

Degenerins

At the Core of the Metazoan Mechanotransducer?

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ABSTRACT: Mechanosensory signaling, believed to be mediated by mechanically gated ion channels, constitutes the basis for the senses of touch and hearing, and contributes fundamentally to the development and homeostasis of all organisms. Despite this profound importance in biology, little is known of the molecular identities or functional requirements of mechanically gated ion channels. Genetic analyses of touch sensation and locomotion in *Caenorhabditis elegans* have implicated a new class of ion channels, the degenerins (DEG) in nematode mechanotransduction. Related fly and vertebrate proteins, the epithelial sodium channel (ENaC) family, have been implicated in several important processes, including transduction of mechanical stimuli, pain sensation, gametogenesis, sodium reabsorption, and blood pressure regulation. Still-to-be-discovered DEG/ENaC proteins may compose the core of the elusive human mechanotransducer.

KEYWORDS: *Caenorhabditis elegans*; Degenerin; ENaC; Ion channel; Mechanosensation; Neurodegeneration; Proprioception

INTRODUCTION

Cell-volume regulation, gravitaxis, proprioception, touch sensation, and auditory transduction all depend on the conversion of mechanical energy into cellular responses.^{1,2} Still, little is known about the molecular properties of ion channels specialized for mechanotransduction. Genetic studies in the nematode *Caenorhabditis elegans* were the first to identify eukaryotic genes that encode subunits of candidate mechanically gated ion channels involved in mediating touch transduction, proprioception, and coordinated locomotion.³⁻⁶ 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 degeneration^{3,7} (see DEGENERINS AND DEGENERATION below).

C. elegans degenerins exhibit approximately 25–30% sequence identity to subunits of the vertebrate amiloride-sensitive epithelial Na⁺ channels (ENaCs; see Ref. 8), which are required for ion transport across epithelia.⁹ Together the *C. elegans* and vertebrate proteins define the degenerin/epithelial sodium channel (DEG/ENaC) superfamily of ion channels.^{10,11} DEG/ENaC proteins range from about 550 to 950

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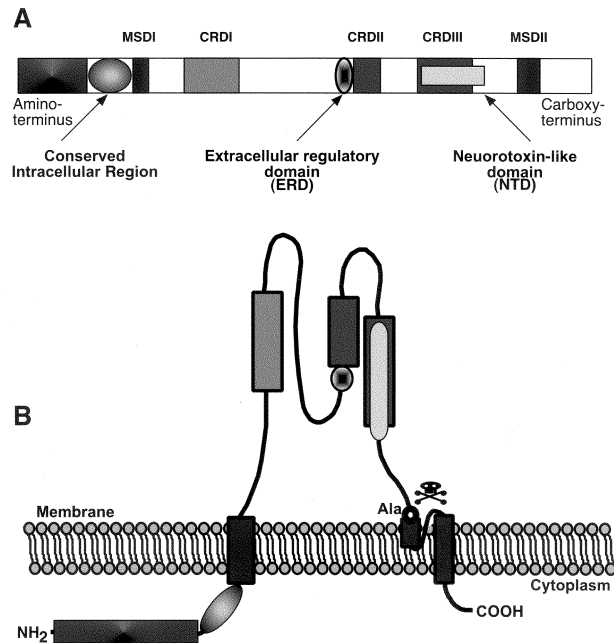


FIGURE 1. Schematic representation of DEG/ENaC ion-channel subunit structure and topology. **(A)** General features: *Shaded boxes* indicate defined channel modules. These include the two membrane-spanning domains (MSDs), and the three cysteine-rich domains (CRDs; the first CRD is absent in mammalian channels). The *small oval* depicts the putative extracellular regulatory domain (ERD) identified by García-Añoveros and co-workers in *C. elegans* degenerins.³⁰ The *box* overlapping with CRDIII denotes the neurotoxin-related domain (NTD; see Ref. 12). The conserved intracellular region is also shown. **(B)** Transmembrane topology: Both terminals are intracellular, with the largest part of the protein situated outside the cell. The *dot* near MSDII represents the amino-acid position (Alanine 713 in MEC-4) affected in dominant, toxic degenerin mutants.

