates with the dose of the toxic allele and on average can take ~8 h (165). These observations are consistent with the hypothesis that a threshold ion influx is needed to initiate the degenerative process. When viewed using the light microscope, the nucleus and cell body of the affected cell first appear distorted and then the cell swells to several times its normal cell diameter (70). Eventually the swollen cell disappears, often after shrinking but sometimes as a consequence of cell lysis. Ultrastructural analysis has established that degeneration is more than the burst of a cell in response to osmotic imbalance. Death initiates with striking infolding of the plasma membrane. Small tightly wrapped membranous whorls are the first indications of pathology. These whorls are internalized and appear to coalesce into large electron-dense membranous structures (Fig. 18*B*; Refs. 165, 399). Subsequently, internalized whorls grow in size and large vacuoles appear. The striking membranous inclusions suggest that intracellular trafficking may contribute to degeneration. Cell body volume can increase 100-fold during this process. The nucleus becomes distorted and chromatin clumps before internal degradation of cell contents occurs (165). Organelles and cytoplasmic contents are degraded, usually leaving a membrane-enclosed shell. Finally, corpse debris is removed in a process that requires the activities of the corpse-engulfment *ced* genes, such as *ced-2*, *ced-5*, and *ced-10* (81). Thus, although mechanisms of killing are distinct in programmed and degenerin-induced cell death, corpse recognition and removal mechanisms share common steps.

What is the cause of cell death? Na^+ influx may alter the osmotic balance of the cell or energetically exhausting the cell by overloading Na^+-K^+-ATPases. Alternatively, a reduction of the membrane Na^+ gradient could diminish the activity of the Na^+/H^+ exchanger or of the Na^+/Ca^{2+} exchanger leading to accumulation of Ca^{2+}, which subsequently triggers degeneration. The formation of whorls and vacuoles may represent an attempt of the cell to reduce leakage; the cell internalizes plasma membranes, and channels are incorporated into intracellular whorls,

where the cationic influx may induce their swelling and vacuolation. Damaged mitochondria observed in a few terminally degenerating cells may be due to the dilution of the cytosol, which in turn water down the mitochondrial intermembrane space and compress the mitochondrial matrix. Damage to mitochondria may also ensue from excessive demand for ATP needed to pump Na^+ from the cytosol (140).

The initiation and execution of degenerative cell death in *C. elegans* and its general neuropathology are reminiscent of elements of excitotoxic cell death and other necrotic-like cell death in higher organisms (78, 274, 399, 403). Excitotoxic neuronal death mediated via glutamate receptors channels in cell culture or in vivo in response to ischemia that occurs after injury, or in stroke, is an example of this type of cell death (275, 359). It is also interesting that there are many reported instances, in animals as diverse as flies, mice, and humans, in which neurons degenerating due to genetic lesions exhibit morphological changes similar to those induced by *mec-4(d)* and other hyperactivated degenerins (202, 399, 468). For example, in some mammalian degenerative conditions such as neuronal ceroid lipofuscinosis (Batten disease; the *mnd* mouse) and that occurring in the *wobbler* mouse, cells develop vacuoles and whorls (fingerprint bodies) that look similar to internalized structures in dying *C. elegans* neurons (85, 319, 399). In addition, the dramatic endocytosis observed during neurodegeneration in *C. elegans* resembles altered intracellular membrane trafficking in Alzheimer's disease and Huntington's disease (45, 173, 325, 399).

The identification of *C. elegans* mutations that cause necrotic-like cell death enables us to exploit the strengths of this model system to gain novel insight into a nonapoptotic death mechanism. The intriguing observation that distinct cellular insults can induce a similar necrotic-like response suggests that *C. elegans* cells may respond to various injuries by a common process, which can lead to cell death. Given that apoptotic death mechanisms are conserved between nematodes and humans (290), it can be hypothesized that various cell injuries, environmentally or genetically introduced, converge to activate a degenerative death process that involves common biochemical steps. At present, the question of common mechanisms remains an intriguing but open question. If specific genes enact different steps of the degenerative process, then such genes should be identifiable by mutation in *C. elegans*. Indeed, suppressor mutations in several genes that block *mec-4(d)*-induced degeneration have been isolated (70, 369, 472). Although some suppressor mutations affect channel function (for example, mutations in *mec-6*; Refs. 70, 369), others are more generally involved in the death process (400, 472). Analysis of such genes should result in the description of a genetic pathway for degenerative cell death. Perhaps, as has proven to be the case for the analysis of *C. elegans* programmed cell death mechanisms, elaboration of an injury-induced death pathway in *C. elegans* may provide insight into neurodegenerative death mechanisms in higher organisms.

C. An All-Purpose Model for the Mechanotransducer in *C. elegans* Touch Receptor Neurons, Motorneurons, and Muscle

The features of cloned touch receptor cell, motorneuron, and muscle structural genes, together with genetic molecular and electrophysiological data that suggest interactions between them, constitute the basis of a model for the nematode mechanotransducing complex (Fig. 20; Refs. 58, 103, 138, 141, 258). The architecture of this mechanotransducer complies with the general principle

