Mechanosensation: **Touch at the molecular level**

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The cloning of genes needed for gentle-touch sensitivity in the nematode *Caenorhabditis elegans* has provided new molecular details about a proposed mechanosensory ion channel complex.

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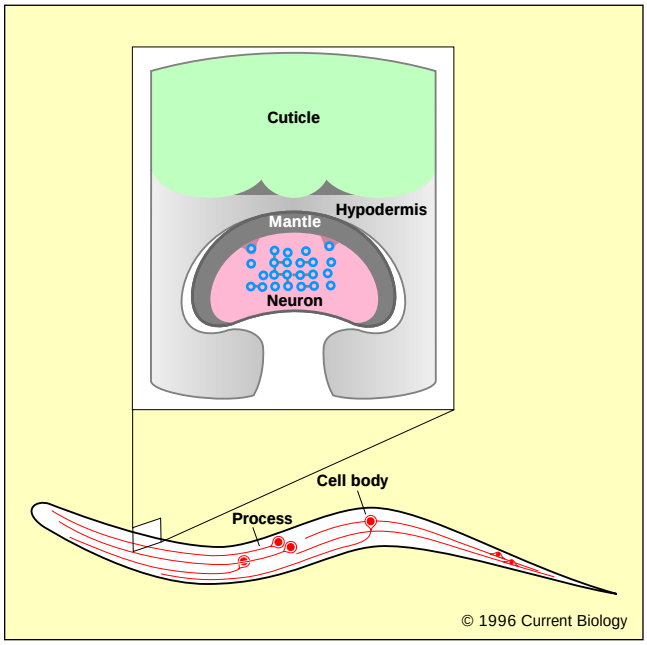
Touch and the related sense of proprioception are the most ubiquitous, yet the least understood, of the senses. In vertebrates, the sensory terminals of touch receptors and proprioreceptors are far from the cell bodies, dispersed throughout the body and embedded in other tissues, impeding the electrophysiological recording of mechanoreceptor potentials and biochemical purification of components of the molecular machinery that mediates mechanosensation. An alternative approach to understanding mechanotransduction has been to isolate touch-insensitive mutants in genetically tractable organisms. This approach is beginning to bear fruit, as evidenced by the recent cloning of genes needed for gentle touch sensitivity in the nematode *Caenorhabditis elegans*, which seem to encode components of a mechanosensory ion channel complex.

In *C. elegans*, six neurons mediate the sensation of gentle touch applied throughout most of the body. A saturated screen for touch-insensitive mutants — termed the Mec phenotype, for mechanosensory deficient — led to the identification of 12 genes needed for the function of these cells (in addition to other genes needed for their development) [1–3]. Two of these 12 genes, *mec-4* [4] and *mec-10* [5], encode similar proteins which have been called ‘degenerins’, because gain-of-function mutations in them cause cells to swell and die. Degenerins belong to a protein superfamily that includes amiloride-sensitive sodium channels, and are likely to form mechanosensitive ion channels.

Although no recordings have been made of the putative channels formed by degenerins, glimpses into their regulation have been obtained by studying gain-of-function mutations that cause degeneration. Thus, degenerins were found [6] to have an extracellular inhibitory domain that is absent in related ion channels that are not believed to be mechanosensitive, and it was suggested [6] that degenerin ion channels are regulated by extracellular attachment, mediated by the inhibitory domain. The type of regulation envisaged is analogous to the ‘tip-link’ model for the mechanosensitive ion channels of hair cells [7], in which fine extracellular links between the tips of hair-cell stereocilia physically pull channels open when sound of the appropriate frequency deflects a bundle of stereocilia. In the case of *C. elegans* touch-cell processes, the physical association of the mechanosensitive channel may be with an extracellular structure called the ‘mantle’ (Fig. 1). This notion receives some support from the fact that the gene *mec-1* is necessary for formation of the mantle [2].

Recently two other *mec* genes, *mec-5* and *mec-9*, have been cloned and found to encode secreted proteins [8]. MEC-5 is a novel collagen, having the characteristic proline-rich Gly–X–Y tripeptide repeat that coils the triple-helical collagen molecules, but its amino-terminal and carboxy-terminal tails are unlike those in any other collagen. All 18 missense *mec-5* mutations that have been sequenced disrupt residues of the Gly–X–Y, often affecting glycines towards the carboxy-terminal end of the repeat region.