amino acids in length and share several distinguishing blocks of sequence similarity¹² (FIGURE 1A). Subunit topology is invariable: all members of the DEG/ENaC superfamily have two membrane-spanning domains with cysteine-rich domains (CRDs; the most conserved is designated CRDIII) situated between the transmembrane segments.^{13,14} N- and C-terminals project into the intracellular cytoplasm, while most of the protein, including the CRDs, is extracellular (FIGURE 1B).

Members of the DEG/ENaC superfamily have been identified from nematodes, snails, flies, and many vertebrates, including humans, and are expressed in tissues as diverse as kidney 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 25 members of the DEG/ENaC superfamily have

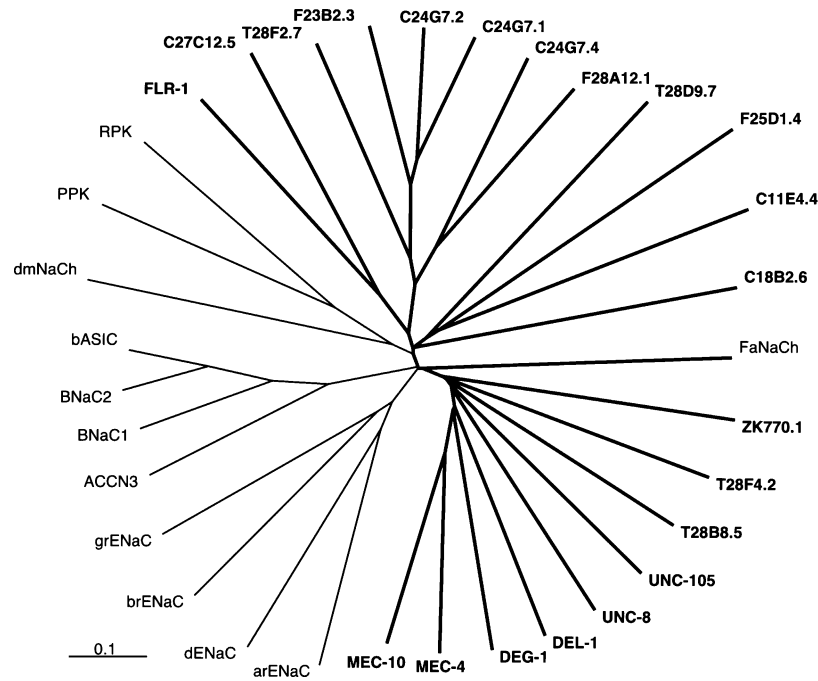


FIGURE 2. Phylogenetic relations between DEG/ENaC proteins. The degenerin content of the complete nematode genome is shown in bold. Other DEG/ENaC proteins from a variety of organisms, ranging from snails to humans, are also included. The *scale bar* denotes evolutionary distance equal to 0.1 nucleotide substitutions per site.

been identified in the *C. elegans* genome (FIGURE 2). An experimental challenge is to decipher the biological functions of all these channel subunits and their mammalian counterparts. Here we review analysis of the *C. elegans* family members directly implicated in mechanotransduction and discuss potential roles of their mammalian counterparts in mechanical signaling.

