Minireview

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Biochemical Basis of Touch Perception: Mechanosensory Function of Degenerin/Epithelial Na^+ Channels*

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Personal experience informs us that receptors in the skin transmit a wealth of tactile information. We can detect the caress of a cheek, the texture of fabric, a fly alighting on our hand, and a pinch of our arm. Yet of all the vertebrate senses, touch is the least understood at the molecular level. For many years it has been postulated that the core components of mechanosensors are ion channels (for a review and references see Ref. 1). Such channels could convert mechanical energy directly into an electrical signal; this could account for the very high speed response of mechanosensors. In contrast, sensory receptors that detect light, odors, and most tastes initiate second messenger cascades, which then activate ion channels to generate electrical activity.

One of the first clues identifying ion channel mechanosensors came from Chalfie and colleagues (2, 3). To identify components of the mechanosensory apparatus in *Caenorhabditis elegans*, they developed a genetic screen, assaying the response to light touch. The screen led to the first members of the degenerin/epithelial Na^+ channel (DEG/ENaC)[1] family. The DEG/ENaC proteins discovered, including MEC-4 and MEC-10, may comprise the core of a multiprotein ion channel complex that opens in response to mechanical stimulation (4, 5). Here we review insights into how DEG/ENaC channels may contribute to mechanosensation.

Anatomical and Physiological Components of Mechanosensation

Previous research has taught us much about the bases for the sense of touch. At the behavioral level, psychometric studies in humans have distinguished several distinct cutaneous sensory inputs (6). At the anatomic level, investigators have identified a variety of specialized cutaneous sensory structures; some examples include Meissner corpuscles, Merkel cell-neurite complexes, lanceolate and pilo-Ruffini fibers surrounding hair follicles, and free nerve endings (Fig. 1*A*) (7). The cell bodies for these neurons reside in the dorsal root ganglion (DRG). At the cellular physiologic level, earlier work showed that distinct mechanosensory modalities are served by different classes of sensory neurons. Examples include rapidly adapting (RA) and slowly adapting (SA) low threshold mechanoreceptors, D-hair receptors, A-fiber mechano-nociceptors (AM) and C-fiber mechano-nociceptors (6, 8). Each class of neurons displays distinct physiologic properties. For example, RA mechanoreceptors possess large myelinated fibers and respond to very light (low threshold) touch. When a supramaximal constant stimulus is applied, they respond briskly during movement of the skin but show no sustained activity in the continued presence of the stimulus (Fig. 1*B*). RA mechanoreceptors include nerve endings in Meissner corpuscles and lanceolate fibers. In contrast, AM fibers respond to high threshold mechanical stimuli, such as a pinch, and adapt only slowly to a constant stimulus (Fig. 1*B*). AM fibers include some free nerve endings.

Yet despite this rich description of mechanosensation, only recently has there been insight into the actual molecules that may convert mechanical stimuli into electrical signals.

The DEG/ENaC Cation Channel Family

DEG/ENaC proteins share a common topology although only a few regions of protein show sequence similarity (Fig. 2). Members have short intracellular N and C termini, two membrane-spanning sequences (M1 and M2), and an extracellular loop (9). The large extracellular loop represents a particularly striking feature of the family; its 14 conserved cysteine residues, which form intrachain disulfide bonds (10), suggest a highly ordered, potentially rigid structure. Compare this microscopic architecture to that of some macroscopic mechanosensors. For example, the antenna of a mosquito is built with a large, relatively rigid external structure that transmits tiny mechanical deflections to a narrow base. Perhaps a similar

