Insect mechanoreception: **What a long, strange TRP it's been**

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Insect bristles are model mechanosensory organs. An ion channel of the TRP superfamily has recently been identified which is required for production of mechanoreceptor currents by insect bristles, and seems likely to represent a new kind of mechanically gated channel.

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Mechanosensitive channels open or close in response to mechanical forces. It is probable that most cells have channels of this kind, and use them to respond to stretch generated during basic cellular processes, such as movement or changes in volume. Multicellular organisms, however, also have cells that are specialized for detecting mechanical forces generated by touch, the stretching of body parts, gravity or sounds. A well-studied mechanosensory organ is the insect bristle. Walker, Willingham, and Zuker [1] have now identified a novel member of the TRP superfamily of ion channels — NOMPC, for '*no*

and thus is most probably a mechanosensitive channel.

Insects have several types of cuticular mechanosensory sensilla, including bristles (which usually respond to touch), trichobothria (which respond to air currents and sound), campaniform sensilla (which respond to cuticle deformation), and chordotonal organs (which respond to stretching). Each type of sensillum has a different type of cuticular specialization which transmits mechanical stimuli to the ciliated dendritic terminal of a sensory neuron [2]. For example, deflection of the bristle compresses the dendrite tip, resulting in a depolarizing mechanoreceptor current carried by a poorly selective cation channel. After reaching peak amplitude, the response decays in two phases [3,4].

The insect bristle has long been studied as a model mechanosensory organ, in part because of its striking biophysical similarities with hair cells of the vertebrate inner ear [1,5]. For example, the receptor potential response has a very short latency of about 0.1 milliseconds, which is of the same order of magnitude as the latency in frog vestibular hair cells and the latency of voltage-gated ion channels. These latencies are significantly shorter than those of the fastest known second-messenger-based forms of transduction, and support the belief that the transduction channel is directly gated by mechanical stimulation [5,6]. In addition, the sensilla can be highly sensitive, responding well to less than a micrometer of deflection. The receptor potential undergoes adaptation upon prolonged deflection, which shifts the sensitive range and restores the dynamic range of response. The responses also show directionality, which is related to the morphology of the sensillum [1,3,7].

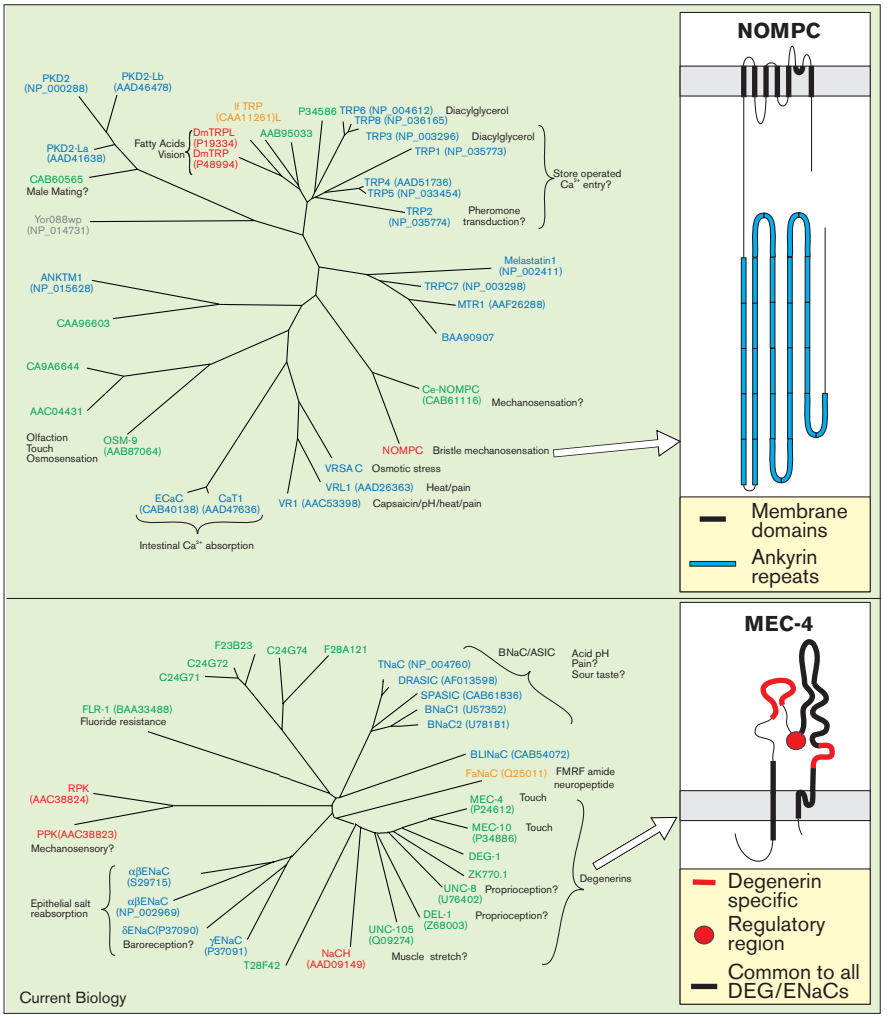
Recognizing the advantage of a combined genetic and physiological approach, Kernan, Cowan and Zuker [8] performed a genetic screen of the *Drosophila* X chromosome for mutants with defective larval touch responses, and then characterized the macrochaete bristle function of these mutants. Mutants with developmental defects were not pursued. Two mutants, *unc* and *uncl*, displayed no mechanoreceptor potentials upon deflection of their bristles — the *nomp* phenotype. In addition to this larval touch defect, the *unc* and *uncl* mutants were found to display adult uncoordination. This additional phenotype was used as the basis of a new, less laborious screen, which, again, was followed by electrophysiological characterization of bristle mechanoreceptor potentials.