FEATURES OF THE *C. elegans* MODEL SYSTEM: ADVANTAGES FOR GENETIC AND MOLECULAR STUDIES OF MECHANICAL SIGNALING

C. elegans is a small (1-mm) free-living hermaphroditic nematode that completes a life cycle in 2.5 days at 25°C. Mutations can be easily induced and large screens can be performed to isolate mutants having specific phenotypes. The simple body plan and transparent nature of both the egg and the cuticle of this nematode have facilitated an exceptionally detailed developmental 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 are known.¹⁵

One considerable advantage of the *C. elegans* system is that it is the first metazoan for which the genome was sequenced to completion.¹⁶ Investigators can take advantage of genome data to perform “reverse genetics,” directly knocking out genes. In addition, a novel method of generating mutant phenocopies, called double-stranded RNA-mediated interference (RNAi), enables probable loss-of-function phenotypes to be rapidly evaluated.¹⁷ Another advantage of this system is that construction of transgenic animals is rapid; DNA injected into the hermaphrodite gonad concatamerizes and is packaged into embryos, hundreds of which can be obtained within a few days of the injection.¹⁸

The anatomical characterization and understanding of neuronal connectivity in *C. elegans* are unparalleled in the metazoan world. Serial section electron microscopy has identified the pattern of synaptic connections made by each of the 302 neurons of the animal (including 5000 chemical synapses, 600 gap junctions, and 2000 neuromuscular junctions), so that the full “wiring diagram” of the animal is known.¹⁹ Although the overall number of neurons is small, 118 different neuronal classes, including many neuronal types present in mammals, can be distinguished. Other animal model systems contain many more neurons of each class (there are about 10,000 more neurons in *Drosophila* with approximately the same repertoire of neuronal types). Overall, the broad range of genetic and molecular techniques applicable in the *C. elegans* model system allow a unique line of investigation into fundamental problems in biology, such as mechanical signaling.

BEHAVIORS THAT DEPEND UPON MECHANOTRANSDUCTION IN *C. elegans*

Mechanical stimuli regulate many *C. elegans* behaviors, including locomotion, foraging, egg laying, feeding (pharyngeal pumping), and defecation. The mechanosensitive response best characterized at the cellular, genetic, and molecular levels is the movement away from a light touch delivered to the body with an eyelash hair referred to as body touch sensation.²⁰ 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 (reviewed in Ref. 21). 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 mechanosensory neurons; see Ref. 22) or to tap (a diffuse stimulus as delivered by a tap on the plate on which worms are reared; Ref. 23), have been less extensively studied at the genetic level.

CELLULAR REQUIREMENTS FOR BODY TOUCH SENSATION

In the laboratory, *C. elegans* moves through a bacterial lawn on a petri plate with a readily observed sinusoidal motion. When gently touched with an eyelash hair (typically attached to a toothpick) on the posterior, an animal will move forward; when touched on the anterior body, it will move backward (FIGURE 3A). This gentle body touch is sensed by the touch receptor neurons anterior lateral microtubule cell left, right (ALML/R), anterior ventral microtubule cell (AVM), and posterior lateral

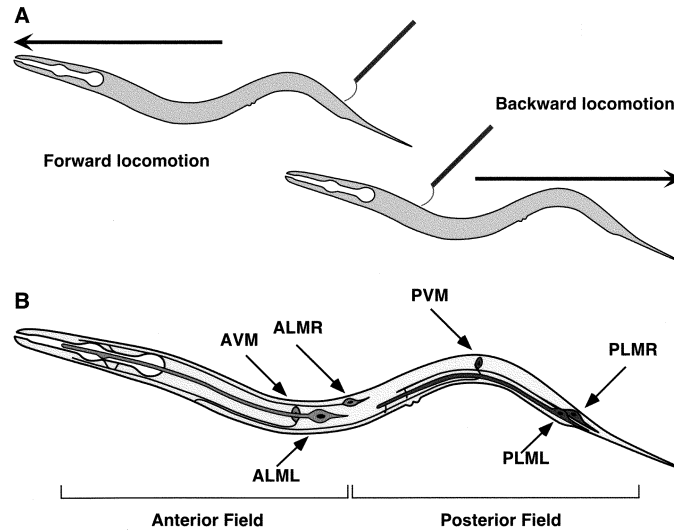


FIGURE 3. The nematode touch response. (A) The behavior. When animals are stimulated with an eyelash hair attached to a toothpick at the anterior field, they respond by moving backwards. Stimulation at the posterior field results in moving to the opposite direction. (B) The neurons: Schematic diagram showing the position of the six touch receptor neurons in the body of the adult nematode. Two fields of touch sensitivity are 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. PVM does not mediate touch response by itself.

