The Force Be With You: A Mechanoreceptor Channel in Proprioception and Touch

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The TRPN1 ion channel has a role in both hearing and bristle mechanosensation in fruit flies and in proprioception in nematodes. In this issue of *Neuron*, two papers present evidence that TRPN1 is also required for proprioception in fruit fly larvae and that it is a bona fide mechanoreceptor channel for nematode feeding behavior.

Animals have evolved a large number of mechanosensory organs responsible for (among others) hearing, balance, touch, osmosensation, proprioception, and somatosensation. Many of these senses are mediated by force-gated ion channels, which open in response to mechanical force directed to the channel protein. Yet it has been remarkably difficult to identify candidate mechanosensory channels and to show that candidates are in fact force-gated (Christensen and Corey, 2007). Two exceptions are the Msc channels of bacteria, which are well characterized but do not have homologs in animals (Kung, 2005), and the MEC-4/MEC-10 channel mediating gentle touch sensation in nematodes (O'Hagan et al., 2005). Other candidates either have not been confirmed or are indeed force-gated but not necessarily as part of their physiological function. Now two papers in this issue (Kang et al., 2010; Cheng et al., 2010) present evidence that the TRPN1 ion channel, also known as nompC, is a force-gated channel mediating mechanosensation in both nematodes and fruit fly larvae.

TRPN1 has had an uneven history over the past decade. The gene was originally isolated in a *Drosophila* screen for mutants that could not escape from their pupal cases and was named nompC (no mechanoreceptor potential) because the mutation virtually eliminates the electrical potential evoked by sensory bristle deflection (Kernan et al., 1994). Subsequently, nompC was found to encode a new member of the TRP ion channel superfamily, now called TRPN1 (Walker et al., 2000). The bristle receptor potential is so fast that the mechanoreceptor channel is probably force-gated (Walker et al., 2000), but it was unclear whether TRPN1 is itself the mechanoreceptor channel or is simply permissive for the function of another channel. No further studies had clarified the role of TRPN1 in *Drosophila* touch sensation.

Nevertheless, a force-gated-channel candidate, from an ion channel family known for a large, nonselective conductance, was just what the vertebrate auditory field was looking for. Working in zebrafish, Sidi et al. (2003) found that morpholino oligonucleotides that target TRPN1 expression inhibited the acoustic startle response, although this observation has not been confirmed by other methods. In *Xenopus*, antibody localization showed TRPN1 to be in hair-cell bundles, but it was associated with the single kinocilium and not the mechanosensitive stereocilia (Shin et al., 2005). Moreover, the TRPN1 gene is not present in reptiles, birds, or mammals, so it cannot be generally involved in vertebrate hearing.

Meanwhile, however, a role for TRPN1 was emerging in Johnston's organ in *Drosophila* (Figure 1). This chordotonal organ detects sound-evoked antennal movements and is the only auditory organ in the fruit fly. Eberl et al. (2000) showed that sound-evoked potentials in the antennal nerve were reduced by half in TRPN1 (nompC) mutants. Spontaneous and sound-evoked antennal movements are also reduced: whereas the antenna is normally more compliant at low sound intensity, the mutant antenna is not and has the same compliance at all intensities (Göpfert et al., 2006). By analogy with the "gating compliance" associated with channel opening in vertebrate hair cells (Howard and Hudspeth, 1988), it was proposed that TRPN1 participates in a force-gated channel in auditory receptor neurons (Göpfert et al., 2006). However, TRPN1 could not be localized to neurons without a specific antibody. The situation became more confusing when two TRPV-family members, Nanchung and Inactive, were also shown to be present in auditory neurons. Eliminating either TRPV completely abolished sound-evoked potentials in the antennal nerve, and in heterologous cells both were shown to be gated by osmotic stretch (Kim et al., 2003; Gong et al., 2004).

Cheng et al. (2010) have now taken a step toward clarification. First, they isolated a TRPN1 transcript longer than that originally reported, and this transcript successfully rescues the mutant phenotypes. A new antibody allowed them to localize the protein to the distal cilium of chordotonal neurons (Figure 1), as was also found by Lee et al. (2010). The Nanchung/Inactive complex, on the other hand, is restricted to the proximal cilium (Gong et al., 2004; Lee et al., 2010). This mismatch poses a puzzle: on the view that TRPN1 is the primary receptor channel in auditory neurons, Nanchung and Inactive have been proposed merely to amplify the force-gated current (Göpfert et al., 2006). However, Nanchung and Inactive are not substantially gated by voltage (Kim et al., 2003; Gong et al.,

