Mechanosensation and the DEG/ENaC Ion Channels

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Mechanosensation—the transformation of mechanical stimuli into electrochemical signals—is ubiquitous in animals; it includes the senses of hearing, balance, touch, proprioception, as well as cellular functions such as volume regulation. The molecular basis of mechanosensitivity has been hard to discern because mechanosensitive organs are small and their constituent proteins are scarce. Recent genetic studies in the nematode *Caenorhabditis elegans* have revealed a dozen candidate proteins for mediating the sense of touch, in the process defining a new superfamily of ion channel proteins. A report in this issue (*1*) now describes two interacting genes (*unc-105* and *let-2*) expressed by C. *elegans* muscle that may be involved in the muscle's response to stretch; *unc-105* is a new member of this channel superfamily and *let-2* encodes a collagen.

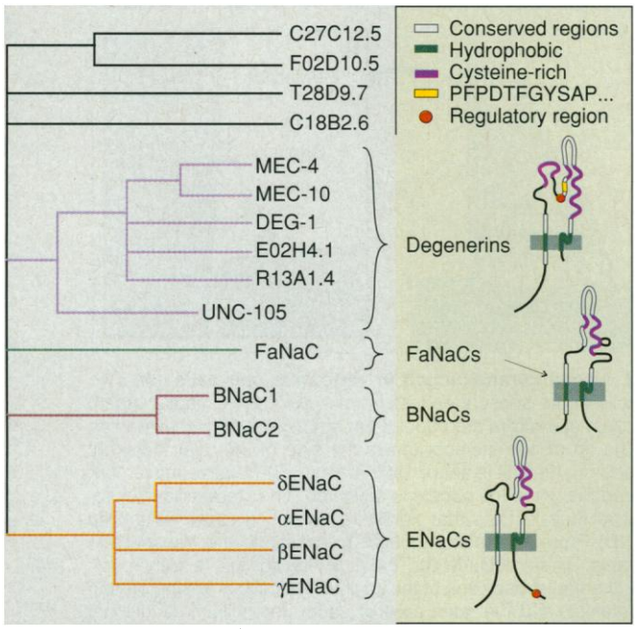

Fig. 1. The DEG/ENaC superfamily. Proteins of the superfamily fall into four distinct branches (degenerins, FaNaC, BNaCs, and ENaCs), with nondegenerin members from *C. elegans* forming other groups. Cartoons illustrate conserved domains of the different branches (*22*).

In C. *elegans*, sensitivity to light touch on most of the body is mediated by six neurons with processes that run along the hypodermis, attached to it by a mantle structure. Twelve genes can be identified that are needed for mechanosensitivity but not for the cells' development; the gene products are candidates for components of a mechanotransduction apparatus (*2*).Two are of particular interest: MEC-4 and MEC-10 are subunits of an ion channel that might be mechanically sensitive. Certain rare mutations in these genes, occurring at or near the pore or in an extracellular site, cause the touch cells to swell and die. The mutations are hypothesized to bias the channels toward being open, causing a continuous ion influx and swelling. These and several closely related proteins in nematodes have consequently been termed degenerins (DEGs) (although mechanosensation is thought to be their normal function because null mutations in some cause the touch-insensitive phenotype). MEC-4 and MEC-10 appear to be subunits of a heteromultimeric channel protein: MEC-10 cannot cause degeneration in the absence of MEC-4, both proteins coimmunoprecipitate in vitro, and neither causes degeneration in the absence of a third possible subunit, MEC-6. Similar experiments suggest that the channel contains more than one copy of both MEC-4 and MEC-10 (*3*, *4*).

Other genes from C. *elegans* encode degenerins. Both *deg-1* and *unc-8* cause no apparent phenotype when deleted, but certain mutations in them cause degeneration of a subset of neurons (*4*, *5*). *unc-105* also produces no phenotype when deleted, but some alleles cause hypercontracted muscles (producing the uncoordinated phenotype) (*6*). In the new report, Liu, Schrank, and Waterston (*1*) have now cloned *unc-105* and identified these mutations, which change extracellular residues near the transmembrane domains.