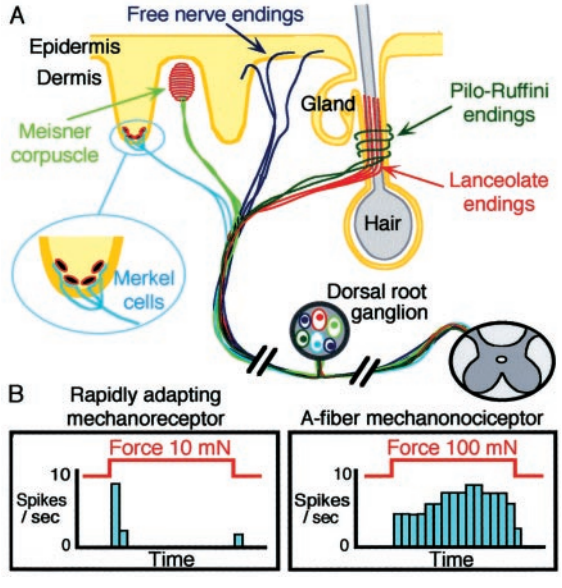

FIG. 1. *A*, some specialized cutaneous mechanosensory structures. *B*, examples of response by two mechanoreceptor fiber types (adapted from Ref. 8).

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[1] The abbreviations used are: DEG/EnaC, degenerin/epithelial Na^+ channel; DRG, dorsal root ganglion; RA, rapidly adapting; SA, slowly adapting; AM, A-fiber mechano-nociceptors.

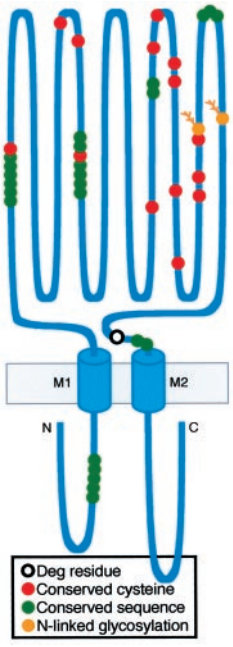

FIG. 2. **Topology of DEG/ENaC channels.** Relative length is approximately that of BNC1. When the small side chain "Deg" residue is mutated to a bulky residue, it induces constitutive activity in some subunits.

construction design is utilized at the microscopic level in mechanosensory DEG/ENaC channels.

Individual DEG/ENaC subunits assemble as homomultimers or heteromultimers to form cation channels. Reports on the number of subunits that form a channel vary, suggesting that 4–9 are involved (11, 12). M2, a portion of the extracellular domain preceding M2, and perhaps M1 contribute to the channel pore (13). The channels are voltage-insensitive with a permeability of $Na^+ \gg K^+$; some channels may also have a limited Ca^{2+} permeability. Extracellular amiloride inhibits current by occluding the pore; however, very high doses are required to block some channels, and amiloride inhibition may not turn out to be a defining property of this family.

When expressed in heterologous cells, some DEG/ENaC members generate constitutively active channels; an example is the channel formed by coexpression of α-, β-, and γENaC (14). Other members open with application of extracellular ligands; examples are the FMRF amide-activated Na^+ channel (FaNaCh) from *Helix aspersa* (15) and three extracellular acid-activated mammalian channels, BNC1, ASIC, and DRASIC (16–18) (Table I). Still other members such as the *Drosophila* Pickpocket (19) have not yet been shown to conduct ions. We presume such proteins could form ion channels if studied under the proper, but yet undetermined, conditions. Sequence analysis suggests that the *C. elegans* genome encodes 20 DEG/ENaC proteins, the *Drosophila* genome encodes 30, and mammals have 9 (Table I), but none have been discovered in unicellular organisms such as bacteria or yeast.

DEG/ENaC Channels Are Located at the Site of Mechanosensation

Several DEG/ENaC channels reside in mechanosensory neurons. In *C. elegans,* six neurons with mechanoreceptor function express MEC-4 and MEC-10 (2–5). In *Drosophila* embryos and larvae, three multidendritic neurons per hemisegment express Pickpocket (Fig. 3*A*) (19). The sensory processes of these neurons course beneath the epidermis where they sense touch and changes in body shape. Pickpocket localizes to dendritic varicosities, the site of specialized contact between neuron and

TABLE I
Nine mammalian DEG/ENaC channels

BNC1 and ASIC each have alternatively spliced isoforms. Despite its name, ASIC4 is not H^+-gated.