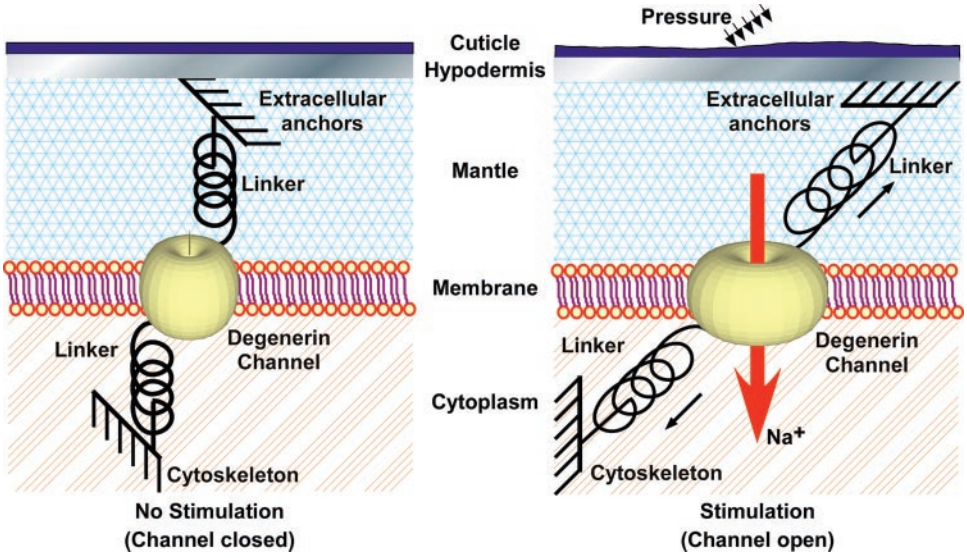

FIG. 20. A mechanotransducing complex in *C. elegans* touch receptor neurons. In the absence of mechanical stimulation, the channel is closed, and therefore, the sensory neuron is idle. Application of a mechanical force to the body of the animal results in distortion of a network of interacting molecules that opens the degenerin channel. Na^+ influx depolarizes the neuron initiating the preceptory integration of the stimulus (138, 141, 402, 404, 405).

of the tethered mechanosensitive ion channel discussed in section II*A*. The central component of the mechanotransduction apparatus is the putative mechanosensitive ion channel that includes multiple MEC-4 and MEC-10 subunits in the case of touch receptor neurons, UNC-8 and DEL-1 subunits in the case of motorneurons, and UNC-105 in the muscle (reviewed in Refs. 405, 402). These subunits assemble to form a channel pore that is lined by the hydrophilic residues of membrane-spanning domain II (189). Subunits adopt a topology in which the cysteine-rich and neurotoxin-related domains extend into the specialized extracellular matrix outside the touch cell and the amino and carboxy termini project into the cytoplasm. Regulated gating depends on mechanical forces exerted on the channel. Tension is delivered by tethering the extracellular channel domains to the specialized extracellular matrix and anchoring intracellular domains to the microtubule cytoskeleton. Outside the cell, channel subunits may contact extracellular matrix components (such as MEC-1, MEC-5, and/or MEC-9 in the case of the touch receptor mantle, and type IV collagen LET-2/SUP-20 in the muscle; Refs. 104, 109, 141, 162, 258). Inside the cell, channel subunits may interact with the cytoskeleton either directly or via protein links (such as MEC-2 in the touch receptor neurons or UNC-1 in motorneurons; Refs. 155, 162, 194, 329, 330). A touch stimulus either could deform the microtubule network or could perturb the mantle connections to deliver the gating stimulus. In both scenarios, Na^+ influx would activate the touch receptor to signal the appropriate locomotory response. This is an attractive hypothesis, but confirmation has been stonewalled by the technical challenge of stimulating and recording directly from the *C. elegans* touch neurons, which are tiny (soma on the order of 1 μm) and embedded in the hypodermis. Furthermore, reconstitution of the mechanotransducing complex in a heterologous system is likely to require both channel expression and regeneration of gating contacts, which would be no small feat. Nonetheless, ongoing efforts to surmount technical difficulties in direct recording from nematode sensory neurons may soon provide decisive information. For example, the phenomenon of fluorescence resonance energy transfer (FRET) has been exploited to develop Ca^{2+} reporter GFP fusions (cameleons; Refs. 225, 294). Such reporters have been used to monitor physiological neuronal responses to touch stimuli in living animals, through in vivo optical imaging (398).

The model proposed for mechanotransduction in the touch receptor neurons and motorneurons of *C. elegans* shares the same underlying principle and features of the proposed gating mechanism of mechanosensory ion channels in *Drosophila* sensory bristles (discussed below in sect. V*B*) and the channels that respond to auditory stimuli in the hair cells of the vertebrate inner ear (121, 152, 168, 195, 196, 204, 317, 318, 452). Hair cells have bundles of a few hundred stereocilia on their apical surface, which mediate sensory transduction. Stereocilia are connected at their distal ends to neighboring stereocilia by filaments called tip links. The integrity of the tip links is essential for channel opening, and the mechanosensitive channels appear to be situated at the ends of the stereocilia, near the connecting tip links. These channels are presumed to be anchored to the actin cytoskeleton of the stereocilium via a specialized myosin isoform (myosin VIIa; Refs. 150, 242). Directional deflection of the stereocilia relative to each other introduces tension on the tip links, which is proposed to open the mechanosensitive hair cell channels directly.

V. MECHANOTRANSDUCTION IN *DROSOPHILA MELANOGASTER*

Drosophila melanogaster offers unique advantages for investigating mechanotransduction that complement studies in *C. elegans*. In addition to the sequenced genome and powerful genetic techniques, the fly allows application of electrophysiological methodologies to monitor the function of mechanoreceptor neurons (223). This capability is decisive for deciphering and dissecting the molecular mechanisms of mechanotransduction and is still largely lacking in the worm. For this reason, it is expected that *Drosophila* studies will contribute significantly to the advancement of our understanding of mechanotransduction across phylogeny (437).

A. *Drosophila* Mechanosensory Organs

Many different mechanosensory organs mediate touch, proprioception, balance, and hearing in *Drosophila* (224, 417–419). There are two main classes of mechanosensors that transduce mechanical stimuli into membrane potential changes: type I and type II. The first class can be further divided into two distinct subtypes: the external sensory (es) and the chordotonal (ch) organs (205). Type I sensory organs or sensilla comprise one or more bipolar neurons, each bearing a ciliated sensory process surrounded by three specialized supporting cells, whereas type II sensory cells are single, nonciliated, multidendritic, or bipolar neurons (205). In external sensory organs, such as mechanosensory bristles, the two outer support cells create external cuticular structures, which can be deflected or deformed by touch, airflow, or proprioceptive stimulation. Each mechanosensory bristle organ is composed of a hallow hair shaft and three cells: the socket cell, the sheath cell, and a ciliated mechanosensory neuron (219). Deflections of the external bristle in the shaft compress the neuron's dendritic tip and gate the transduction channels, causing depolarization of the cell and promoting neurotransmitter release. Insect bristles

and the vertebrate cochlea share the unusual property of secreting a K^+-rich extracellular fluid, which provides additional driving force for neuron firing (89, 160). In contrast, the chordotonal organs have no associated external structures. They lie under the cuticle and respond to stretching by flexion of the joint between two segments. Their characteristic feature is the scolopale, a spindle-shaped cage enclosing an extracellular cavity, into which the ciliary outer segment extends (113, 219).