microtubule cell left, right (PLML/R); FIGURE 3B).^{20,24} Posterior ventral microtubule (PVM) is a neuron that is morphologically similar to the touch receptor neurons and expresses genes specific for touch receptor neurons but has been shown to be incapable of mediating normal touch response by itself. The touch receptors are situated so that their processes run longitudinally along the body wall embedded in the hypodermis adjacent to the cuticle. The position of the processes along the body axis correlates with the sensory field of the touch cell. Laser ablation of AVM and the ALMs, which have sensory receptor processes in the anterior half of the body, eliminates anterior touch sensitivity, and laser ablation of the PLMs, which have posterior dendritic processes, eliminates posterior touch sensitivity. 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 called the mantle, which appears to attach the cell to the cuticle. Second, they are filled with unusual 15-protofilament microtubules.²⁰ Genetic studies suggest that both features are critical for the function of these neurons as receptors of body touch (reviewed in Ref. 21).

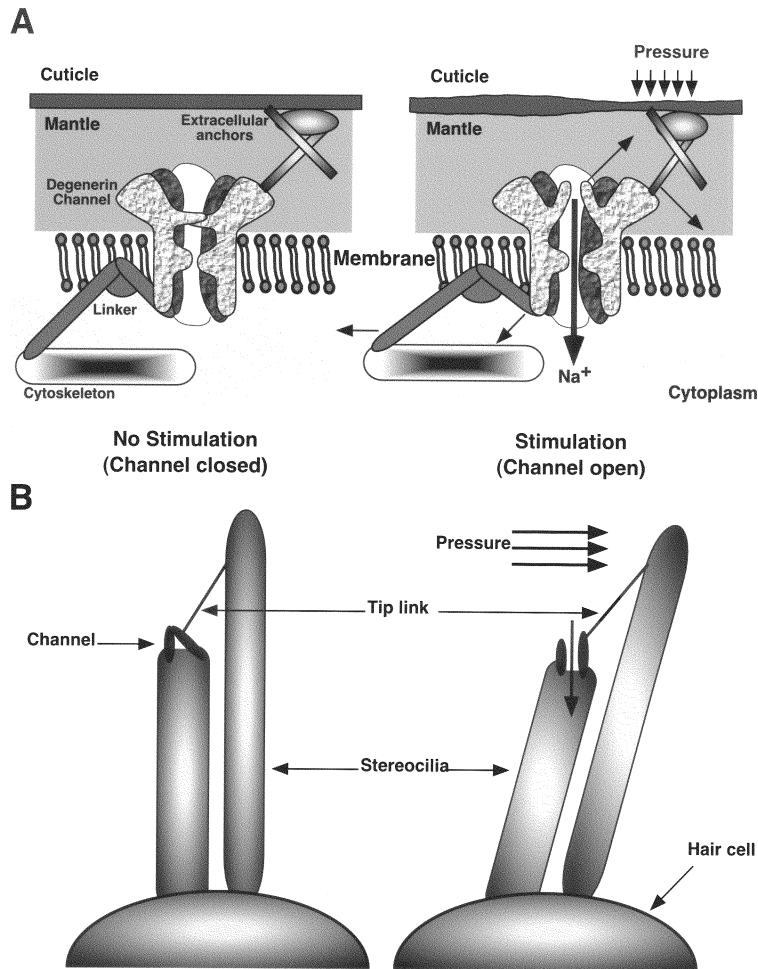


FIGURE 4. Models of mechanotransduction: **(A)** A touch transducing complex in *C. elegans* touch receptor neurons. In the absence of mechanical stimulation the channel is closed, and therefore the sensory neuron is idle. Application of a mechanical force to the body of the animal results in distortion 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 stereocilia tips are pulled open by the tip-link when stereocilia are deflected. (Adapted from Pickles and Corey.⁴⁰ Reproduced by permission.)

