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ORIGINAL ARTICLE

Mechanotransduction in *Caenorhabditis elegans*

The Role of DEG/ENaC Ion Channels

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Abstract

One of the looming mysteries in signal transduction today is the question of how mechanical signals, such as pressure or mechanical force delivered to a cell, are interpreted to direct biological responses. All living organisms, and probably all cells, have the ability to sense and respond to mechanical stimuli. At the single-cell level, mechanical signaling underlies cell-volume control and specialized responses such as the prevention of poly-spermy in fertilization. At the level of the whole organism, mechanotransduction underlies processes as diverse as stretch-activated reflexes in vascular epithelium and smooth muscle; gravitaxis and turgor control in plants; tissue development and morphogenesis; and the senses of touch, hearing, and balance. Intense genetic, molecular, and elecrophysiological studies in organisms ranging from nematodes to mammals have highlighted members of the recently discovered DEG/ENaC family of ion channels as strong candidates for the elusive metazoan mechanotransducer. Here, we discuss the evidence that links DEG/ENaC ion channels to mechanotransduction and review the function of *Caenorhabditis elegans* members of this family called degenerins and their role in mediating mechanosensitive behaviors in the worm.

Index Entries: Degenerin; epithelial sodium channel; mechanosensation; neurodegeneration; proprioception.

INTRODUCTION

Mechanotransduction is the conversion of a mechanical stimulus such as a minute stretch force into a cellular response and plays a central role in a broad range of biological processes *(1,2)*. Cell-volume regulation, fertilization, gravitaxis, involuntary movement, and the senses of touch, balance, and hearing all rely on mechanical transduction. Despite the widespread importance of mechanical signaling in biology, remarkably little is known about the nature of the molecules that mediate mechanotransduction. Elegant electrophysiological studies in several systems have estab-

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lished that mechanically gated ion channels are the mediators of the response. For years, however, these channels have eluded intense cloning efforts. Why are these channels so particularly resistant to our exploitation? These channels are rare. In skin pads, mechanoreceptors are spread out so there are only 17,000 in the finger and palm skin pad *(3).* This is an extremely low concentration. In the specialized hair cells of our ears, only a few hundred mechanically gated channels may exist. To make our prospects of directly encountering them even more slim, mechanosensory channel are embedded and intertwined with materials that attach them to the surrounding environment, contacts probably critical to function that are hard or even impossible to reconstitute or mimic in a heterologous system such as *Xenopus* oocytes. Finally, there are no known biochemical reagents that interact with the mechanically gated channels with high specificity and high affinity, thwarting efforts at biochemical purification. Biochemical purification of an *Escherichia coli* mechanosensitve channel, *MscL,* has been accomplished *(4),* but until recently, eukaryotic mechanosensitive ion channels have eluded cloning efforts and thus little is understood of their structures and functions.

An alternative approach toward identifying the molecules that are involved in mechanotransduction is to identify them genetically. This approach has been particularly fruitful in the simple nematode, *Caenorhabditis elegans (5,6).* Genetic dissection of touch transduction in this worm has led to the identification of several molecules that are likely to assemble into a mechanotransducing complex. These genetic studies revealed several genes that encode subunits of candidate mechanically gated ion channels involved in mediating touch transduction, proprioception, and coordinated locomotion *(7–10).* These channel subunits belong to a large family of related proteins in *C. elegans* referred to as degenerins, because unusual gain-of-function mutations in several family members induce swelling or cell *(7–9,11,12).*

C. elegans degenerins exhibit approx 25–30% sequence identity to subunits of the vertebrate amiloride sensitive, epithelial Na^+ channels (ENaC) *(13,14),* which are required for ion transport across epithelia *(15)* and acid-sensing ion channels that may contribute to pain perception (ASICs) *(16).* Together the *C. elegans* and vertebrate proteins define the DEG/ENaC (degenerin/epithelial sodium channel) family of ion channels *(17).* Additional members of this large group of proteins are the snail FMRF-amide gated channel FaNaC *(18),* the *Drosophila* ripped pocket (RPK) and pickpocket (PPK) *(19,20),* and *C. elegans flr-1 (21).* To summarize, members of the DEG/ENaC family have now been identified in organisms ranging from nematodes, snails, files, and many vertebrates including humans, and are expressed in tissues as diverse as kidney and lung epithelia, muscle, and neurons.

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, 21 members of the DEG/ENaC protein family have been identified in the *C. elegans* genome (Fig. 1). An experimental challenge is to decipher the biological functions of all these channel subunits and their mammalian counterparts. Here we discuss the studies that led to the identification of the nemadode degenerins and discuss a molecular model of touch transduction in the worm. This model resembles the one proposed for mechanotransduction in the vertebrate ear.

THE *C. elegans* MODEL SYSTEM

C. elegans is a small (1 mm) free-living nematode that completes a life cycle in 2.5 d at 25°C, progressing from a fertilized embryo through four larval stages to become an egg-laying adult, and lives for about 2 wk (Fig. 2A). Under adverse conditions such as starvation, overcrowding, or high temperature, larvae can enter an alternative life stage (called the dauer-enduring-larva), during which they move but do not feed. The dauer larva is a nonaging