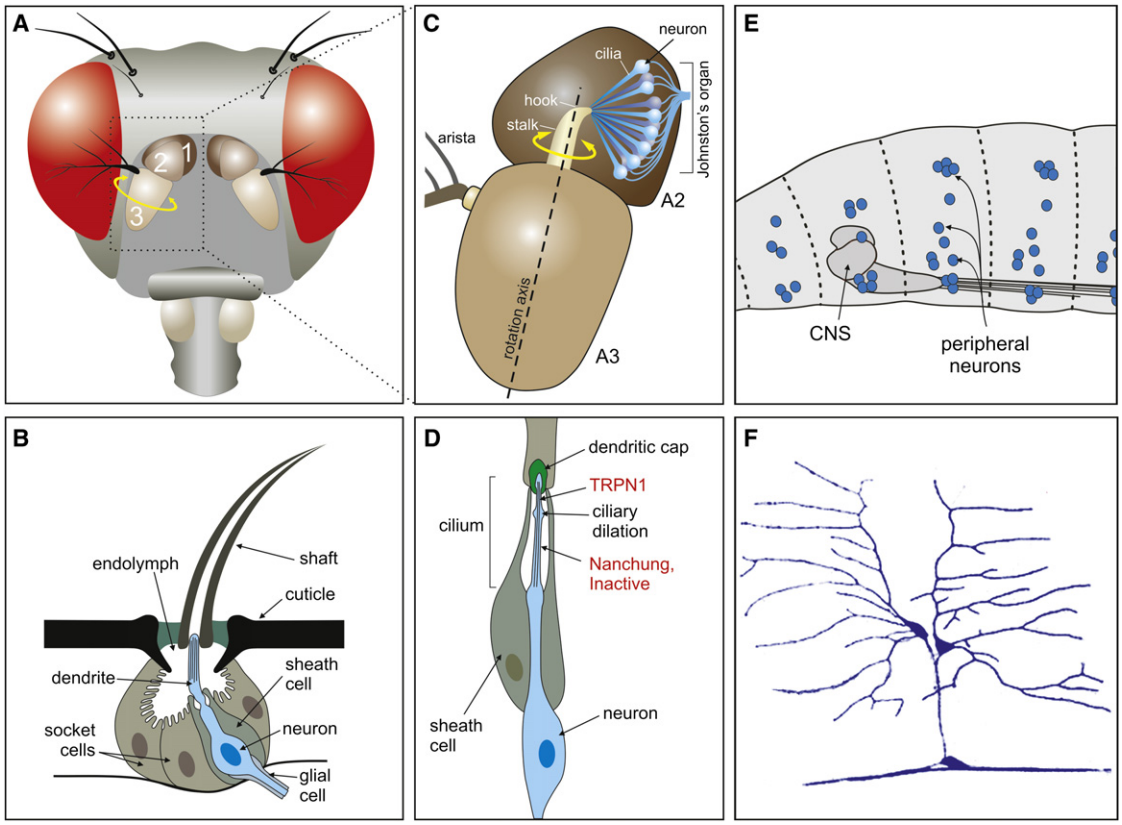

Figure 1. Sensory Organs in Adult and Larval *Drosophila*

(A) Auditory stimuli move the feathery arista of the antenna, causing rotation between the third and second antennal segments. The head also carries both large and small bristle mechanoreceptors.

(B) The sensory neuron of a bristle receptor extends a ciliated ending into the shaft.

(C) Rotation of the third antennal segment causes the hook region to stimulate sensory neurons of Johnston's organ.

(D) Neurons of Johnston's organ extend a cilium toward the dendritic cap near the hook. TRPN1 is located distal to a ciliary dilation, whereas Nanchung and Inactive are proximal.

(E) Sensory neurons of the peripheral nervous system in larvae are distributed in each segment.

(F) Among these are the nonciliated multidendritic neurons required for locomotion.

2004), and so it is unclear how they could rapidly sense and amplify the force-gated signal if they are not colocalized with that channel.

The study of behavior in TRPN1 mutants has been hindered by the fact that adult flies are extremely sick and uncoordinated. Cheng et al. instead focused on the larval stage. They show that although mutant larvae are healthy, the mutants crawl more slowly than normal, suggesting a defect in proprioception. They also report that TRPN1 is expressed in multidendritic mechanosensory neurons in the larval body wall and is required for the responses of these neurons to spontaneous body contractions, further evidence that this molecule can function in both ciliated mechanosensors (chordotonal and bristle neurons) and nonciliated mechanosensors (multidendritic neurons).