More distant relatives of the degenerins make up channels involved in ion transport in kidney, colon, and lung—the amiloride-sensitive epithelial sodium channels (ENaCs) (*7*). The native ENaC channel apparently occurs as a multimer of at least three subunits (α, β, and γ) (*8*). As has been demonstrated for αENaC and MEC-4, each subunit may have two transmembrane domains and a large extracellular loop (see Fig. 1).

In the last 7 months, this DEG/ENaC sodium-channel superfamily has been expanded by at least two more branches (see Fig. 1). A FRMF amide-activated Na^+ channel (FaNaC) (*10*), cloned from the snail *Helix aspersa*, can function when expressed by itself in oocytes, but, like aENaC, might produce larger currents if expressed with other subunits. Three groups (*11–13*), all chasing a whiff of channel sequence from the database of expressed sequence tags, have recently cloned an amiloride-sensitive sodium channel from human brain (BNaC1, for brain Na^+ channel). A second member of this branch (BNaC2) has also been identified (*13*); both proteins are expressed in most neurons, but their function has not been elucidated. Importantly, mutations introduced into BNaC1 corresponding to those producing degeneration in DEG-1, MEC-4, and MEC-10 cause BNaC1 to be open much of the time and result in cell death (*12*), strongly supporting the interpretation of the degeneration phenotype in C. *elegans* (*3–5*). Lastly, the C. *elegans* genome project has revealed other members of the DEG/ENaC superfamily that are not members of the degenerin branch, which is defined by three conserved domains in the extracellular loop, including an almost perfectly conserved 19–amino acid segment (PFPDTFGYSAPTGFISSFG) (*14*) and an inhibitory region (*4*). Control of gating in this superfamily may be mediated by the extracellular loop, through either ligand binding or mechanical tension.

If MEC-4, MEC-6, MEC-10, and other degenerins form mechanically gated channels, how might a mechanosensitive transduction apparatus be put together? Some ideas have come from studies of transduction by inner-ear hair cells, the receptor cells of the vertebrate auditory and vestibular systems (*15*) (see Fig. 2). Each hair cell bears on its apical surface a cluster of 30 to 300 stereocilia, in a staircase arrangement of increasing height. Short extracellular filaments called tip links connect the tips of stereocilia (*16*). Deflection of the bundles toward the tallest stereocilia opens nonselective cation channels in the stereocilia (*17*). Although most of the constituent proteins of the transduction apparatus have not been identified in hair

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cells, the cells' highly specialized morphology and relative accessibility for physiology have nevertheless enabled a model for how the channels might be activated. It is thought that a mechanically gated transduction channel is connected to each end of each tip link in a bundle (*16*, *18*). Deflection toward the tallest stereocilia would stretch the tip links and increase tension on the channels, causing them to open. It is also supposed that each channel is connected to the actin core of its stereocilium by some intracellular link, so that tip-link tension does not pull it out of the membrane. If this general model is broadly applicable, a mechanosensory apparatus must include a rigid cytoskeleton, an intracellular linking protein, a mechanosensitive channel, and an extracellular linking protein. (Of course, if forces are directed parallel to the membrane, two intracellular or two extracellular links could work equally well.)