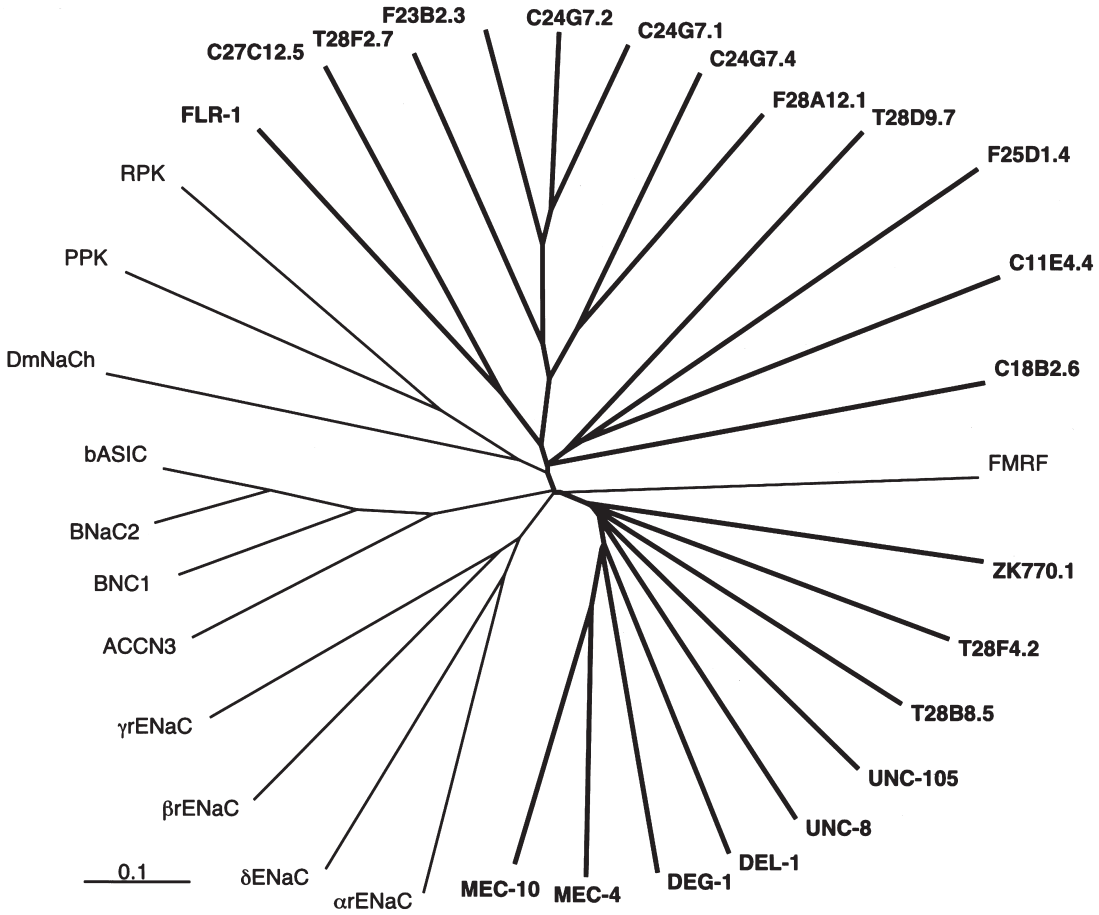

Fig. 1. Phylogenetic relations between DEG/ENaC proteins. Nematode degenerins are shown in bold. Representative DEG/ENaC proteins from a variety of organisms, ranging from snails to humans, are also included. The scale bar denotes evolutionary distance equal to 0.1 nucleotide substitutions per site.

stage and dauers can survive for weeks or even months *(22).* When favorable conditions are detected, dauers re-enter the lifecycle at the L4 larval stage and go on to reproduce and live normally the rest of their lifespan. The most common sexual form is the hermaphrodite (XX), although males (X0) can be propagated and used to construct strains carrying multiple mutations. 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. 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 (Fig. 2B) *(23,24).* In addition, the pattern of synaptic connections made by each of the 302 neurons of the animals has been described, so that the full "wiring diagram" of the animal is known *(25).* Researchers can take advantage of the fact that every cell is an identified cell and can perform laser microsurgery in which individual cells are killed by a laser microbeam *(26).*