Mechanosensory bristles are readily suitable for electrophysiological recordings in the intact animal, allowing coupling of electrophysiological studies of mechanotransduction in situ, with genetic studies of mechanosensory cells and behaviors. Fly mechanoreceptor potentials, recorded as changes in transepithelial potential evoked by mechanical stimuli, show latencies of ~200 μS, a response time >100 times faster than the fastest known second messenger cascade, indicating that fly mechanosensory transduction is rather directly gated by mechanical stimulation than through second messengers (438). In addition, small transduction currents were elicited by stimuli of only 100 nm, with the corresponding displacement at the base of the bristle to be estimated ~50-fold less, or 2 nm, indicating the high level of sensitivity of these neurons (438). The adaptation properties of mechanosensory bristles closely resemble those of vertebrate hair cells (88, 317). Such adaptation permits mechanoreceptors to continuously adjust their range of responsiveness, thus enabling the cell to detect new displacements in the presence of an existing stimulus. This suggests that the core transduction components in fly bristles and vertebrate hair cells are functionally related.

B. Genetics of Mechanotransduction in *Drosophila*

1. Mechanosensory mutants

Numerous *Drosophila* mutants with defects in mechanosensory behavior and electrophysiology have been isolated that have resulted in the identification of many genes required for mechanotransduction in external sensory organs (114, 223). Two genes, *uncoordinated* (*unc*) and *uncoordinated-like* (*uncl*), were identified mutations in a genetic screen for defective touch response in *Drosophila* larvae (223). Subsequent screens for uncoordinated flies yielded several mutants with mechanosensory defects that have been classified into two major groups: the *nomp* mutants (no mechanoreceptor potential) and the *remp* mutants (reduced mechanoreceptor potential). Electrophysiological recording showed that *unc*, *uncl*, and *nomp* mutations eliminate or reduce bristle mechanosensory receptor potentials, resulting in mutant flies that are touch insensitive and uncoordinated (223). Interestingly, all these mutations eliminate or reduce the sound-evoked potentials from the antennal nerves, indicating that they can also affect transduction by the sensory neurons in the chordotonal Johnston's organ, a *Drosophila* auditory receptor. Such evidence suggests that related transduction mechanisms operate in external sensory and the chordotonal organs, which require the function of *unc*, *uncl*, and *nomp* gene products (115). In addition, mutations specifically affecting chordotonal organs have been identified, such as *beethoven* (*btv*) and *touch-insensitive-larva B* (*tilB*). These mutants show defects in the axonemal cytoskeleton, which suggests a role for ciliary action in mechanotransduction by chordotonal neurons (114, 115).

2. The nompC gene

Cloning and characterization of the *nompC* gene revealed that it encodes a new, distant member of the *Drosophila* TRP (transient receptor potential) family of cation channels (438). In *Drosophila*, the TRP and TRP-like (TRPL) family members form Ca^{2+}-permeable channels, which are expressed almost exclusively in photoreceptor cells and mediate responses to light (292, 298). Recent reports also suggest a role of TRP channels in olfactory system development. Specifically, analysis of *trp* gene expression and temperature-shift analysis of temperature-sensitive *trp* mutants indicate that *trp* function is required during development for normal olfactory adaptation but not olfactory sensation, while being dispensable in the mature antenna (385).

nompC specifies a long protein with six transmembrane domains and an unusual amino terminus containing 29 ankyrin (ANK) motifs, rather than the more usual two to four. nompC defines the TRPN branch of the TRP superfamily, along with the nematode and zebrafish NompC proteins. Loss-of-function mutations in *nompC* gene largely reduce mechanosensory responses leading to severe uncoordinated phenotype, whereas a missense mutation in an extracellular loop between two transmembrane domains alters the fine characteristics (adaptation properties) of mechanically induced currents. In situ hybridization revealed that *nompC* is selectively expressed in ciliated mechanosensory organs in *Drosophila*, such as bristles and chordotonal organs (438). Hence, evidence strongly suggests that NompC is a mechanosensitive ion channel in *Drosophila* sensilia. Like other TRP family channels, NompC might form heteromultimers with another subunit. This could explain the residual mechanoreceptor currents in *nompC* null mutants. Alternatively, NompC might be a necessary regulator of the channel rather than the channel itself (110). The presence of the long ANK repeats is intriguing. It is tempting to speculate that they serve to anchor the mechanosensory channel to the cytoskeleton in a fashion similar to the general mechanotransduction model that has been formulated for the *C. elegans* touch receptor and vertebrate hair cell mech-

anotransducer. Therefore, even though the underlying principles pertinent to mechanotransduction might be similar for the two systems, they are implemented via different, specialized sensory ion channels.

Sequence similarity searches of the *C. elegans* genome identified 24 genes predicted to encode TRP proteins, which are likely to have diverse cellular functions. They fall into seven subfamilies, six of which were also represented in *Drosophila*, *Fugu rubripes*, and humans, whereas one appeared to be nematode specific (157). The six conserved subfamilies of TRP channels encompass three subfamilies, which are more related to *Drosophila* TRPs, TRP-canonical (TRPC), TRP-vanilloid (TRPV), TRP-melastatin (TRPM), and the three more divergent TRP-NompC (TPRN), TRP-polycystin (TRPP), and TRP-mucolipidin (TRPML) (299).