		Ref.
ENaC	α, β, γ, and δ subunits of epithelial Na^+ channel	14, 41
BNC1	Brain Na^+ channel 1; also called MDEG, BNaC1, and ASIC2	16, 26, 42, 43
ASIC	Acid-sensing ion channel; also called BNaC2, ASIC1	17, 26, 43, 44
DRASIC	Dorsal root acid-sensing ion channel; also called ASIC3	18, 26
BLINaC	Brain-liver-intestine amiloride-sensitive Na^+ channel	45
ASIC4	Acid-sensing ion channel 4; also called SPASIC	46, 47

epidermis, and the likely site of mechanotransduction. Several studies have detected transcripts and/or protein of β- and γENaC, BNC1, ASIC, and DRASIC in large diameter DRG mechanosensory neurons. More to the point, β- and γENaC subunits localize to specialized cutaneous mechanosensory structures including the Merkel cell-neurite complex (Fig. 3*B*) (20, 21). BNC1 also lies in several cutaneous mechanosensory structures, including pacinian corpuscles, Meissner corpuscles, Merkel cell-neurite complexes, and lanceolate nerve endings surrounding the hair shaft (Fig. 3*C*) (22, 23). Thus, their location positions DEG/ENaC channels where they can detect tactile stimuli.

Disruption of DEG/ENaC Genes Impairs Normal Mechanosensation

When a probe touches the lateral body wall, *C. elegans* shows a characteristic response, moving away from the stimulus. Mutations in the genes encoding MEC-4 and MEC-10 disrupt this behavior, suggesting that these channels contribute to mechanosensation (2, 3). Two other *C. elegans* DEG/ENaC proteins, UNC-105 and UNC-8, may also have mechanosensory functions, sensing stretch in body wall muscle and/or functioning in proprioception (24, 25).

To investigate the mechanosensory role of a mammalian DEG/ENaC channel, the mouse BNC1 gene was disrupted (22). Then the response of single sensory fibers to mechanical stimuli was recorded using an *in vitro* skin-nerve preparation. In wild type animals, increasing the strength of a mechanical stimulus increased the number of action potentials in RA mechanoreceptors (Fig. 4). In BNC1 null mice, RA mechanoreceptors still responded to mechanical stimuli, but the stimulus-response relationship revealed a flattened discharge frequency. The stimulus-response function of SA mechanoreceptors showed a smaller but significant shift. However, other fiber types responded normally to touch, acid, and noxious heat. Thus BNC1 was specifically required for the sensitivity of low threshold mechanoreceptors. The dynamic sensitivity of these mechanoreceptors is critically important for the perception and discrimination of touch sensation (6).

Relationship between Acid Activation and Mechanosensation

The finding that protons activate some mammalian DEG/ENaC channels (BNC1, ASIC, and DRASIC) suggested that they contribute to acid-evoked nociception (26). Indeed, DRASIC may play this role in cardiac afferents (27), and DEG/ENaC channels may contribute to nociception in some cutaneous neurons. However, BNC1, DRASIC, and ASIC are also expressed in the soma and peripheral extensions of large diameter DRG neurons, *i.e.* low threshold mechanosensors. Moreover, DEG/ENaC channels generate H^+-gated currents in

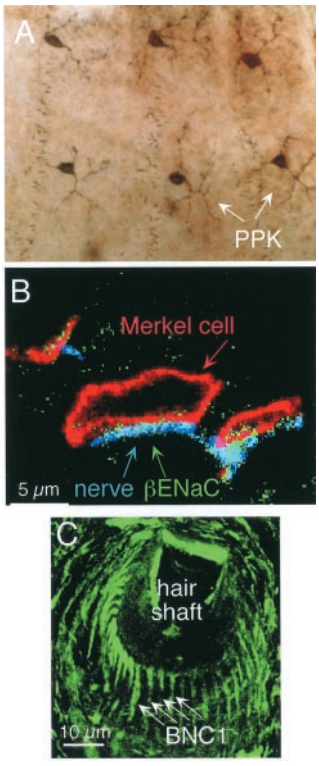

FIG. 3. **Immunohistochemical localization of DEG/ENaC subunits.** *A*, Pickpocket (*PPK*) in *Drosophila* larvae multidendritic neurons. *B*, βENaC in a rat Merkel cell-neurite complex. *Aquamarine* color indicates colocalization of βENaC and nerve cell markers. *C*, BNC1 in mouse lanceolate fibers surrounding guard hair shaft. From Refs. 19, 20, and 22 with permission.