C. elegans is well-established as a powerful genetic system *(27).* When a hermaphrodite

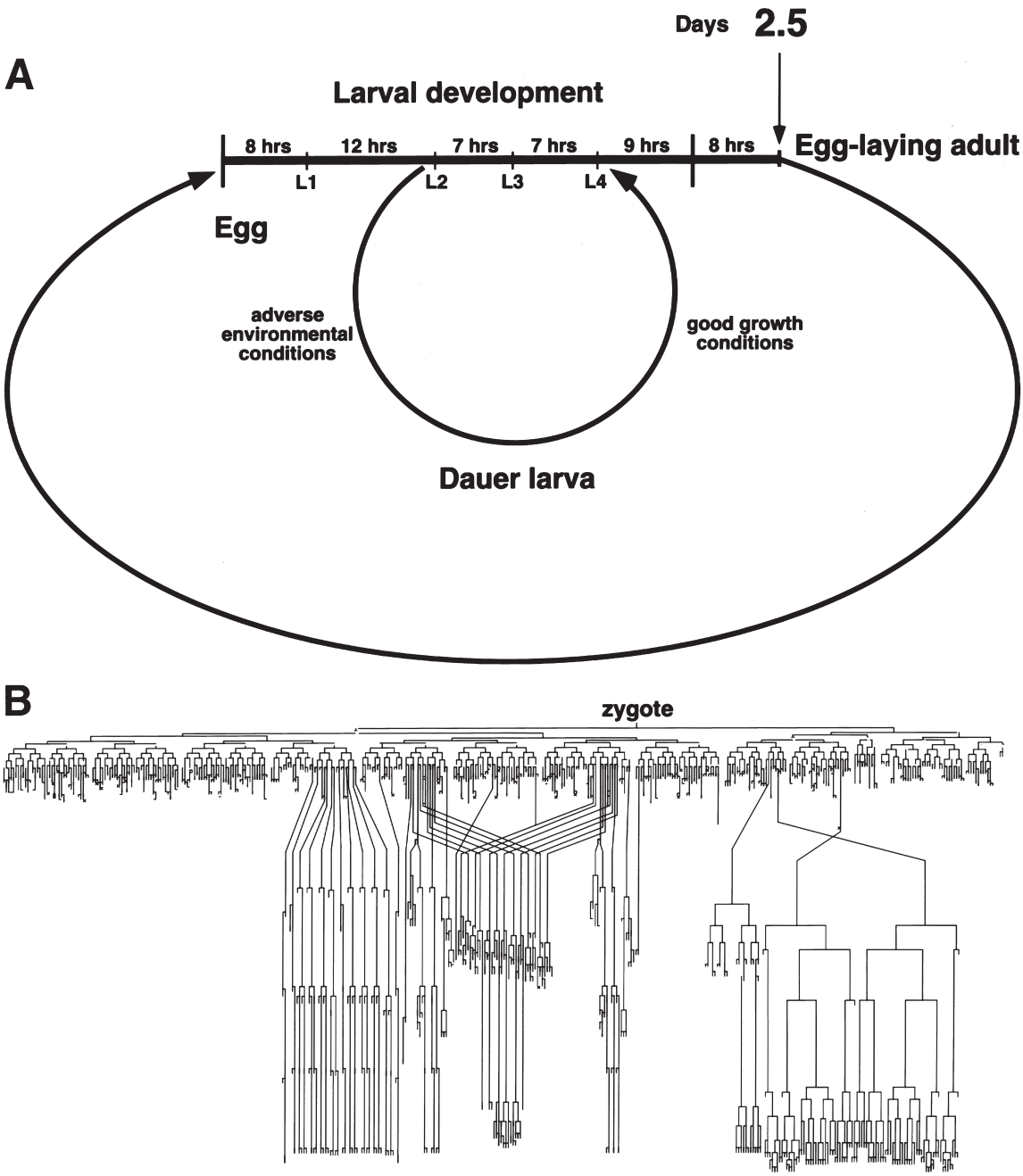

Fig. 2. The *C. elegans* life cycle and cell lineage. **(A)** Following hatching, worms progress through four larval stages before reaching adulthood *(27).* The duration of each stage at 25°C is shown in hours. Adult nematodes lay eggs for about 5 d. The average lifespan of animals is about 15 d. **(B)** 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 *(23,24).* Drawing adapted with permission from Nick Rhind (http://elegans.swmed.edu/parts/lineage.gif).

parent is subjected to a mutagenizing agent, the F1 progeny self-fertilize to produce F2 animals that are homozygous for recessive mutations. 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.

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 *(28,29).* Sequencing of the total *C. elegans* genome has been accomplished *(28,30,31).* In addition, ongoing efforts to obtain Expressed Sequence Tags for all *C. elegans* genes will soon provide a complete collection of the cDNAs of the nematode (Y. Kohara, National Institute of Genetics, Mishima, Japan, personal communication). *C. elegans* is also particularly amenable to reverse genetics studies. Investigators can take advantage of genome data to perform "reverse genetics," directly knocking out genes *(32).* A novel method of generating mutant phenocopies, called double-stranded RNA mediated interference (dsRNAi), enables probable loss-of-function phenotypes to be rapidly evaluated *(33).*

DNA manipulated in vitro can be microinjected back into animals for functional assays *(34).* Vectors are available for identification of transformants, cell-specific expression, and generation of fusions to marker genes such as *E. coli* β-galactosidase *(35)* and the jellyfish Green Fluorescent Protein (GFP) *(36)* so that individual cells can be visualized in stained or living animals.

C. elegans AND MECHANOTRANSDUCTION

C. elegans normally moves in a sinusoidal motion along a petri plate with solid agar medium and feeds on a thin layer of bacteria spread on top of the agar. If gently touched with an eyelash hair (typically attached to a toothpick) on the posterior, an animal will move forward; if touched on the anterior body, it will move backward. Both genetic and laser ablation studies have established that the mechanosensory component of this touch-sensitive behavior is mediated by six mechanosensory neurons, called the touch receptor neurons *(37–39).* The six touch receptors are visualized in live animals using a GFP reporter gene in Fig. 3A.

Touch-receptor neurons are situated laterally along the body wall. Three touch-receptor neurons are positioned in the posterior of the animal (these are named PLML, PLMR, and PVM). Three touch-receptor neurons are situated anteriorly (ALML, ALMR, and AVM). All send processes in an anterior direction. The positioning of touch cell processes is correlated with their sensory fields (Fig. 3B). For example, if all but the PLM cells are removed by laser ablation, the animal will remain touch-sensitive in the posterior, but not in the anterior *(37–40).* In addition to mediating touch avoidance, the touch-receptor neurons appear to control the spontaneous rate of locomotion since animals that lack functional touch cells are lethargic. The mechanical stimuli that drive spontaneous locomotion are unknown but could include encounters with objects in their environments or body stretch induced by locomotion itself.

Touch-receptor neurons have two distinguishing features. First, they are surrounded by a specialized extracellular matrix (ECM) called the mantle that appears to attach the cell to the cuticle *(37).* Second, they are filled with unusual 15-protofilament microtubules (Fig. 4). Genetic studies suggest that both features are critical for the function of these neurons as receptors of body touch *(5,41).*

As noted earlier, the complete description of the pattern of all synapses made by *C. elegans* neurons has been described *(25).* The neuronal circuit for touch sensitivity requires the combined action of the mechanosensory touch-receptor neurons, interneurons, and the motorneurons that drive locomotion (Fig. 5).