Members of the TRPM subfamily in *C. elegans* are the *gon-2* (gonadogenesis abnormal) and *ced-11* (abnormal programmed cell death) gene products, which may mediate Ca^{2+} influx and control cell division and apoptosis, respectively. A TRPC homolog in *C. elegans* (TRP-3) is required for sperm-egg interactions during fertilization (473). Members of the TRPV subfamily are the *osm-9* and *ocr* gene products, whereas TRPP subfamily comprises the LOV-1 and PKD-2 proteins. LOV-1 (for location of vulva) and PDK-2 are the homologs of mammalian PDK1 and PDK2, respectively (86). Mutations in PDK1 or PDK2 genes result in a very common inherited disease, the autosomal dominant polycystic kidney disease (ADPKD). PDK1 and PDK2 form a Ca^{2+}-permeable ion channel, which is activated by bending of the apical cilium and sensing fluid flow in certain epithelial cells (301). LOV-1 and PDK-2 act in nematode mating; males deficient in either or both *lov-1* and *pdk-2* are dramatically less successful in the recognition of hermaphrodites and locating the vulva, resulting in decreased male mating efficiencies. Both proteins are localized to the ends of sensory neurons in male tails and to the CEM neurons in the head, consistent with a chemo- or mechanosensory function (20, 212).

A singe, previously uncharacterized, member of the TRPN subfamily has been identified in the *C. elegans* genome (*Ce*-NompC). *Ce*-NompC is expressed in two interneurons of the nerve ring and in the sensory dendrites of CEPV, CEPD, and ADE ciliated neurons, which play a role in osmosensation (438). However, *Ce*-NompC is not expressed in the ciliated neurons that mediate the nose-touch response, or in the touch-receptor neurons that mediate the gentle body touch response (110).

An additional nematode gene, *osm-9*, that encodes a member of the TRPV family class of ion channels has been implicated in mechanotransduction. *osm-9* is expressed in the ciliated, polymodal ASH neurons, where it appears to mediate osmosensory and mechanosensory responses to osmotic and nose-touch stimuli, respectively (84). OSM-9 might be directly gated by mechanical stimuli, or it could be regulated by G protein-coupled receptors. Alternatively, OSM-9 could indirectly regulate touch responses, for example, by regulating the ionic concentration in sensory cilia. Interestingly, *osm-9* is also expressed in olfactory neurons where it mediates chemosensory responses, together with other members of the TRPV family encoded by the *ocr* genes (*osm-9*/capsaicin receptor related; Ref. 422). The *ocr* genes (*ocr-1* to *ocr-4*) are expressed in many but not all of the neurons expressing *osm-9*. This suggests that OSM-9 serves different functions in different neurons depending on the OCR subunit (422). For example, OSM-9/OCR-2 activity has been related to social behavior in *C. elegans* as mutations in these genes abolish animal aggregation and group feeding (96).

3. nompA

Mutations in the *nompA* gene, which eliminate mechanotransduction in *Drosophila* bristles, were shown to disrupt contacts between neuronal sensory endings and cuticular sensory structures (82). Cloning and characterization of *nompA* revealed that it encodes a large transmembrane protein, exclusively expressed in type I sensory support cells of the peripheral nervous system (82). It has a modular extracellular segment that includes a zona pellucida (ZP) domain and several plasminogen amino-terminal (PAN) modules. The NompA extracellular domain is localized specifically to the dendritic cap, an extracellular matrix that connects the sensory cilia to cuticular structures (in external sensory organs), or to attachment cells (in chordotonal organs), and transmits mechanical stimuli to the transduction apparatus (82). This suggests that NompA creates a mechanical linkage required for mechanotransduction in sensory neurons.

4. Painless

Painless represents another member of the TRPN gene family in *Drosophila* that is required for sensing both high temperature and strong mechanical stimuli, but not for sensing light touch. It is expressed in the chordotonal and multidendritic sensory neurons, suggesting that these neurons are involved in nociceptive signaling (423).

5. Nanchung

Nanchung (Nan) is one of the two members of the TRPV family in *Drosophila* and is most similar to *C. elegans* OCR-4. In Chinese hamster ovary (CHO) cells expressing Nan, hyposmotic solutions elicit calcium influx and cation currents. In *Drosophila* embryos and adults, Nan is specifically expressed in chordotonal neurons and is localized to their ciliated endings. Fly mutants lacking Nan show abnormal sedentary behavior and no sound-evoked potentials, although no structural or ultrastructural defects are seen in these mutants. Nan may

form part of a chordotonal transducer channel in the Johnston's organ of *Drosophila*, which is activated by hypotonic stress (but not by capsaicin and high temperature) similarly to the mammalian TRPV4 channel (227).

6. *Candidate DEG/ENaC mechanosensitive channels in Drosophila*

Pickpocket (PPK), a member of the DEG/ENaC family of ion channels, has been implicated in mechanotransduction. PPK was found in the sensory dendrites of a subset of peripheral neurons in late-stage embryos and early larvae. In insects, such multiple dendritic neurons play key roles in touch sensation and proprioception, and their morphology resembles human mechanosensory free nerve endings. These results suggest that PPK may be a channel subunit involved in mechanosensation (1). Recent studies indicate that several *ppk* genes are expressed in the *Drosophila* tracheal system, with distinct temporal and spatial expression patterns during development. Inhibition of PPK function, by either amiloride treatment or RNA interference, suggests that PPK proteins play a role in tracheal liquid clearance. Interestingly, in mammalian airways, the ENaC channel contributes to salt and liquid absorption, thus maintaining gas-filled airways (260). One *ppk* gene (*ppk1*) is expressed in multiple dendritic (md) neurons tiling the larval body wall and a small number of bipolar neurons in the upper brain. Loss of PPK1 function caused enhanced larval locomotion, which implies a role of PPK1 in controlling rhythmic locomotion (3).

VI. SENSORY MECHANOTRANSDUCTION IN VERTEBRATES

Despite enormous progress on the illumination of vertebrate mechanosensory cell biology achieved in recent years, there is still a striking gap between the biophysical information that has accumulated and our understanding of the molecular aspects of mechanosensation. Sophisticated experiments in mice and humans revealed many genes involved in the development and function of the mammalian cochlea and have culminated in the formulation of the gating-spring model for hair cell mechanotransduction (149, 152). However, many pieces of the mechanotransducing apparatus puzzle are still missing. Work in lower vertebrates such as birds, amphibians, and fish has also contributed significantly in complementing and extending the studies with mammals. In these animals mechanosensory structures are often much easier to access, follow, and monitor providing large potential for investigating the molecular basis of auditory transduction (9, 375). In this section we focus on two vertebrate model organisms, the fish *Danio rerio* (zebrafish) and the mouse, which have been used successfully in efforts to molecularly dissect mechanotransduction.