Figure 1



Longitudinal view of nematode *Caenorhabditis elegans*, showing all the mechanosensory neurons, with an enlarged cross-section through the process of one touch receptor neuron. The 15-protofilament microtubules of a bundle appear connected through bridges and terminate in apparent association with the membrane. The neuronal process is surrounded by the mantle and embedded into the hypodermal syncytium (the ‘worm skin’). Densely staining material in the hypodermis extends from the mantle to the cuticle.

These mutations are likely to interfere with triple-helix formation, which starts assembling from the carboxyl terminus. About half of the *mec-5* mutations are temperature-sensitive, including two that substitute prolines, probably because collagen triple helices are temperature labile and their stability depends on hydroxylated prolines.

The *mec-5* gene is expressed in the hypodermis, the skin of the worm which is closely associated with the touch cell processes (Fig. 1). The sensory nerve endings of certain vertebrate cutaneous receptors are embedded in collagenous tissues, but collagens are the most abundant proteins in the animal kingdom, so this coincidence is hardly surprising. A role for collagens in degenerin regulation may be common, however, as rare dominant mutations in *unc-105*, another nematode degenerin gene, are completely suppressed by specific mutations in *sup-20* (also known as *let-2*) (J. Liu, B. Shrank and R. Waterston, personal communication), which encodes a type IV basement collagen [9].

MEC-9 contains an unusual combination of EGF repeats and Kunitz-like serine-protease inhibitor domains [8]. Both types of domain seem important for MEC-9 function, as numerous missense mutations disrupt residues in them. A similar combination of EGF repeats and serine-protease inhibitor domains is found in the protein agrin, which clusters acetylcholine receptor channels. Furthermore, two snake venom peptides, dendrotoxin and calcicludine, that each consist of a Kunitz domain, inhibit voltage-dependent K^+ channels and L-type Ca^{2+} channels, respectively. Therefore, it is not unlikely that MEC-9 associates with, and regulates, degenerin ion channels.

Intuitively, it seems that for a structure to pull or push open a channel, a second structure must hold the channel in place. The hair-cell channels mentioned above, for instance, are thought to be pulled on by the extracellular tip links but anchored to the actin cytoskeleton by myosin molecules. Two *mec* genes, *mec-12* (M. Hamelin, M. Chou and J. Culotti, personal communication) and *mec-7* [10], encode the α and β tubulins necessary for the formation of the large diameter microtubules that are found only in the processes of the touch cells (Fig. 1). In most *mec-7* and *mec-12* mutant animals, these 15-protofilament microtubules are replaced by a smaller number of the 11-protofilament microtubules that are normally present in all other cells. As a result, the touch cell processes extend as in wild-type nematodes, but fail to sense touch.

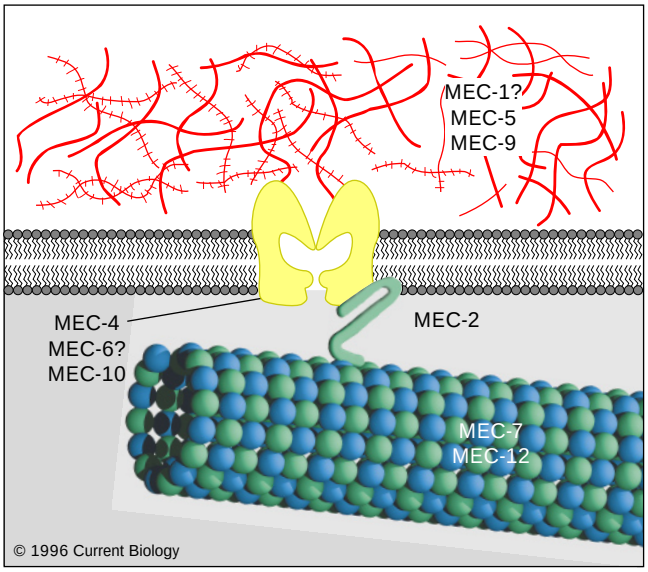
These unusual 15-protofilament microtubules are only 2–5 % as long as the neuronal process, and seem to run obliquely to its axis, terminating very near the membrane in association with a diffusely staining material [11] (Fig. 1). Certain insect mechanoreceptors also contain specialized microtubules associated with membrane particles, which themselves associate with the extracellular cuticle [12]. Thurm [12] found that compressing the cuticle visibly displaces the microtubules and causes a mechanoreceptor potential, and suggested that the membrane particles constitute the 'sensors'. A similar mechanism may operate in the touch cells of the nematode by an attachment of the microtubules to the degenerin channels.