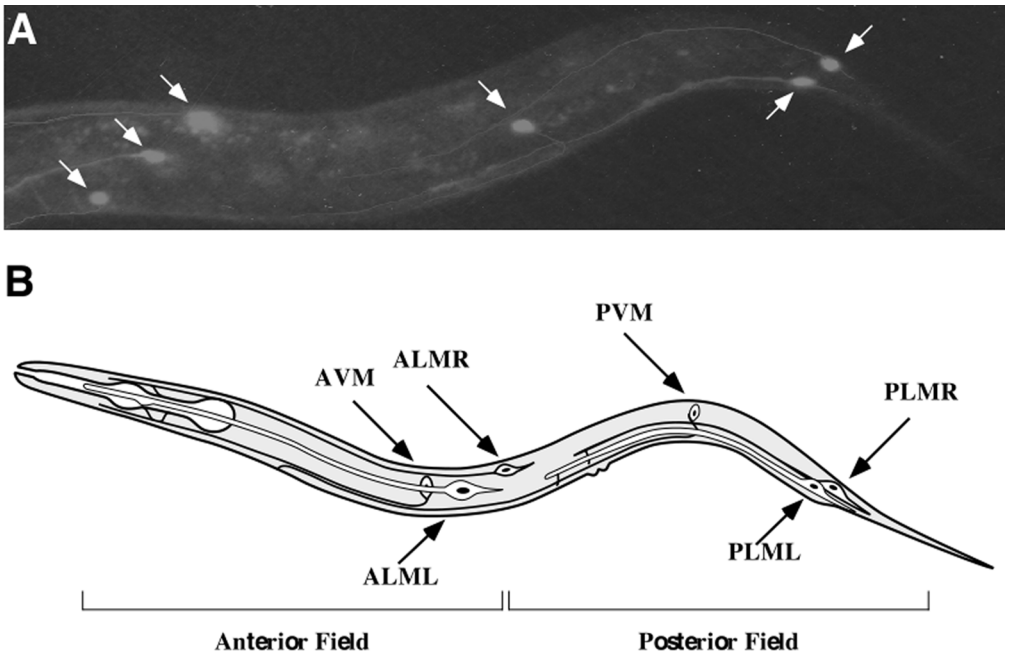

Fig. 3. The *C. elegans* touch receptor neurons. **(A)** Visualization of touch receptors. Worms are expressing the Green Fluorescent Protein (GFP) under the control of the *mec-4* promoter, which is active only in the six touch-receptor neurons. Arrows indicate touch-receptor cell bodies. Some touch-receptor axons are apparent. **(B)** Schematic diagram, showing the position of the six touch-receptor neurons in the body of the adult nematode. Note the two fields of touch sensitivity defined by the arrangement of these neurons along the body axis. The ALMs and AVM mediate the response to touch over the anterior field, whereas PLMs mediate the response to touch over the posterior field. Drawing adapted with permission from Nick Rhind (http://elegans.swmed.edu/parts/lineage.gif).

The likely relay circuit for mechanotransduction has been worked out by examining the connectivity patterns and testing relationships using laser ablation *(38).*

Mechanical stimuli regulate many other *C. elegans* behaviors including locomotion, foraging, egg laying, feeding (pharyngeal pumping), and defecation *(41).* Another behavioral paradigm that has been elegantly utilized to study mechanosensory control of locomotion is the response to nose touch—the reversal of direction as a consequence of head-on collision or a light touch on the side of the nose *(5,42).* Other touch-mediated locomotory responses such as a reaction to harsh touch (a strong prod with a metal wire best assayed in the absence of gentle-touch touch mechanosensory neurons) *(43),* or to tap (a diffuse stimulus as delivered by a tap on the plate on which worms are reared) *(44)* have been less extensively studied at the genetic level.

DEGENERINS ARE REQUIRED FOR MECHANOTRANSDUCTION IN *C. elegans*

Extensive genetic mutant screens have identified hundreds of mutations that specifically disrupt body-touch sensation (designated *mec* mutations for the *mec*hanosensory abnormal phenotype of the mutants). These mutations define at least nine structural genes that encode proteins hypothesized to participate in a touch-

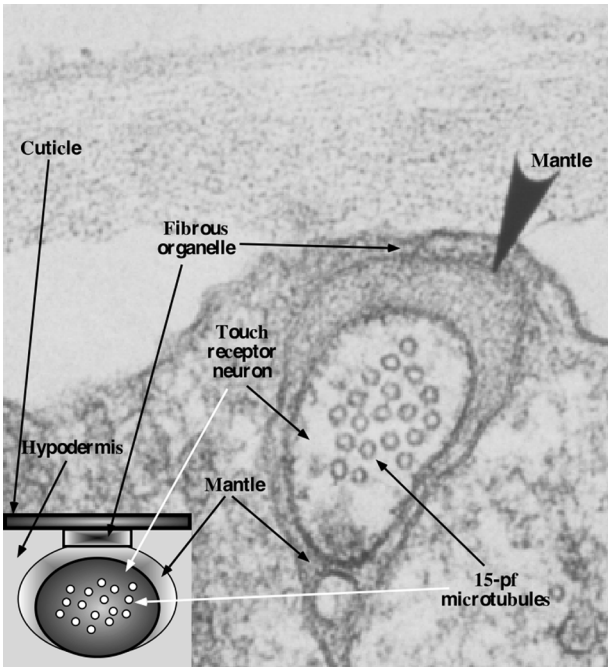

Fig. 4. Ultrastructural features of the touch-receptor neurons. An electron micrograph of a cross-section of a touch-receptor neuron process is shown. The touch-cell process is filled with 15-pf microtubules. The process is embedded in the hypodermis and surrounded by the mantle (indicated by the large black arrowhead). Schematic representation of a touch receptor-neuron cross-section is embedded for clarity. A darkly staining region labeled fibrous organelle is depicted here as a bar-shaded rectangle connecting the mantle and the cuticle. Such specializations appear periodically along the length of the touch-receptor process and may serve to attach the process to the cuticle.