A. Zebrafish Mutants With Mechanosensory Defects

The zebrafish (*Danio rerio*) is a promising vertebrate model organism for studying ear development and hair cell mechanosensitivity, having the benefit of rapid genetic analysis with the convenience of following the mechanosensory structures in the transparent embryo (164). The zebrafish has two major organs for sensing mechanical stimuli: the inner ear for hearing and balance and the lateral line for sensing water movements (302). Although the inner ear of the zebrafish does not feature a cochlea, it encompasses several specific structures such as otoliths, stereocilia, and hair cells for detecting vibrations and the direction of the gravity vector as well as semicircular canals for detecting dynamic rotation in three axes and acceleration (9).

In a large screen for mutants with auditory defects, 58 mutations affecting development of the inner ear have been identified, and analysis of these mutants revealed that many of the ear structures could develop independently of each other (457). Several mutants with specific defects in embryonic and larval motility have been identified in additional genetic screens, using simple behavior tests for selection (158). In 63 motility mutants, obvious defects in muscle development were detected, defining 18 genes that function in myoblast differentiation, muscle fiber development, or muscle tissue maintenance. A total of 105 motility mutants, defining at least 30 genes, had no visible defects in muscle formation but represent a diverse collection of behavioral mutants with no or reduced motility, mechanosensory defects, "spastic" motility, circling behavior, and motor circuit deficits (158). These behavioral mutants include 15 morphologically normal mutants with defective balance, indicating defects within the mechanosensory organs of the auditory-vestibular system (302). Analysis of behavior, anatomy, and physiology of these "circler" mutants classified them in five groups, with each one likely to affect auditory-vestibular mechanosensation at a different step. Elegant electrophysiological measurements of the "microphonic" currents that flow through the transducer channels during deflection of the hair bundle in small groups of cells further classified the mutations in relation to the sequence of events during mechanotransduction. These events include displacement of hair cell bundle, mechanoelectrical transduction, and events subsequent to transduction (302).

The first group contains mutations in the *sputnik* and *mariner* genes, which appear to affect hair bundle integrity. Microphonic or extracellular potentials were either absent or too weak in these mutants, due to a defect in the extracellular linkages resulting in loss of sensitivity to mechanical stimulation (302). Interestingly, *mariner* encodes a zebrafish homolog of the unconventional myosin

VIIA. This myosin is expressed in the sensory hair cells of the inner ear, and in humans it is responsible for many hearing disorders such as the Usher 1B syndrome, DFNA11, and DFNB2 (178, 179, 262, 450, 451). Moreover, *mariner* hair cells resemble those of the *shaker-1* mouse, which is also defective in myosin VIIA, suggesting conservation in mechanisms underlying auditory perception (117, 148). A second group of mutants that includes *orbiter*, *mercury*, and *gemini* have normal hair cell morphology and synaptic transmission but do not show any hair cell microphonic currents (302). This suggests a defect in components of the transduction apparatus. The remaining three mutant groups define one gene each, the *astronaut*, *cosmonaut*, and *skylab* (302). The mutants *astronaut* and *cosmonaut* have nearly normal hair cell microphonic currents, although they have a greatly reduced response to vibrational stimuli. Therefore, these two mutations appear to affect events downstream of mechanotransduction, such as synaptic transmission of neural signals (302). The *skylab* mutant is the only one that shows degeneration of the sensory neuroepithelium, suggesting that this gene product may be involved in the maintenance or survival of hair cells (302). Hence, the characterization of zebrafish circler mutants offers a unique opportunity to dissect the phenomenon of hair cell transduction, and identification of the corresponding genes will advance our knowledge of the molecular basis of mechanotransduction.

Three additional touch-insensitive mutants, *macho*, *alligator*, and *steifftier*, were isolated during the genetic screen for zebrafish motility mutants. Mutant embryos do not respond to touch, although they are motile and can swim spontaneously (158). This behavioral phenotype suggests a defect in the mechanosensory system. Whole cell electrophysiological recordings in semi-intact preparations of *macho*, *alligator*, and *steifftier* mutant embryos revealed defects in specific mechanosensory neurons, the Rohon-Beard primary cells (341). These cells, which are required for normal response to touch, either fail to fire or fire abnormal action potentials, resulting in reduced rapid inward currents. The lack or reduction of overshooting impulses is accompanied by a specific reduction in the amplitude of whole cell voltage-dependent sodium currents (341). The reduction in cellular excitability in these mutants appears responsible for their behavioral phenotypes (341). Therefore, it is conceivable that *macho*, *alligator*, and *steifftier* may encode structural subunits of sodium channels as well as components that developmentally regulate or localize such channels.

1. The zebrafish NompC

The zebrafish ortholog of *Drosophila* NompC TRP cation channel belongs to the subgroup of TRP channels (TRPN) that also includes the nematode *Ce*-NompC (372). It is mainly expressed in embryonic and larval sensory hair cells, and loss of *nompC* function results in deafness and imbalance phenotypes in larvae. These phenotypes are correlated with the absence of mechanotransduction-dependent apical endocytosis and microphonic responses. The findings provide evidence that nompC is required for vertebrate sensory hair cell mechanotransduction, similarly to the *Drosophila* NompC protein.