Recently, the *mec-2* gene was cloned [13]. More than half of the MEC-2 sequence is 75 % identical to stomatin, a widely expressed integral membrane protein, also known in erythrocytes as band 7.2b. Stomatin has intracellular amino and carboxyl termini, and binds to the cytoskeleton through its carboxy-terminal region [14]. It has been confirmed by experiments using β-galactosidase fusion proteins that MEC-2 has a similar topology to stomatin. Localization of a MEC-2–β-galactosidase fusion protein to neuronal processes is reduced to a variable extent in *mec-7* and *mec-12* mutants (the remaining partial localization might be accounted for by the regular microtubules that replace the 15-protofilament microtubules in some of these mutants). Localization of MEC-2 to neuronal processes seems therefore to involve microtubules, but it is not clear whether MEC-2 binds microtubules or is simply transported along them. Stomatin probably does not bind microtubules, for the simple reason that red blood cells have none; MEC-2, however, contains additional sequences at both its intracellular ends which may bind microtubules or their associated proteins. It would be of interest to determine whether the diffusely staining material adjacent to the membranes at the end of microtubules is present in *mec-2* mutants, and whether antibodies against MEC-2, MEC-4 or MEC-10 appear in electron microscopic sections near the ends of microtubules.

Certain mutations in *mec-2*, like others in *mec-12*, *mec-4*, *mec-14* and *mec-15*, somewhat reduce the amount of neuronal degeneration caused by a *mec-10* gain-of-function mutation. Although very indirect effects may account for this mild suppression, one possibility is that the products of these genes regulate the degenerin channel [5]. Stomatin, which is normally expressed in most cell types, is absent from the red blood cells of patients with stomatocytosis, a disease characterized by 'mouth'-shaped red blood cells with unusually low K^+ and high Na^+ levels. However, neither the coding sequence nor the mRNA expression of the stomatin gene is altered in patients, and the cause of the disease is probably a mutation in another gene that affects both membrane conductance and stomatin protein levels [14]. So there is little evidence that stomatin or MEC-2 regulates any ion channel, although the possibility remains appealing.

Despite the lack of direct evidence bearing on their precise activities, most MEC proteins can be incorporated into a working model of how a mechanosensory apparatus might be assembled and operate, mostly on the basis of

Figure 2



A possible arrangement for MEC proteins in which touch-induced displacement of microtubules or shearing of the extracellular matrix would lead to channel opening. The genes *mec-1* and *mec-6* have not yet been cloned, but their mutant phenotypes suggest their proposed roles (see text).

similarities to known proteins (Fig. 2). It has recently been demonstrated (C. Lai and M. Driscoll, personal communication) that MEC-4 and MEC-10 interact *in vitro*. Genetic interactions also suggest that the channel is a oligomer of MEC-4, MEC-10 and a third subunit, MEC6 (in the same way that the related epithelial sodium channel is made of three homologous subunits [15]). There is, however, no direct evidence that any of the other MEC proteins interact with the channel.

Further interactions between many of the *mec* genes have been described. Temperature-sensitive alleles of *mec-5* are dominantly enhanced by several normally recessive alleles (some actually null) of *mec-9*, *mec-4* or *mec-10* [8], and another temperature-sensitive mutation in *mec-4* is dominantly enhanced by recessive mutations in *mec-2*, *mec-7* or *mec-12* [13]. The synthetic Mec phenotype observed in all these double mutants is to be expected for genes that participate in the same process, but does not further suggest that their products directly interact. Allele-specific suppressions of the kind that would suggest a physical interaction between gene products are rare, and have not been reported to occur between any two *mec* genes. Perhaps the best approach to test for interactions among the MEC proteins is through biochemical experiments such as co-immunoprecipitation after *in vitro* translation in the presence of microsomes (as done by C. Lai and M. Driscoll to prove an interaction between MEC-4 and MEC-10) or the yeast two-hybrid system.

Another obstacle to understanding mechanotransduction at the molecular level is that any of the *mec* gene products may not contribute to a mechanosensory channel complex but have other roles such as signaling to interneurons (which the touch cells can do with gap junctions) or maintaining a particular physiological state needed for cell function. The recent success in recording currents from *C. elegans* neurons [16] creates the means to find which *mec* genes are needed for the generation of mechanoreceptor potentials. Finally, a complete arrangement of MEC proteins awaits the cloning of five more genes: *mec-1*, *mec-6*, *mec-14*, *mec-15* and *mec-18*. Despite these limitations, characterization of the *C. elegans mec* genes is beginning to reveal the molecular structure of a mechanosensitive channel complex, complementing biophysical and anatomical models developed in other systems.

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