IDENTIFICATION OF PROTEINS REQUIRED SPECIFICALLY FOR BODY TOUCH

Elegant genetic analysis has identified approximately 15 genes that, when mutated, specifically disrupt gentle body touch sensation, and are therefore thought to encode candidate mediators of touch sensitivity (these genes were named *mec* genes, since when they are defective, animals are *mechanosensory abnormal*²⁵). Many of the *mec* genes have now been molecularly identified and most of them encode proteins postulated to make up a touch-transducing complex.^{26,27} The core elements of this mechanosensory complex are the channel subunits MEC-4 and MEC-10,^{27,28} which can interact genetically and physically.^{28–30} Both these proteins are DEG/ENaC family members.⁸ Genetic arguments support that at least two MEC-4 and at least two MEC-10 subunits are assembled in the heteromeric touch-transducing channel.⁴ The MEC-4 and MEC-10 extracellular domains are postulated to be linked to a touch cell-specific specialized extracellular matrix.³¹ Channel intracellular domains are hypothesized to be tied to the cytoskeleton.³² The tethering of channel subunits to the extracellular matrix and the intracellular cytoskeleton is postulated to confer channel gating tension. In this model, the minute mechanical deflection produced by light touch causes a conformational change in the channel, which is stretched between two attachment points, directly opening a gate for ion flow.^{27,28} Below, we discuss a model accommodating the information available on the structure of a nematode mechanotransducing complex (see section on modeling mechanical signaling in *C. elegans* and FIGURE 4).

DEGENERINS AND PROPRIOCEPTION

Unusual, *semidominant* gain-of-function mutations in another degenerin gene, *unc-8*, [*unc-8(sd)*] induce transient neuronal swelling³³ and severe uncoordination.^{34,35} *unc-8* encodes a degenerin expressed in several motor neuron classes and in some interneurons and nose touch sensory neurons.⁶ Interestingly, *semidominant 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^{6,30} (see also FIGURE 1A). 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. Another degenerin family member, *del-1* (for *degenerin-like*) is coexpressed 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.⁶

What function does the UNC-8 degenerin channel serve in motoneurons? *unc-8* null mutants have a subtle locomotion defect.⁶ 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; see more detailed discussion in Refs. 16 and 22). *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 indicates that the UNC-8 degenerin channel functions to modulate the locomotory trajectory of the animal.

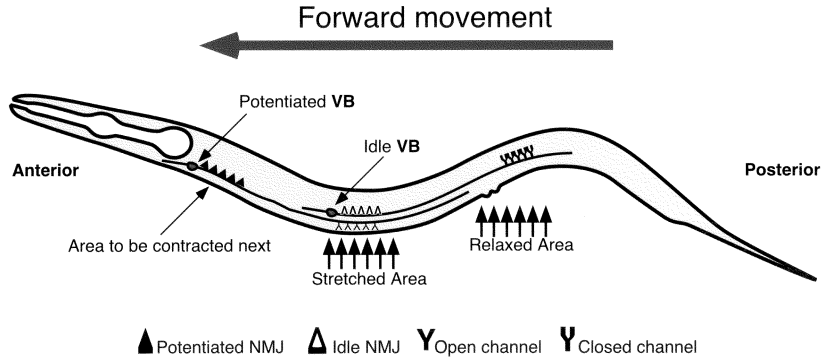


FIGURE 5. Model for modulation of locomotion by stretch-responsive channels in motor neurons. Two VB motor neurons in the ventral nerve cord are shown with stretch-sensitive channels postulated to be situated in their undifferentiated processes. The anterior VB signal to muscle is potentiated by the opening of ion channels in its process that experiences stretch due 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).