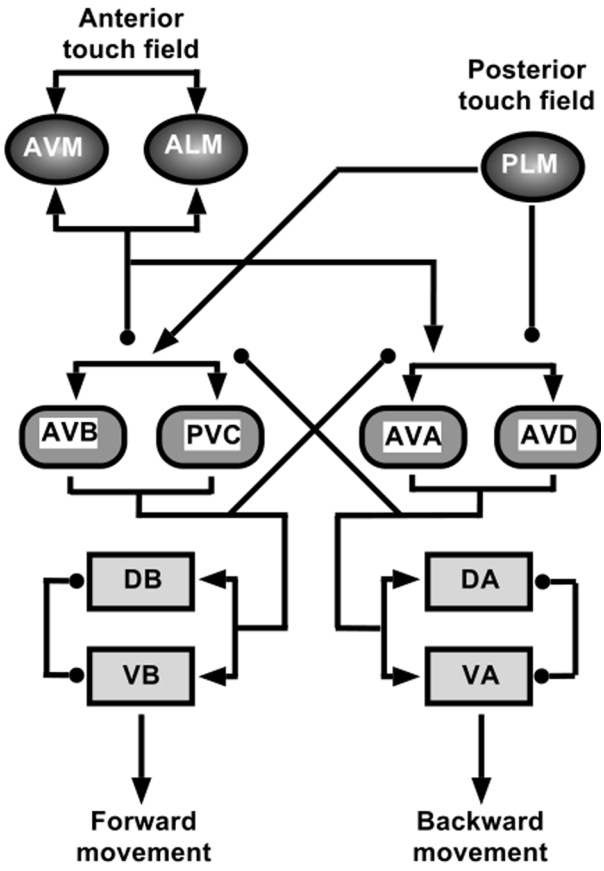

Fig. 5. Neuronal circuitry for locomotion in response to gentle body touch. Interconnections between sensory neurons (ALMs, AVM, PLMs), interneurons (AVB, PVC, AVA, AVD), and motor neurons (DB, VB, DA, VA) are shown. Arrowheads represent stimulatory connections and dark circles represent inhibitory connections. Sensory input from the anterior-touch field inhibits forward movement and stimulates backward movement. Sensory input from the posterior field produces the opposite effect (*see* ref. *38*).

transducing molecular complex *(6,13,41).* The core molecules in the complex are the degenerins MEC-4 *(7,45)* and MEC-10 *(8).* These proteins are postulated to be subunits of a mechanically gated touch-transducing channel.

Unusual, semi-dominant, gain-of-function mutations in another degenerin gene, *unc-8,* induce transient neuronal swelling *(12)* and severe uncoordination *(27,46). unc-8* null mutants have a subtle locomotion defect *(10).* Wild-type animals move through an *E. coli* lawn with a characteristic sinusoidal pattern (this occurs by localized alternating contraction and relaxation of body-wall muscles) *(5,25). unc-8* null mutants inscribe a path in an *E. coli* lawn that is markedly reduced in both wavelength and amplitude as compared to wild-type. This phenotype suggests that the

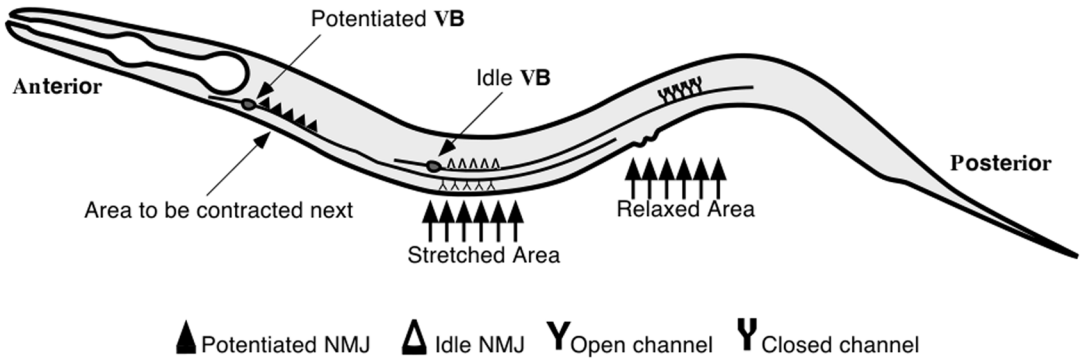

Fig. 6. Model for modulation of locomotion by stretch-responsive channels in motor neurons. Two VB motor neurons in the ventral-nerve cord are shown with stretch-sensitive channels postulated to be situated in their undifferentiated processes. The anterior VB signal to muscle is potentiated by the opening of ion channels in its process that experiences stretch owing to local body bend. This motor neuron will signal to the anterior muscles to then become fully contracted. At the same time, another motor neuron in the middle of the body remains idle because its process does not receive a stretch stimulus. Sequential activation of motor neurons that are distributed along the ventral-nerve cord and signal nonoverlapping groups of muscles, amplifies and propagates the sinusoidal body wave (NMJ, neuromuscular junction).

UNC-8 degenerin channel functions to modulate the locomotory trajectory of the animal.

unc-8 encodes a degenerin expressed in several motor-neuron classes and in some interneurons and nose touch-sensory neurons *(10).* Another degenerin family member, *del-1*(for *de*generin-*l*ike) is co-expressed in a subset of neurons that express *unc-8* (the VA and VB motor neurons) and is likely to assemble into a channel complex with UNC-8 in these cells. How does the UNC-8 motor-neuron channel influence locomotion? One highly interesting morphological feature of some motorneurons (in particular, the VA and VB motorneurons that co-express *unc-8* and *del-1*) is that their processes include extended regions that do not participate in neuromuscular junctions or neuronal synapses. These "undifferentiated" process regions have been hypothesized to be stretch-sensitive *(25).* Given the morphological features of certain motor neurons and the sequence similarity of UNC-8 and DEL-1 to candidate mechanically gated channels, we have proposed that these subunits co-assemble into a stretch-sensitive channel that might be localized to the undifferentiated regions of the motor-neuron process *(10).* When activated by the localized body stretch that occurs during locomotion, this motor-neuron channel potentiates signaling at the neuromuscular junction, which is situated at a distance from the site of stretch stimulus (Fig. 6). The stretch signal enhances motor-neuron 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 believe that the output of such an oscillator is further enhanced and modulated by stretch-sensitive motorneurons. One important corollary of the

Table 1
DEG/ENaC Proteins Implicated in Mechanotransduction

Protein	Expression pattern	Postulated function	Organism
MEC-4	Touch-receptor neurons	Touch sensitivity	*C. elegans*
MEC-10	Touch-receptor neurons Other sensory neurons	Touch sensitivity	*C. elegans*
DEG-1	Interneurons Sensory neurons Muscle Hypodermis	Harsh-touch sensitivity?	*C. elegans*
UNC-105	Muscle	Stretch sensitivity	*C. elegans*
UNC-8	Motor neurons Interneurons Sensory neurons	Stretch sensitivity Proprioception	*C. elegans*
DEL-1	Motor neurons Sensory neurons	Stretch sensitivity Proprioception	*C. elegans*
PPK	Sensory dendrites of peripheral neurons	Touch sensitivity Proprioception	*D. melanogaster*
γENaC	Baroreceptor-nerve terminals innervating the aortic arch and carotid sinus	Pressure sensitivity	*Rattus norvegicus*
BNC1	Lanceolate nerve endings that surround the hair follicle	Touch sensitivity	*Mus musculus*

unc-8 mutant studies is that the UNC-8 channel does not appear to be essential for motor-neuron 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.