C. Zuker, personal communication). Cloning of these genes is likely to provide valuable information about the molecular mechanisms of mechanotransduction in insect sensilla. In the nematode *Caenorhabditis elegans*, twelve genes have been identified that are necessary for mechanosensation by a set of six touch-sensitive mechanoreceptors [9]. In addition to ion channels, these genes encode components of the extracellular matrix and the cytoskeleton [10]. The evidence suggests that mechanotransduction in specialized mechanoreceptors involves groups of proteins, and is consistent with the morphological specializations observed in mechanoreceptor cells at proposed sites of transduction [7,10].

Then began the long, arduous journey of finding an ion channel gene among those defective in these mutants. Walker *et al.* [1] have now reported that one of the defective genes, *nompC*, encodes a 1619 amino-acid protein with six transmembrane domains and homology to the TRP superfamily of ion channels (Figure 1). From the topology of other TRP channel proteins, the amino and carboxy termini of the NOMPC protein are predicted to be intracellular. NOMPC is a very long, unusual member of the TRP family, as the amino terminus contains 29 ankyrin

Figure 1

Dendrograms of the TRP (top) and DEG/ENaC (bottom) superfamilies of ion channel subunits, showing to the right schematic representations of the NOMPC and MEC-4 proteins. Yeast, nematode, insect, molluscan and mammalian members of these superfamilies are indicated in grey, green, red, yellow and blue text, respectively. The channels formed by these proteins are activated by mechanical, thermal or chemical stimuli, and serve various physiological roles. When protein names are indicated, accession numbers are in parentheses.



motifs, rather than the more usual two to four. Null mutants of *nompC* show an approximately 90% reduction in macrochaete bristle mechanoreceptor currents. Another mutant, *nompC*^{4}, contains a missense mutation in an extracellular loop between transmembrane domains 3 and 4, and has mechanoreceptor currents of wild-type amplitude but with faster decay and altered adaptation time constants. *In situ* hybridization revealed *nompC* expression selectively in ciliated mechanosensory organs, in particular in bristles and chordotonal organs. Further localization studies are now needed to show whether *nompC* is, as expected, expressed specifically in the mechanosensory neurons and to determine whether the NOMPC protein is localized in the mechanoreceptor dendrites.

This study [1] also identified a *nompC* homologue, *Ce-NOMPC*, in *C. elegans*. A reporter construct coding for a fusion protein consisting of Ce-NOMPC linked to the green fluorescent protein (GFP) was found to be expressed in two interneurons, but also in the sensory dendrites of three pairs of ciliated neurons, which might be mechanosensory because their endings are embedded in the cuticle in association with the extracellular matrix. The *Ce-NOMPC::GFP* construct was not, however, found to be expressed in the ciliated neurons which mediate the response of the animal to nose-touch, or in the non-ciliated mechanoreceptors that sense touch to the body. It appears, therefore, that *NOMPC* is expressed in subsets of ciliated mechanosensory neurons/organs in *C. elegans* and *Drosophila*, but not in many other mechanoreceptor neurons. This clearly suggests the existence of other mechanosensitive ion channels.