these neurons as evidenced by altered H^+-gated currents in knockout mice.[2] Thus, it seems paradoxical that acid fails to elicit activity from the peripheral extensions of these neurons; instead they are tuned to detect innocuous tactile stimuli (28, 29). Why is this? We speculate that in mechanosensory nerve endings, DEG/ENaC channels are tethered to extracellular proteins that confer touch sensitivity but mask pH-responsive sites. We suggest that proton activation is a signature of DEG/ENaC function in cells where protons are not the physiologic ligand.

If this is the case, then perhaps the biophysical properties observed with H^+ activation will predict the response of these channels as mechanosensors. For example, individual DEG/ENaC channels evince different rates of desensitization during a continued acid stimulus (27). Perhaps the desensitization rate parallels the rate of adaptation to a constant mechanical stimulus. We wonder whether different combinations of DEG/ENaC subunits in RA and AM mechanoreceptors might explain, at least in part, their different adaptation to a constant tactile stimulus (Fig. 1*B*).

Contribution of DEG/ENaC Channels to Mechanotransduction in Other Tissues

DEG/ENaC channels have been considered candidates for the mechanosensitive ion channels in cochlear hair cells. However, studies of knockout mice indicate that αENaC and BNC1 are not required for hearing (30).[2] Perhaps auditory mechanosensitive channels are related to Trp channels involved in cilium-bearing mechanoreceptors (31).

Mechanosensors in aortic arch baroreceptors detect arterial wall stretch, initiating reflexes that buffer acute arterial pressure fluctuations. Neurons innervating the aortic arch contain β- and γ- but not αENaC transcripts, and γENaC resides in their corkscrew, spiraling terminals (32). This location plus blunting of baroreceptor nerve activity by an amiloride analog suggests DEG/ENaC subunits might detect vessel stretch. Because β and γ subunits require αENaC to generate a constitutively active channel, these data suggest that β and γ subunits must have a different functional role in baroreceptors than they do in epithelia.

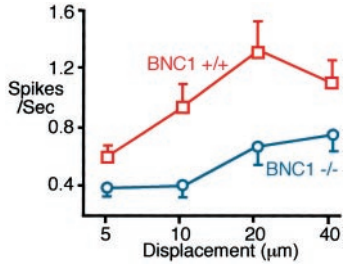

FIG. 4. **Response of RA mechanoreceptors from BNC1 +/+ and −/− mice.** From Ref. 22 with permission.

Are DEG/ENaC Channels Mechanosensors?

Although the jury is still out on this question, we think the genetic data, the localization, and the functional abnormalities in knockout animals suggest that the answer is yes. Nevertheless, it is interesting that the loss of BNC1 causes only modest, albeit specific defects in mechanosensation (22). Likewise *C. elegans* that are UNC-8 null show only subtle locomotion defects, and a mechanosensory defect has not yet been reported in UNC-105 null worms (24, 25). We speculate that in these null animals other DEG/ENaC subunits contribute to mechanosensation, compensating in part for the missing subunit.

What additional data would help determine whether DEG/ENaC channels are mechanosensors? Receptor potential measurements taken at the precise site of mechanosensation in wild type and null animals would be useful. Although graded changes in receptor potential in response to graded stimuli would be informative, the tiny size of the nerve endings makes this a formidable challenge. It will also be interesting to study animals missing multiple DEG/ENaC subunits. In addition, it would be important to reconstitute the system, supplying all the parts to produce a mechanosensor *in vitro* or in a heterologous cell. However, before the system can be reconstituted, it may be necessary to know more about how the channels work.