Two additional members of the nematode degenerin family, *deg-1* and *unc-105*, have been implicated in mechanical signaling. *deg-1* is expressed in neurons required for harsh-touch response and might participate in sensing extreme mechanical forces applied during harsh-touch tests that involve prodding nematodes with a metal wire *(11). unc-105* is expressed in muscles and postulated to facilitate muscle-stretch feedback *(9,47,48).*

MECHANOTRANSDUCTION AND DEG/ENaC PROTEINS IN OTHER ORGANISMS

An increasing amount of evidence suggests that some DEG/ENaC proteins may be mechanically gated similarly to their nematode counterparts (*see* Table 1 for a list of all DEG/ENaC proteins implicated in mechanotransduction). The vertebrate epithelial Na^+ channel (ENaC) is composed of three subunits similar in aminoacid sequence (α, β, γENaC). In mammals, γENaC is localized to baroreceptor-nerve endings that detect acute fluctuations in arterial pressure. These baroreceptor-nerve terminals innervate the aortic arch and carotid sinus and are involved in blood-pressure regulation. An amiloride analog that is a strong and specific inhibitor of ENaC channels blocks

baroreceptor nerve activity and baroreflex control of blood pressure. These data suggest that ENaC subunits may be components of the baroreceptor mechanotransducer *(49).*

In rodent, hairy-skin mechanoreceptor neurons are excited by hair movement. The mammalian brain Na^+ channel (BNC1) that belongs to the ASIC sub-family of DEG/ENaC ion channels is expressed in the lanceolate nerve endings that lie adjacent to and surround the hair follicle. Although BNC1 has been proposed to have a role in pH sensing, the acid-evoked current in cultured sensory neurons and the response of acid-stimulated nociceptors were normal in BNC1 null mice. Remarkably however, disrupting the mouse BNC1 gene markedly reduces the sensitivity of specific mechanoreceptors found in lanceolate nerve endings. These results directly implicate the BNC1 channel in the mechanical signaling that underlies the sense of touch *(50,51).*

In *Drosophila melanogaster,* a novel DEG/ENaC protein, PPK, was found in 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 *(19,20).*

DEG/ENaC STRUCTURE, TOPOLOGY, AND FEATURES

DEG/ENaC family members exhibit about 25–35% identity and 45–65% similarity over their lengths and share distinct blocks of sequence that are highly conserved among all family members. 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 Cys-rich domains (CRDs), an extracellular regulatory domain and a neurotoxin-related domain (NTD) before predicted transmembrane domain II (Fig. 7) *(52,53).* Degenerins are situated in the membrane such that amino and carboxy termini are intracellular and a single large domain is positioned outside the cell *(45,54)* (Fig. 7). The more N-terminal of the two membrane-spanning domains (MSDI) is generally hydrophobic, whereas the more C-terminal of these (MSDII) is amphipathic. In the region immediately preceding MSDII is a key residue that influences channel activation. Large side-chain amino acid substitutions for a conserved small residue situated close to MSDII lock the channel in an open conformation (alanine 713 for MEC-4) *(7,52,55,56).* In *C. elegans,* this genetically induced channel hyperactivation can induce necrotic-like cell death of cells expressing the mutant genes (Fig. 8) *(7,11,57,58).*

Amino acids on the polar face of amphipathic transmembrane MSDII are highly conserved and are essential for *mec-4* function *(59–62).* 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 *(59).* 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 *(55,60).*

Other alterations in degenerins can also induce neurodegeneration: *mec-4* alleles harboring a missense mutation (A404T) or a small deletion (Δ399–407) in the extracellular region induce degeneration in transgenic animals *(52),* an observation consistent with the idea that these mutations disrupt a channel closing domain that is situated on the extracelluar 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, semi-dominant *unc-8* alleles that induce transient swelling of motor neurons alter an amino acid in the corresponding region.

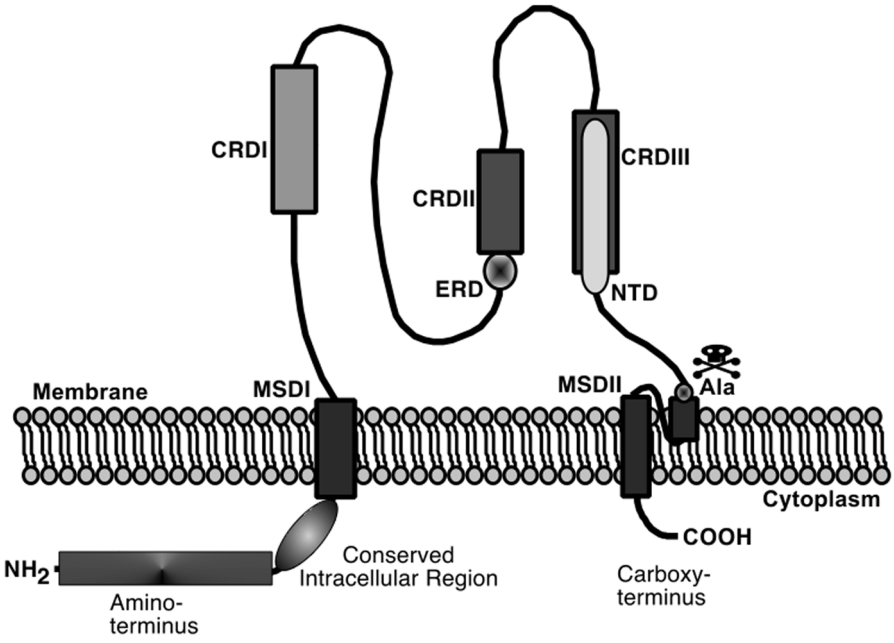

Fig. 7. Schematic representation of DEG/ENaC ion-channel subunit structure and topology. Shaded boxes indicate defined channel modules. These include the two membrane-spanning domains (MSDs), and the three cysteine-rich domains (CRDs; the first CRD is absent in mammalian channels). The small oval depicts the putative extracellular regulatory domain (ERD) identified by García-Añoveros and coworkers in *C. elegans* degenerins *(52).* The box overlapping with CRDIII denotes the neurotoxin-related domain (NTD) *(53).* The conserved intracellular region at the amino terminus is also shown. Both termini are intracellular with the largest part of the protein situated outside the cell. The dot near MSDll represents the amino-acid position (Alanine 713 in MEC-4) affected in dominant, toxic degenerin mutants.