The significant reduction of mechanoreceptor current in *nompC* mutants, combined with expression of *NOMPC* in mechanosensory organs, strongly suggests that NOMPC is a mechanically gated ion channel of *Drosophila* sensilla. This inference is further supported by the fact that other TRP family members form non-selective cation

channels, and insect bristle mechanoreceptor currents are known to be carried by non-selective cation channels [4]. The residual receptor currents of about 10% of the wild-type amplitude which are present in *nompC* null mutants point to the existence of another mechanosensitive ion channel in *Drosophila* sensilla [1]. Other TRP family channels are known to form heteromultimers [11,12], and so it is possible that NOMPC and another channel subunit together generate the receptor currents. Nevertheless, it remains possible, although probably unlikely, that *nompC* is a necessary regulator of the mechanoreceptor channel rather than the channel itself. Direct mechanical activation of NOMPC in a heterologous expression system may be difficult to achieve, but would provide definitive proof.

NOMPC adds a new member to the list of known or suspected types of mechanosensory channel: the bacterial MscL [13] and MscS [14], the yeast Mid1 [15], the K^+ channel TREK-1 [16], and some DEG/ENaC proteins of animals [17]. Each one of these channels belongs to a different protein family, phylogenetically unrelated to the others, and most of these families are restricted to only animals, bacteria, or yeast. Most of these families include channels not thought to be mechanosensitive (Figure 1). For instance, the TRP superfamily contains the capsaicin receptor — physiologically activated by heat, acid pH or anandamide [18,19] — and several members that are activated by fatty acids or diaclgycerol [20,21]. Likewise, although some DEG/ENaC channels have been implicated in several forms of mechanosensation — most notably the nematode degenerins MEC-4 and MEC-10, needed for lateral touch sensation — other DEG/ENaC channels may be activated by acid pH or peptide ligands. So it appears clear that mechanosensitive channels evolved independently, several times, and perhaps from channels originally gated by non-mechanical stimuli, such as ligands, second messengers or heat.

All channel proteins are necessarily in contact with the lipids of the membrane; they are often bound to the cytoskeleton, and occasionally to the extracellular matrix. Mechanosensory channels may simply require attachments to structures that appropriately convey tension to them. Only a slight rearrangement could position these connections such that force would gate the channels [10]. Transduction models have been formulated for the mechanosensory structures for hearing and touch, in which a channel is attached at two or more sites to the cytoskeleton, the extracellular matrix, or both. Tension between these structures would be conveyed to the channel and force it to open. The 29 ankyrin repeats of NOMPC — the most any known protein contains — suit it well for attachments to the cytoskeleton. Likewise, the large extracellular loop of degenerin proteins is appropriate for binding to the matrix.

What mechanosensory channels mediate vertebrate hearing, balance, proprioception, and touch? PPK, a *Drosophila* DEG/ENaC protein, is expressed in free cutaneous endings, which are presumably mechanosensory [22]. Thus, both TRP and DEG/ENaC channels may participate in mechanosensation in both nematodes and insects (Figure 1). Might vertebrate members of these families constitute the mechanotransducing channels of hair cells or of touch-sensitive dorsal root ganglion neurons? Some DEG/ENaC channels of the BNaC/ASIC branch are expressed in mammalian dorsal root ganglia. BNaC1, in particular, is expressed by the subset of these neurons that are mechanosensory, and is located in their sensory terminals in the skin (our unpublished data). Although channels of this branch are opened by acid pH and there is a suggestion that some may sense painful tissue acidosis, they may also mediate the sense of touch in vertebrates.

Most DEG/ENaC channels pass Na^+ but not Ca^{2+} ions, and in this ionic selectivity, their small conductance and their mode of block by amiloride, they differ from the mechanotransducing channels of hair cells [17]. By contrast, the TRP channels have a high Ca^{2+} permeability and large conductance, like the hair cell transduction channel. Indeed, we and others have found several TRP channels expressed in the inner ear (unpublished data); one of these, VR-SAC, is opened by osmotic stress when expressed in cultured cells (S. Heller and A.J. Hudspeth, personal communication; see also [23]). A channel of this family may well be the long-sought transduction channel of the inner ear.

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