How Might DEG/ENaC Channels Work as Mechanosensors?

There are three main hypotheses about how movement activates mechanosensors (1). First, bilayer tension could directly activate the channel (Fig. 5*A*). This occurs with prokaryotic stretch-activated channels such as MscL (33, 34). Whether membrane thinning, curvature, and/or another factor is responsible is not yet certain, but no associated proteins are required. Some mammalian channels that are functionally defined by stretch activation may also be directly mechanically gated (1). There are conflicting data about whether ENaC can be gated by this mechanism, but at present how this mechanism functions *in vivo* in multicellular organisms is uncertain.

Second, mechanosensation could involve release of an extracellular ligand that activates a channel (Fig. 5*B*) (1). A ligand, for example ATP (35), might be released from a mechanosensitive cell itself or an associated cell. It would seem that such a mechanism would not be sufficiently fast to account for the very high speed of mechanosensation, for example with a tactile vibratory stimulus of 1–2 kHz (36). However, Hamill and Martinac (1) note that the rapid response of mechanosensors

[2] M. J. Welsh, M. P. Price, and J. Xie, unpublished results.

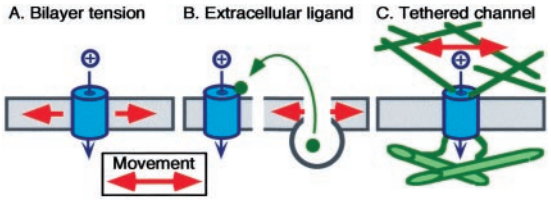

FIG. 5. **Potential mechanisms by which a channel responds to a tactile stimulus (*red arrows*).**

may not be sufficient to exclude this hypothesis. Intriguingly, some mechanosensory nerve endings, including pacinian corpuscles and Merkel cells, contain what appear to be synaptic vesicles sitting adjacent to nerve endings (36, 37). These endings also contain synaptic proteins (38). Yet some data suggest that destruction of the Merkel cell but not the adjacent nerve fails to abolish mechanosensation (39). We wonder if vesicles near the mechanosensory site might release ligands that modulate mechanosensory channel function rather than serving as the mechanosensory mechanism itself.

The third mechanism, a tethered model (Fig. 5*C*) (1), is the one we favor for DEG/ENaC channels. In this model, the channel binds the extracellular matrix and/or the intracellular cytoskeleton (1, 4, 9, 31). Movement of this complex structure transmits and perhaps amplifies applied stresses to gate the channel. Elegant genetic screens identified several genes required for normal tactile responses in *C. elegans* (4). The large extracellular loop of MEC-4 and MEC-10 channel subunits may interact with MEC-9 and MEC-5, an extracellular collagen. At the intracellular surface, the α-tubulin MEC-12 and the β-tubulin MEC-7 may link to the mechanosensory complex via a stomatin-related protein MEC-2. Genetic data also suggest that UNC-105 interacts with a type IV collagen (24).

For mammalian DEG/ENaC channels, no interacting matrix proteins have yet been identified. Nedd4, α-spectrin, and syntaxin 1A bind intracellular domains of ENaC subunits (13), and BNC1 and ASIC bind PICK1 (40); however, the consequences for mechanosensation are unknown. A tethered mechanism may also apply to Trp channel family members proposed to function as mechanotransduction channels (31). Identifying proteins that interact with DEG/ENaC proteins in neurons should help us understand how mechanical stimuli gate these channels.

Conclusion

We speculate that mechanical deformation of sensory nerve endings activates an ion channel complex that includes DEG/ENaC subunits as the core component. Activation would elicit a depolarizing cation current that triggers action potentials. The diversity of molecular components, including varied DEG/ENaC subunits and associated scaffolding and matrix proteins, offers the opportunity to construct sensory receptors with substantial functional heterogeneity. Heterogeneity may allow tuning of this evolutionarily conserved channel family to generate, in part, the rich diversity we experience in our sense of touch.

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