Sequence analysis of recessive loss-of-function *mec-4* alleles has highlighted two other regions of MEC-4 that appear especially important in channel function *(63).* Amino acid substitutions that disrupt MEC-4 function cluster within a conserved region that is situated on the intracellular side, close to MSDI. This region of the channel could interact with cytoskeletal proteins. Interestingly, the effects of semi-dominant alleles of *unc-8* can be completely blocked by mutations in this conserved region, highlighting its functional importance. 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 co-expressed genes, as observed in heterozygote animals carrying a semi-dominant allele on one chromosome and a mutation in the conserved intracellular amino terminal region on the other). Such a pattern of genetic suppression suggests that UNC-8 proteins interact to form a dimeric or multimeric complex where more than one molecules associate to form a channel. The conserved intracellular amino terminal region could play a role in facilitating such interactions. A second hot-spot for channel-inactivating substitutions is situated near and within CRDII. This is a candidate region for interaction of the channel with the ECM.

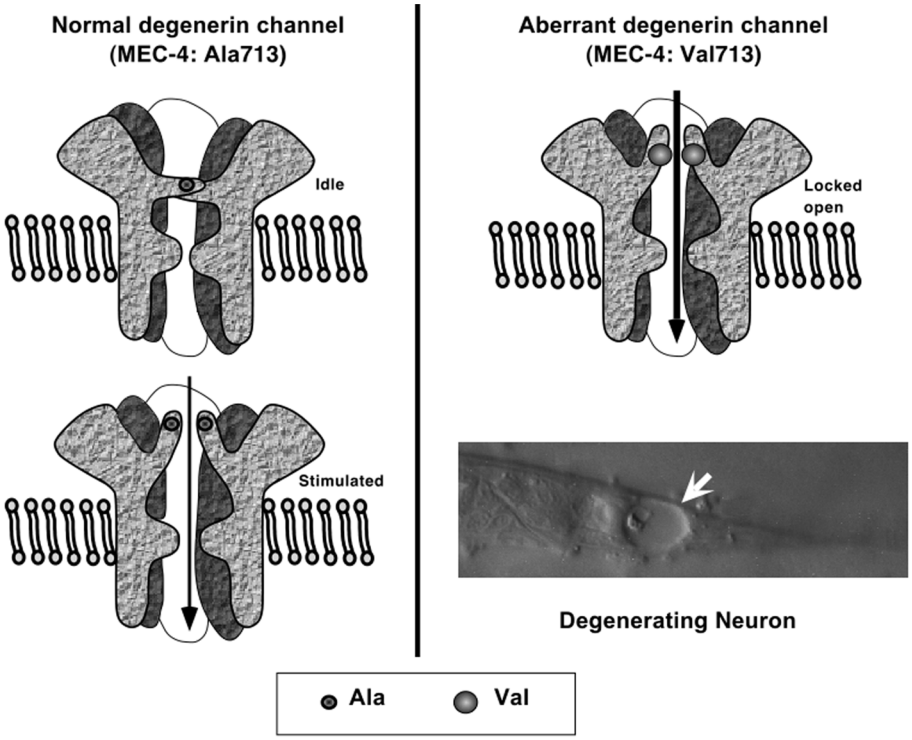

Fig. 8. Degenerin-induced necrotic-like cell death. Gain-of-function mutations in the degenerin gene *mec-4* encode substitutions for a conserved alanine adjacent to MSDll and result in neuronal degeneration. Amino acids with bulkier side chains at this position are thought to lock the channel in an open conformation by causing steric hindrance, resulting in Na^+ influx that triggers the necrotic-like cell death shown at the bottom right panel.

A MODEL FOR MECHANOTRANSDUCTION IN *C. elegans*

The molecular features of cloned touch-cell and motor-neuron structural genes required for mechanical signaling, together with genetic data, that suggest interactions between them constitute the basis of a model for the nematode mechanotransducing complex (Fig. 9A) *(5,6,41,64).* The central component of this model is the candidate mechanosensitive ion channel that includes multiple MEC-4 and MEC-10 subunits in the case of touch-receptor neurons, and UNC-8 and DEL-1 in the case of motor neurons. These subunits assemble to form a channel pore that is lined by hydrophilic residues in MSDII. Subunits adopt a topology in which the Cys-rich and neurotoxin domains extend into the specialized ECM outside the touch cell and the amino- and carboxy-termini project into the cytoplasm.

Regulated gating is expected to depend on mechanical forces exerted on the channel. Tension is hypothesized to be delivered by tethering the extracellular-channel domains to the specialized ECM and anchoring intracellular domains to the microtubule cytoskeleton. Outside the cell, channel subunits may contact ECM components. Inside the cell, channel subunits may interact with the cytoskeleton either directly of via protein links. A touch stimulus could deform the microtubule network, or could perturb the mantle connections to deliver the gating stim-