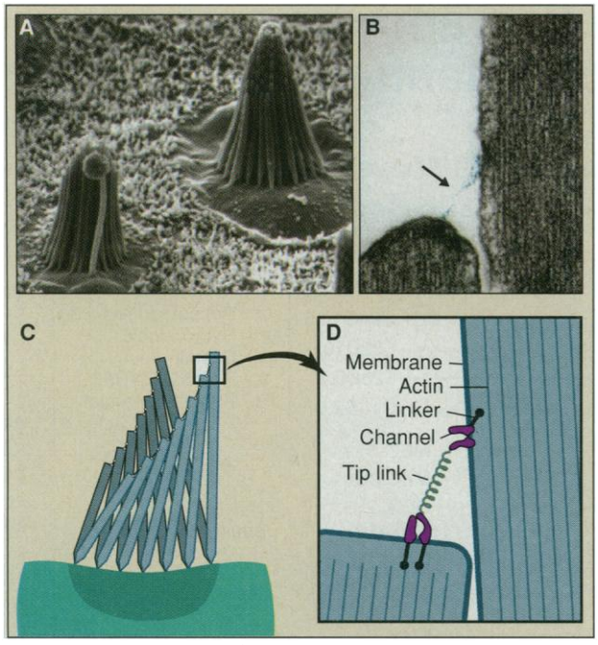

Fig. 2. Mechanotransduction in vertebrate hair cells. (**A**) Two hair cells in the sensory epithelium of a bullfrog vestibular organ. The tallest stereocilia are about 8 μm high. (**B**) A tip link extending from the tip of one stereocilium to the side of the taller adjacent stereocilium; the link is about 150 nm long. (**C**) Relative movement of stereocilia when the bundle is deflected. The stereocilia stick together at their tips, so that rightward deflection stretches the tip links. (**D**) Protein elements thought to constitute the transduction apparatus. These include the extracellular tip link, a transmembrane channel at each end of the tip link, intracellular linking protein (or proteins), and the actin core of each stereocilium. Additional constituents of an adaptation mechanism are not shown.

Candidates for all of these elements have emerged from studies of the touch cells in C. *elegans*. The *mec-12* and *mec-7* genes encode α- and β-tubulins, which form unique 15-protofilament microtubules in the touch cell processes. *mec-2* encodes a protein also present within the touch cell processes; it is homologous to stomatin, a membrane-associated cytoskeletal protein in erythrocytes. Although there is good evidence that MEC-4 and MEC-10 are channel subunits, the evidence that they are mechanically gated is largely by default; no other channel candidates have emerged from a saturating screen. Two other genes, *mec-5* and *mec-9*, encode secreted proteins. MEC-5 is a novel collagen expressed by the hypodermis, which is associated with the touch cell processes. MEC-9 has a series of EGF repeats and Kunitz-like serine protease domains (*19*). The *mec-1* gene is essential for formation of the mantle, which links the processes to the hypodermis (*2*).

Now, Liu *et al.* show that *unc-105*, expressed in C. *elegans* muscle, interacts with *let-2*, which encodes a collagen IVa2: The contraction phenotype caused by mutations in UNC-105 can be suppressed by certain mutations near the COOH-terminus of LET-2 (*1*, *20*). One interpretation is that UNC-105 is a mechanically gated channel and that LET-2 normally carries tension to UNC-105 when the muscle is stretched. The mutations in UNC-105 might increase the channel opening, causing contraction, whereas the suppression mutation in LET-2 might disconnect the collagen from the channel. In both touch cells and muscle of C. *elegans*, much work remains to clarify the connections among these proteins.

Do the C. *elegans* proteins also play a role in vertebrate mechanotransduction? At the moment, there is no evidence for such conservation. No homolog of the degenerin branch of the DEG/ENaCs has been found outside of C. *elegans* (Fig. 1). The hair cell tip link is unlikely to be related to the collagens MEC-5 or LET-2, because it is relatively resistant to collagenase. A bacterial channel that is clearly mechanically sensitive, MscL, has no sequence similarity to the degenerins and senses membrane tension in the absence of any protein linkers (*21*). These points all argue against a universal apparatus. It seems possible, instead, that mechanosensation may have evolved independently in different systems. Many kinds of ion channels are attached to the cytoskeleton, if only to keep them in the right place in the cell. It is a small step to make two attachments that can pull relative to one another, so that the channel responds to distortion of a tissue. If mechanosensation has arisen more by analogy than homology, a long road lies ahead to figure out the molecular basis of mechanotransduction in each system.

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14. Abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; F, Phe; G, Gly; I, Ile; P, Pro; S, Ser; T, Thr; and Y, Tyr.
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22. The conserved regions of all members (174 amino acids, including the transmembrane domains) were aligned with CLUSTALW and relationships calculated with PAUP version 3.1 with 2000 iterations. GenBank accession numbers for representative sequences are as follows: MEC-4 (U53669), MEC-10 (P34886), DEG-1 (L34414), E02H4.1 (Z68003), R13A1.4 (U40798), UNC-105 (Z48045), F02D10.5 (Z67990), C27C12.5 (Z67883), C18B2.6 (U40413), T28D9.7 (U28738), BNaC1 (U57352), FaNaC (X92113), αENaC (X70497), βENaC (X77932), γENaC (X77933), δENaC (U38254).

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