B. Candidate Mammalian Mechanosensitive Ion Channels

1. γ-ENaC

An increasing amount of evidence suggests that some mammalian DEG/ENaC proteins may play a role in mechanosensation similarly to their nematode counterparts. In mammals, there are strong indications that ENaC subunits may be components of the baroreceptor mechanotransducer, one of the most potent regulators of anterial pressure and neurohumoral control of the circulation (106, 107). Baroreceptors innervate the aortic arch and carotid sinuses and are activated by pressure-induced vessel wall stretch. It has been shown that γ-ENaC is localized to baroreceptor-nerve terminals that detect acute fluctuations in arterial pressure (106). In addition, an amiloride analog, which inhibits DEG/ENaC channels, also blocks baroreceptor-nerve activity (106). Although these observations suggest that an ENaC subunit is a component of the mechanosensitive channel responsible for baroreceptor function, it should be noted that amiloride and its analogs also inhibit other types of channels. The trimeric epithelial Na^+ channel itself does not appear to be the mechanosensor; the α-subunit, which is required for constitutive channel function, is not present in the baroreceptor neurons (105). It seems more likely that other, as yet unidentified subunits multimerize with γ- and possibly β-subunits to form the mechanosensor, or these subunits may have a different role in baroreceptor neurons than in epithelia tissue. Furthermore, it has been shown that β- and γ-ENaC, but not α-ENaC, are located in tactile sensory receptors in the hairless skin of the rat paw, suggesting that these subunits may be components of a mechanosensory receptor for touch (105). ENaC immunoreactivity was also detected in mechanosensory lanceolate nerve endings of the rat mystacial pad in the vibrassae (whisker). It is intriguing that stomatin-like immunoreactivity has also been observed in the same lanceolate endings of the vibrissal follicle complex, signifying that not just the channel proteins, but an entire complex analogous to the *C. elegans* touch-transducing complex, might be assembled in mammalian touch sensory neurons (131). We note here that β- and γ-ENaC knockout mice die of severe metabolic abnormalities within 1–2 days after birth, precluding a physiological

analysis (221, 281, 284). Tissue-specific or conditional disruption of these genes in mice may allow a detailed physiological assessment of their role in the future. Humans with pseudohypoaldosteronism (PHA) type I have mutations in β- and γ-ENaC subunits, without apparent associated defects in mechanosensation (159, 346, 348). Nonetheless, there is likely to be significant redundancy in mechanosensors, and person to person differences in sensation may be difficult to detect. Furthermore, subtle defects might go undetected without a detailed electrophysiological analysis, which is not possible in humans.

2. BNC1

Members of the acid-sensing ion channel (ASIC) subgroup of the DEG/ENaC family have been implicated in mechanotransduction in mammals. BNC1 (brain Na^+ channel; also known as MDEG, BNaC1, ASIC2; Refs. 139, 328, 433, 434) has emerged as a promising candidate for a mechanosensitive channel; it is the ASIC member most similar in amino acid sequence to nematode MEC-10 (as determined by computational analysis) and can be genetically altered analogously to MEC-4 and MEC-10 to generate hyperactive, toxic channels (2, 71, 433). There exist two splice variants (isoforms) of BNC1 (α and β; also known as MDEG1 and MDEG2; Refs. 255, 328, 432) that share a common carboxy-terminal half.

In rodent hairy skin, several specialized nerve termini function as mechanoreceptors, including rapidly adapting (RA), slowly adapting (SA), and D-hair receptors (239). Antisera raised against BNC1 identifies large numbers of central nervous system neurons, but also reveals that BNC1 specifically localizes to the palisades of lanceolate nerve terminals, fine parallel processes projected in the hair follicle and surrounding the hair shaft, a likely site for sensation of hair movement (326). The BNC1α isoform is exclusively expressed in large-diameter dorsal root ganglion (DRG) neurons and in many specialized touch cell endings, in addition to palisades (142). These nerve terminals house one type of rapidly adapting mechanoreceptor. Interestingly, in these studies BNC1 immunoreactivity is not prevalent in other nerve termini intimately associated with the hair follicle and implicated in mechanotransduction, such as the pilo-Ruffini endings that also circle the hair shaft terminal, or other mechanoreceptors or nociceptors (326). The specific subcellular localization is striking in that many large- and small-diameter dorsal root ganglion neurons express messages homologous to BNC1, yet the protein is localized to only a few mechanosensory termini (326). Broad transcript expression in large- and small-diameter neurons, but rare localization of the protein in nerve termini, has been observed for ENaCs in the dorsal root ganglion and in baroceptor neurons (74, 105, 142, 434). Such specificity indicates that mechanisms for localized or selective positioning of DEG/ENaC channels are operative in peripheral neurons. Alternatively, channel proteins may not be sufficiently concentrated to be easily detected by immunological methods, a characteristic of typical mechanoreceptor channels. It is also possible that in situ hybridization probes detect additional isoform transcripts, which escape detection by antibodies that are isoform specific.

Does BNC1 play a role in mechanosensation or nociception? Either (or both) is plausible, since BNC1 is detectable in both large-diameter neurons (mostly mechanosensitive neurons) and small-diameter neurons (mostly nociceptors) of the dorsal root ganglion (326, 434). Generation of a BNC1 mouse knockout enabled testing of these possibilities. Both splice variants of BNC1 were eliminated in this mouse (326). At a gross level, the BNC1 null mice appear generally normal in development, size, fertility, and behavior (326). To address a potential function in mechanotransduction, detailed characterization of skin sensory neurons was performed on a skin-nerve preparation in which nerve terminals are tested for response to applied displacement force (326). This hairy skin preparation houses all five specialized mechanoreceptor types, classified based on their electrophysiological properties: rapidly adapting (RA) low-threshold mechanoreceptors, slowly adapting (SA) low-threshold mechanoreceptors, D-hair mechanoreceptors, A-fiber mechanonociceptors, and polymodal C-fiber mechanonociceptors (239). In $BNC1^{-/-}$ animals, neither the stimulus-response curves nor the median force required to activate D-hair mechanoreceptors, A-fiber mechanonociceptors, and C-fiber mechanonociceptors is altered, compared with $BNC1^{+/+}$ controls (326). Likewise, all efforts to test for changes in acid-induced responses and nociception in dorsal root ganglion neurons and polymodal C fibers failed to indicate an essential role for BNC1 in modulating H^+-gated currents in these cells (326). In contrast, a significant change in the function of RA and SA low-threshold mechanoreceptors was observed in the BNC1 null mutant (10 and 50%, respectively). Although the minimal force detectable for activation of these mechanoreceptors remains the same, the stimulus-response curve for RA, and to a lesser extent SA, $BCN1^{-/-}$ neurons is significantly different (326). In wild-type nerve terminals, increasing the force exerted on the fiber elicits increasing numbers of action potentials. Mutant neurons still respond to displacement, but produce fewer action potentials over a comparable range of stimuli. Interestingly, the effects on the action potential do not appear to result from developmental defects in the neurons involved. There are no apparent differences in the proportion of RA and SA fibers in skin preparations of wild-type and mutant mice. In addition, the number and morphology of lanceolate fibers (one, but not the only, type of RA receptor) is similar in $BNC1^{+/+}$ and $BNC1^{-/-}$ animals (326). Similarly, the nematode touch receptor neurons can develop nor-