TRPN1 is also in nematodes, where it is called TRP-4. Using a GFP fusion protein, Walker et al. (2000) found that TRPN1 is expressed in the CEP, ADE, and PDE neurons, eight dopaminergic neurons that have ciliated sensory endings (reviewed in Goodman, 2006). These neurons (Figure 2) mediate the "basal slowing response," a decrease in locomotion speed upon encountering food that is thought to be mediated by mechanosensation of food particles (Goodman, 2006). The response is distinct from a related behavior, withdrawal upon nose touch, which is mediated instead by the ASH, OLQ, and FLP ciliated sensory neurons (Goodman, 2006). A third set of 36 ciliated mechanosensory neurons in males innervates the posterior sensory rays and is required for mating (Goodman, 2006).

Studies of these ciliated mechanosensory neurons have been somewhat eclipsed by a large body of work by Chalfie and colleagues on a fourth set of mechanosensory cells: the nonciliated neurons that sense touch to the lateral body wall (Goodman, 2006). There are 12 *mec* genes required for gentle touch sensation but not essential for touch-cell development. Of these, *mec-4* and *mec-10* are likely to encode pore-forming subunits of the mechanoreceptor ion channel: the receptor current activates on a millisecond timescale, suggesting a force-gated channel, and mutation of either subunit's putative selectivity filter changed the ionic selectivity of the receptor current (O'Hagan et al., 2005),

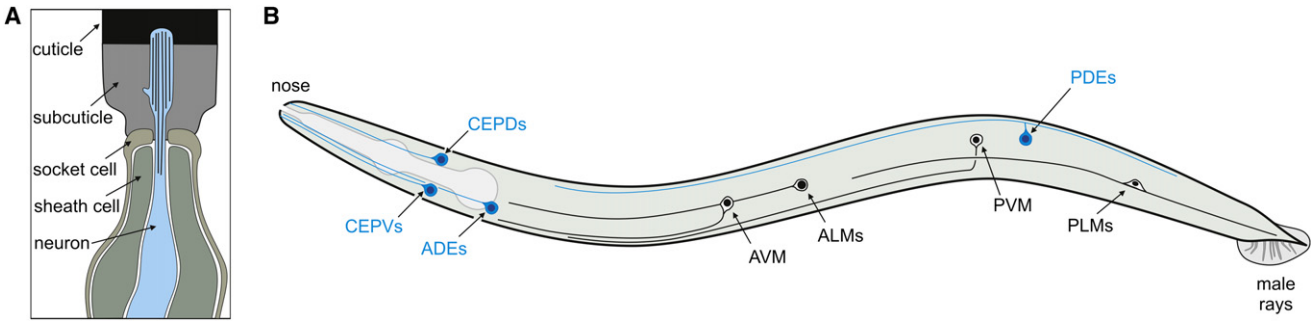

Figure 2. Mechanoreceptor Neurons in Nematodes

(A) Ultrastructure of the CEP neurons. Mechanical stimuli to the cuticle near the ciliated process produce mechanoreceptor currents recorded in the CEP neuron. (B) Location of the eight dopaminergic neurons (blue) and the six gentle touch neurons (black). Neurons are bilaterally symmetric as noted. Not shown are the nose-touch neurons and neurons innervating the male's rays.

passing a critical test for a channel candidate (Christensen and Corey, 2007).

Now Kang et al. (2010) have returned to TRPN1 and the basal slowing response. By removing a small piece of cuticle, they were able to patch-clamp the CEP neurons and record receptor currents while delivering mechanical stimuli to the ciliary ending. CEP neurons are sensitive (submicrometer) and fast (submillisecond), arguing for direct activation of a force-gated channel. Importantly, both the receptor current in CEP neurons and the basal slowing response were absent in TRPN1 mutants but were rescued by expression of TRPN1 in dopamine neurons. Is TRPN1 the channel that carries the mechanoreceptor current, or simply required for transduction by another channel? Like most TRP channels but unlike MEC channels, the CEP receptor conductance is not selective among alkali cations. Kang et al. (2010) mutated acidic residues of the predicted pore domain of TRPN1 and rescued with these mutant TRPN1 channels. Glycine or alanine substitution at just two of the seven acidic residues in this region changed the ionic selectivity of the receptor current without substantially reducing the current or the basal slowing response. This demonstration that TRPN1 carries the mechanoreceptor current and that the receptor current is very fast strongly supports the idea that TRPN1 is a force-gated sensory channel.

TRPN1's function in both ciliated and nonciliated mechanoreceptors indicates a broad role in invertebrate mechanosensation. Although the absence of TRPN from most vertebrate genomes is a disappointment to vertebrate auditory researchers, the power of fruit fly and nematode genetics will accelerate an understanding of eukaryotic force-gated channels, and may generate fundamental insights into the biophysical principles of this gating mechanism.

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