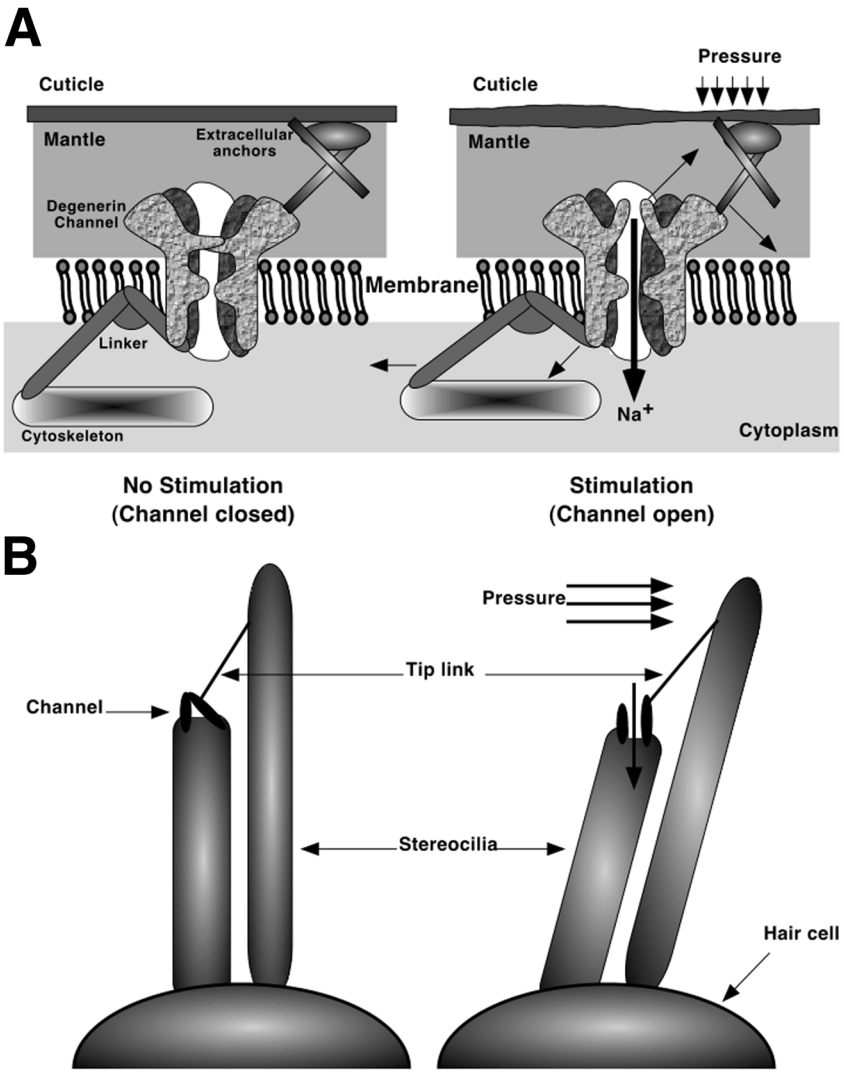

Fig. 9. Models of mechanotransduction. **(A)** A touch-transducing complex in *C. elegans* touch receptor neurons. In the absence of mechanical stimulation, the channel is closed and therefore the sensory neuron is idle. Application of a mechanical force to the body of the animal results in distortion of a network of interacting molecules that opens the degenerin channel. Na^+ influx depolarizes the neuron initiating the perceptory integration of the stimulus. **(B)** Mechanical gating of the channels in vertebrate hair cells. Mechanosensory channels situated at the stereocilla tips are pulled open by the tip-link when stereocilia are deflected. Drawing adapted with permission from ref. *41.*

ulus. In either scenario, Na^+ influx would activate the touch receptor to signal the appropriate locomotory response *(13,41).*

Interestingly, the model proposed for mechanotransduction in the touch-receptor neurons shares features of the proposed gating mechanism of mechanosensory channels that respond to auditory stimuli in the hair cells of the vertebrate inner ear (Fig 9B) *(65, 66).* Stereocilia situated on the hair-cell apical

surface are connected at their distal ends to neighboring stereocilia by filaments called tip links. 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.

CONSIDERATIONS AND FUTURE DIRECTIONS

DEG/ENaC proteins have been implicated in diverse cellular functions, among which is the conversion of mechanical stimuli to biological responses. In *C. elegans* neurons, the detailed model for mechanotransduction accommodates genetic data and molecular properties of cloned genes. This model, also based on mutant phenotypes, cell morphologies, and structural features of degenerins, remains to be tested by determining subcellular-channel localization, subunit associations, and, most importantly, channel-gating properties. However, it should be emphasized that no direct interactions between proteins proposed to be present in the mechanotransducing complex have been demonstrated. Given that genes for candidate-interacting genes are in hand, it should now be possible to test hypothesized associations biochemically.

More challenging and most critical, the hypothesis that a degenerin-containing channel is mechanically gated must be addressed. This may be particularly difficult because at present it is not straightforward to record directly from tiny *C. elegans* neurons. Expression of the MEC-4/MEC-10 or (UNC-8/DEL-1) channel in heterologous systems such as *Xenopus* oocytes will be complicated by the presence of the many endogenous mechanically gated ion channels *(67)* and by the likely possibility that not only the multimeric channel, but essential interacting proteins, will have to be assembled to gate the channel. However the development of the necessary technology that will allow direct recordings from nematode neurons *(68,69)* will facilitate electrophysiological studies on degenerin ion channels while they are kept embedded in their natural surroundings. This approach, combined with the powerful genetics of *C. elegans,* will, it is hoped, allow the complete dissection of a metazoan mechanotransducing complex.

A 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 earlier *(50).* 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 proven directly. 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. 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. With the sequencing of the human genome, additional members of the human ENaC family have been identified. Some of these may be the long-sought human mechanosensors.

The previous 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 advent of the human genome sequence will provide the full set of testable DEG/ENaC candidates. In addition, point mutations that do not dramatically incapacitate a candidate channel could be engi-

neered back into animals to then examine how these affect the characteristics of mechanically induced currents.

Recently another strong candidate for a mechanosensory channel in the fly hair bristle, *nompC,* has been identified *(70).* Data is especially convincing because mutations in this channel alter the fine characteristics (adaptation properties) of mechanically induced currents recorded from these cells. Even in this case, however, a role in mechanical signaling is only inferred and not directly proven. An intriguing property of *nompC* is that it contains several ankyrin repeats in the intracellular amino terminus. Such repeats, known to interact with cytoskeletal components, could provide a means of tethering the channel to an intracellular rigid point of reference.

In closing, we feel 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. 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. 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.

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