mally in the absence of MEC-4 channels. Also important is that the defects in action potential firing in the BNC1 mutant appear to affect something other than in the capacity to generate an action potential. Injection currents required to elicit action potentials in cultured low-threshold mechanoreceptor neurons from $BNC1^{+/+}$ and $BNC1^{-/-}$ mice are similar. Since the basic capacity to convert a depolarizing inward current to an action potential appears to be normal in the $BNC1^{-/-}$ sensory neurons, it appears that the problem in $BNC1^{-/-}$ neurons is the actual generation of a mechanically induced depolarizing potential, consistent with the hypothesis that BNC1 participates directly in a mechanosensitive channel.

The consequences of the BNC1 channel deficiency, although somewhat modest at first glance, may be of profound biological importance, since in humans the dynamic sensitivity of RA and SA receptors is thought to be critical for perception and discrimination of touch sensation (206, 207). Why might the response be modified rather than eliminated in mechanosensitive neurons of the BNC1 knockout? One plausible reason is that DEG/ENaC channels are most often heteromultimeric, and BNC1 might act more as a modulatory subunit than as the core of a mechanotransducing complex, much as β- and γ-ENaC are less critical than α-ENaC function in kidney epithelia. Alternatively, different DEG/ENaC channels (or other channels) may perform redundant functions in the same neurons. Consistent with this possibility, ENaC subunits have been immunologically detected in neurons expressing BNC1, suggesting ENaC subunits could be components of mechanotransducing channels in neurons as well.

3. ASIC3 (DRASIC)

ASIC3 or DRASIC (dorsal root ganglion ASIC) is an additional member of the ASIC family of ion channels that similarly to BNC1 has been implicated in mechanotransduction. ASIC3 and BNC1α expression patterns overlap in large-diameter DRG neurons, and it is likely that these proteins participate in the formation of heteromultimeric channels in DRG neurons (29). Animals lacking ASIC3 show abnormal mechanosensitivity (327). Coupled, these results suggest that ASIC3 together with BNC1α and probably other members of the ASIC family form ion channels capable of transducing mechanical stimuli (8).

4. TRPV4

TRPV4 is a mammalian mechanosensitive TRPV channel, similar in sequence to the nematode OSM-9, and is activated by osmotic stress when expressed heterologously (253, 388). It appears to mediate sensitivity of nociceptive neurons of the DRG to hyposmotic stimuli (6). TRPV4 is expressed in a wide variety of tissues, and it is therefore possible to be a multifunctional subunit that serves various functions. Interestingly, a related vacuolar TRP channel in yeast shows mechanosensitivity; it is activated by an osmotic upshock to release Ca^{2+} from the vacuole (483). Thus TRP channels may have a role in sensing cell volume changes, conserved from yeast to mammals (34).

VII. EMERGING THEMES

Investigations on the genetics of sensory mechanotransduction, which were initiated in *C. elegans* and are now also being carried out in *Drosophila* and in vertebrates (zebrafish and mammals), have converged to reveal a limited set of underlying mechanisms (113, 152, 177, 221). This remarkable convergence of independent studies in distant species strongly suggests that different mechanotransducers in different systems have evolved to strictly adhere to the same set of principles. Members of two major ion channel families, the DEG/ENaC and the TRP groups, have emerged as the common denominators within a metazoan mechanosensory apparatus (7, 110, 292, 298, 404, 453). Moreover, in all cases examined, genetic, molecular and physiological data portray a similar architecture for mechanotransducing complexes. This architecture implements variations of the tethered-ion channel concept. It is striking that regardless of the identity of the core ion channel (DEG/ENaC or TRP), both intracellular and extracellular tethers appear to be required to render the core channel mechanosensitive (83, 141, 269). The mechanosensory function of the complex dictates its highly specialized structure. The nematode model of mechanotransduction in touch receptor neurons best illustrates this point, with a unique cytoskeletal network intracellularly, and a dedicated extracellular mantle being essential for mechanosensory transduction. Furthermore, the requirement for anchoring of mechanosensitive ion channels is signified by the presence of exceptionally long ankyrin repeats in the NompC mechanosensory channels of *Drosophila* and zebrafish. The conjecture that mechanotransduction dictates an explicit structure has predictive powers; an arrangement of ion channel proteins and associated components that is aligned with the specifications of the tethered-ion-channel model is likely to have mechanotransducing properties.

VIII. CONCLUDING REMARKS AND FUTURE DIRECTIONS

A. Open Issues

The detailed model for mechanotransduction in *C. elegans* neurons accommodates genetic data and molec-

ular properties of cloned genes. This model also based on mutant phenotypes, cell morphologies, heterologous expression approaches, and degenerin structural features remains to be tested by determining subcellular channel localization, subunit associations, and, most importantly, channel gating properties. It should be emphasized that the proposed direct interactions between proteins that build the mechanotransducing complex remain largely hypothetical and only recently have they begun to be addressed experimentally (73, 155).