How does the UNC-8 motor neuron channel influence locomotion? One highly interesting morphological feature of some motoneurons (in particular, the VA and VB motoneurons that coexpress *unc-8* and *del-1*) is that their processes include extended regions that do not participate in neuromuscular junctions or neuronal synapses (see FIGURE 5). These “undifferentiated” process regions have been hypothesized to be stretch sensitive (discussed in Ref. 36). 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 coassemble into a stretch-sensitive channel that might be localized to the undifferentiated regions of the motor neuron process.⁶ 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 (FIGURE 5). The stretch signal enhances motoneuron 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 motoneurons.

One important corollary of the *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, an-

imals 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 channels, 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.

A MODEL FOR MECHANICAL SIGNALING IN *C. elegans*

The molecular features of cloned touch cell and motorneuron structural genes, together with genetic data that suggest interactions between them, constitute the basis of a model for the nematode mechanotransducing complex (FIGURE 4A; see Refs 26 31, 37, 38 for discussion). The central component of this model is the candidate mechanosensitive ion channel, which 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 motorneurons. These subunits assemble to form a channel pore that is lined by hydrophilic residues in membrane-spanning domain II. Subunits adopt a topology in which the Cys-rich and NTD domains extend into the specialized extracellular matrix 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 extracellular matrix and anchoring intracellular domains to the microtubule cytoskeleton. Outside the cell, channel subunits may contact extracellular matrix components. Inside the cell, channel subunits may interact with the cytoskeleton either directly or via protein links. A touch stimulus could deform the microtubule network, or could perturb the mantle connections to deliver the gating stimulus. In either scenario, Na⁺ influx would activate the touch receptor to signal the appropriate locomotory response.

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 (FIGURE 4B; see Refs. 39 and 40). 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 thought to open the mechanosensitive hair cell channels directly.

DEGENERINS AND DEGENERATION

MEC-4, MEC-10, and several related nematode degenerins have a second, unusual property: specific amino-acid substitutions in these proteins result in aberrant channels that induce the swelling and subsequent necrotic death of the cells in which they are expressed.^{3,7} This pathological property is the reason that proteins of this subfamily were originally called degenerins.⁷

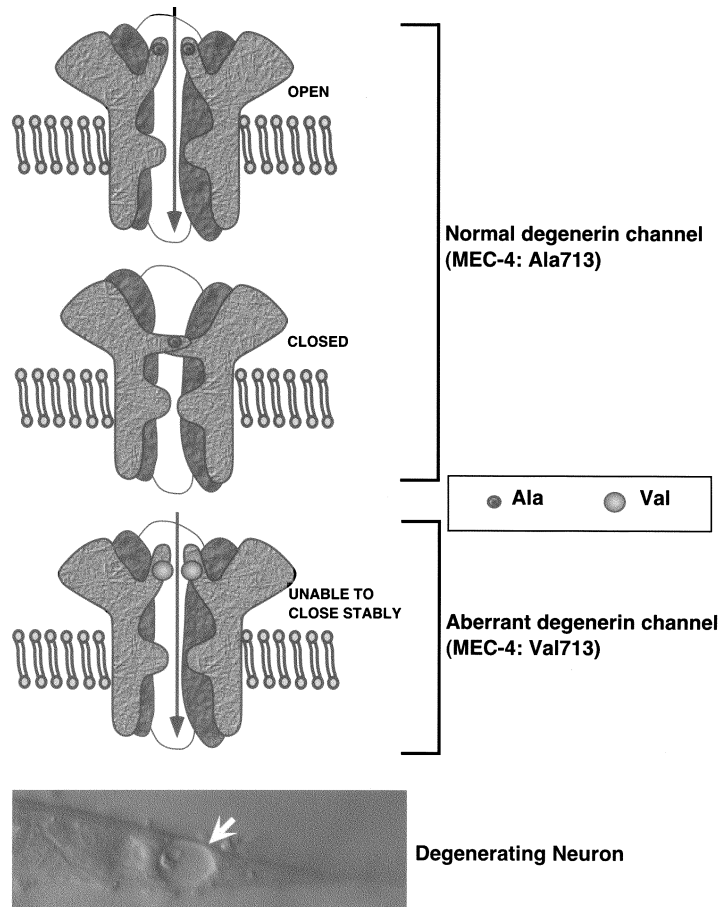


FIGURE 6. Model for degenerin-induced toxicity. MEC-4 is used as a paradigm. Gain-of-function mutations in the degenerin gene *mec-4* encode substitutions for a conserved alanine adjacent to MSDII and result in neuronal degeneration. Amino acids with bulkier side chains at this position are thought to lock the channel in an open conformation by causing steric hindrance, resulting in ion influx (ionic selectivity, for the MEC-4-containing channel has not been established yet, but by analogy it is most likely selective for Na⁺) which triggers the necrotic-like cell death shown at the bottom.

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. In contrast, most *mec-4* mutations are recessive loss-of-function mutations that disrupt body touch sensitivity without affecting touch receptor ultrastructure or viability.⁴¹ *mec-4(d)* alleles encode substitutions for a conserved alanine that is positioned extracellularly, adjacent to the pore-lining membrane-spanning domain (see FIGURE 1B). 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 Ala with a large side-chain amino acid is toxic.³ 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. Steric interference conferred by a bulky amino-acid side chain prevents such an approach, causing the channel to close less effectively. Increased cation influx results, initiating neurodegeneration (FIGURE 6). That ion influx is critical, for degeneration is supported by the fact that amino-acid substitutions that disrupt the channel conducting pore can prevent neurodegeneration when present *in cis* to the A713 substitution. In addition, large side-chain substitutions at the analogous position in some neuronally expressed mammalian superfamily members do markedly increase channel conductance.^{42,43} Interestingly, the cell death that occurs appears to involve more than the burst of a cell in response to osmotic imbalance.⁴⁴ Rather, it appears that the necrotic cell death induced by these channels may activate a death program that is similar in several respects to that associated with the excitotoxic cell death that occurs in higher organisms in response to injury in stroke. Electron microscopy studies of degenerating nematode neurons that express the toxic *mec-4(d)* allele have revealed a series of distinct events that take place during degeneration, involving extensive membrane endocytosis and degradation of cellular components.⁴⁴ Thus the toxic degenerin mutations provide the means with which to examine the molecular genetics of injury-induced cell death in a highly manipulable experimental organism.

FUTURE PROSPECTS: DEGENERINS AND MECHANICAL SIGNALING

DEG/ENaC proteins mediate diverse biological functions and may be gated via several different mechanisms. Beyond this diversity, however, lies a highly conserved subunit structure. This strong conservation across species suggests that DEG/ENaC family members shared a common ancestor 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 remains to be tested by identifying and isolating such structural modules within degenerins.

The detailed model for touch transduction in the *C. elegans* body touch receptor neurons accommodates genetic data and molecular properties of cloned *mec* genes. However, it should be emphasized that, apart from findings that MEC-4 and MEC-10 coimmunoprecipitate *in vitro*, 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 since, 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 (see, for example, Ref. 45) 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 neurons⁴⁶ 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. Indeed, some evidence suggests that related molecules are in the right place at the right time. For example, ENaC immunoreactivity has been found in mechanosensory lanceolate nerve endings of the rat mystacial pad⁴⁷ in the vibrissae (whisker), and γ ENaC immunoreactivity is localized to baroreceptor nerve terminals that innervate the aortic arch and carotid sinus, and mediate blood pressure regulation.⁴⁸ With the sequence of the human genome due to be released in the near future, additional members of the human ENaC family (which we anticipate could include hundreds of members) should be identified. 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.

NOTE ADDED IN PROOF: While this paper was under review, elegant work by Price and coworkers⁴⁹ demonstrated a requirement for the mammalian DEG/ENaC family member BNC1 in normal mechanosensation in the mouse. This finding further supports the involvement of specialized DEG/ENaC ion channels in mechanotransduction in higher organisms.

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