An additional major question that remains to be addressed is whether the mammalian counterparts of the *C. elegans* degenerins play specialized roles in mechanical signaling in humans. A significant step toward addressing this question has been accomplished with the demonstration that BNC1 is involved in mechanosensory signaling in the skin as we have described above. Even though the candidacy of BNC1 for being in the core of a mechanotransducing complex was greatly boosted by these results, a demanding critic would argue that albeit very strong, it still remains just a candidacy. The potential role of BNC1 as part of the core mechanotransducing channel can still only be inferred from these experiments and is not directly proven. It is still possible that BNC1 forms or participates in an auxiliary channel that facilitates the function of the actual mechanotransducing channel. A BNC1 knockout does not completely eliminate the responses to mechanical stimuli (326). The incomplete nature of the BNC1 deficiency effects indicates that even if BNC1 is indeed part of the core mechanosensory channel, it most likely is not the only critical one. Alternatively, there might be more than one, different mechanotransducing complexes within one neuron, with different properties and composition. The above arguments, however, are by no means confined to BNC1. On the same basis, MEC-4/MEC-10 and UNC-8/DEL-1 in *C. elegans* as well as PPK in *Drosophila* might not be parts of the real mechanotransducer but only auxiliary ion channels.

The recent identification of another strong candidate mechanosensory channel, the *Drosophila* NompC, adds to the list of candidate mechanosensitive ion channels (438). NompC is unrelated in amino acid sequence to DEG/ENaC channels and is required for normal mechanosensitive currents in fly hair bristles (438). Evidence implicating NompC in mechanotransduction is especially convincing given the supporting electrophysiological analysis that is feasible in this system, and the availability of mutants with altered properties and intermediate effects (438). Therefore, NompC homologs in other organisms, including humans, emerge putative mechanosensitive ion channels. Even in this case, however, there are caveats; the absence of NompC does not completely eliminate mechanosensitive currents in *Drosophila* hair bristles. Furthermore, the identities and properties of force-generating tethers of NompC in mechanotransducing complexes will need to be determined. Another issue that needs to be addressed is the potential interplay between DEG/ENaC and NompC channels in mechanosensory cells before a clear understanding of mechanotransduction can be achieved.

B. Limitations of Current Methodologies

Genetic analyses in *C. elegans*, *Drosophila*, the zebrafish, and the mouse have been highly successful in identifying genes needed for mechanosensitive behaviors (58, 115, 152, 168, 302, 326). Still, limitations of the genetic approach to dissection of mechanotransduction mechanisms should be mentioned. Genes that encode products needed for the activities of mechanotransducing complexes in multiple cell types or that perform multiple cellular functions might have evaded genetic detection because mutations in such genes would be expected to be severely uncoordinated or even lethal. Indeed, many mutations that affect mechanosensation in *Drosophila* render animals severely uncoordinated and nearly inviable (114, 223). Moreover, genes whose functions are redundantly encoded cannot be readily identified in genetic screens. Thus additional cellular proteins essential for the mechanotransducing complex in the well-studied *C. elegans* body touch receptor neurons may still remain to be identified.

More challenging and most critical, the hypothesis that a degenerin-containing channel is mechanically gated must be addressed. This may be particularly difficult since at present it is not straightforward to record directly from tiny *C. elegans* neurons. Expression of the MEC-4/MEC-10 or the UNC-8/DEL-1 channel in heterologous systems such as *Xenopus* oocytes is complicated by the presence of the many endogenous mechanically gated ion channels (169, 282, 478) and by the likely possibility that not only the multimeric channel but essential interacting proteins will have to be assembled to gate the channel (162, 405).

C. Alternative/Complementary Approaches

Despite the undeniably considerable progress that has been achieved during recent years in all fronts toward dissecting the process of sensory mechanotransduction at the molecular level, several thorny questions are still begging for answers. What is the gating mechanism of mechanosensitive ion channels? How is tension delivered to the mechanotransducing complex? What additional molecules play a part in the biological response to mechanical stimuli? Are human sensory mechanotransducers similar in composition and function to nematode or

Drosophila ones? Are DEG/ENaC and NompC truly the core mechanosensitive channels or are they merely auxiliary channels/components? It is important to emphasize that although specialized ion channels most likely comprise the core of every metazoan mechanotransducer, it is the other physically associated proteins that shape its wonderful properties. It is equally important to seek and identify these. Without them, our understanding of mechanical transduction will never be complete even if the identity of the core ion channel is revealed. Let us keep in mind that mechanical sensation at the molecular level in higher organisms is most likely a property of a complex structure involving many components and contacts and not of any single protein.

Several tools could be employed towards this goal, such as yeast two-hybrid screens and biochemical methods of copurification of channel complexes together with anchoring proteins. The advent of the human genome sequence will provide the full set of testable DEG/ENaC candidates for mechanotransduction in humans. Some of these may be more closely related to nematode proteins specialized for mechanotransduction than currently identified family members and may be the long-sought human mechanosensors. In addition, fine mutations that do not dramatically incapacitate a candidate channel could be engineered back into mice to then examine how these correlate to the characteristics of mechanically induced currents. Characterization of expression patterns of all ASIC and ENaC family members in these animals and genetic knockouts of other candidate mechanotransducer channels will be required to address the question of functional redundancy, work that can be easily pursued in the postgenome era. Obviously such studies should also reveal whether other DEG/ENaC family members are needed for the function of other mechanoreceptors or nociceptors in mouse skin.

A tremendous boost to sensory mechanotransduction studies will be provided when the necessary technology that would allow direct recordings from nematode neurons is achieved. Although electrophysiological recording from some *C. elegans* neurons and muscles are possible, the touch receptor neurons and other sensory neurons are beyond the realm of feasibility given the current state of the art (11, 156, 342). The capacity to perform electrophysiological studies on degenerin or other ion channels while they are kept embedded in their natural surroundings is the currently missing tool. Perhaps the development of noninvasive new monitoring and measurement technologies will be required in the case of the tiny *C. elegans* neurons (43, 48, 49, 225, 226, 309, 398). Direct, nondestructive recordings from touch receptor neurons coupled with the powerful genetics of *C. elegans* will hopefully allow the complete dissection of a metazoan mechanotransducing complex.

We gratefully acknowledge the contributions of numerous investigators that we did not include in this review.

Work in the authors' laboratory is funded by grants from European Molecular Biology Organization (EMBO), the European Union, and Institute of Molecular Biology and Biotechnology. N. Tavernarakis is an EMBO Young Investigator.

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