Molecules and Mechanisms of Mechanotransduction

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To many animals, including humans, some of the best things in life are mechanical. Not only courtship and sex but also simple movements such as walking depend on the ability to transform mechanical energy in the form of touch, sound, and muscle tension into ionic currents. This ability is also essential for control of osmotic balance, bladder function, and blood pressure in mammals. To meet these diverse needs, animals bear numerous sensory organs that contain either ciliated or nonciliated mechanoreceptor cells. Vertebrate hair cells and insect chordotonal neurons are examples of ciliated mechanoreceptor cells; mechanoreceptive neurons that innervate the body surface of nematodes, insects, and mammals are examples of nonciliated mechanoreceptor cells. All of these cells share the ability to signal the presence of mechanical stimuli by opening ion channels. Mechanical energy may activate mechanotransduction channels directly or indirectly. Only now are we beginning to uncover proteins that are likely constituents of mechanotransduction channels and the protein machinery that couples applied forces and channel activation.

The research presented by us at the 2004 Society for Neuroscience meeting is directed toward the discovery of the molecular events that give rise to the senses of touch and hearing (Lumpkin et al., 2004). Progress in this area has resulted from a convergence of genetics, genomics, and electrophysiology in vertebrates and invertebrates. Genetic studies reveal subunits of putative transduction channels. Leading candidates include ion channel subunits of the TRP (transient receptor potential) and the DEG/ENaC (degenerin/epithelial Na channels) superfamilies. TRP channels form cation channels, many of which are gated by ligand binding or temperature changes (Clapham et al., 2003). DEG/ENaCs form voltage-insensitive Na⁺ channels blocked by amiloride (Kellenberger and Schild, 2002). Many members of both families are present in the genomes of vertebrates and invertebrates (Goodman and Schwarz, 2003) and are expressed in a variety of sensory receptor cells.

To date, all proteins proposed to form transduction channels in ciliated mechanosensory cells are members of the TRP channel superfamily. In *Caenorhabditis elegans*, two members of the

TRPV (vanilloid TRP) superfamily, OSM-9 (osmotic avoidance abnormal) and OCR-2 (OSM-9 and capsaicin receptor-related), are needed for avoidance of nose touch and high osmolarity and are coexpressed in ciliated mechanosensory endings (Colbert et al., 1997; Tobin et al., 2002). NOMPC (no mechanoreceptor potential C), a TRPN (NOMPC-like) family member, mediates rapidly adapting receptor currents in bristle mechanoreceptor epithelia and is expressed in *C. elegans* mechanosensory neurons that detect variations in substrate texture (Sawin et al., 2000; Walker et al., 2000). Kim et al. (2003) discussed two additional channel subunits expressed in chordotonal organs, stretch sensors that serve as proprioceptors and as sensory elements in insect hearing organs. Sound-evoked potentials recorded from antennal chordotonal organs are reduced in *nompC* mutants but are completely absent from *nan* (*nanchung*) or *iav* (*inactive*) mutants. The NAN and IAV proteins are the only *Drosophila* members of the TRPV subfamily. When expressed in cell culture, each protein promotes cation currents and intracellular calcium spikes activated by hypo-osmotic saline (Kim et al., 2003). In flies, NAN and IAV are specifically expressed in chordotonal neurons and colocalized to their cilia; each subunit requires the other for stable expression. OSM-9 and OCR-2 in *C. elegans* exhibit a similar interdependence (Tobin et al., 2002). Interestingly, NAN and IAV are restricted to the proximal part of the cilium, constraining models for their activation by stretch of the chordotonal organ.

What about transduction channels in hair cells, the sensory receptors of vertebrate hearing and balance system? By searching the zebrafish genome database, Sidi et al. (2003) found an ortholog of *Drosophila* NOMPC, TRPN1, and showed that it was expressed in hair cells and needed for proper hearing and balance. Morpholino-mediated knock-down of *trpn1* abolishes both startle reflexes and microphonic potentials recorded from hair-cell sensory epithelia (Sidi et al., 2003). Thus, TRPN1 is a promising candidate for the transduction channel in zebrafish hair cells. Alternatively, TRPN1 may play an essential supporting role in sensory signaling. Determining the location of TRPN1 in hair cells will help resolve this uncertainty, as will recordings from single hair cells. TRPN1 is conserved among invertebrates, such as nematodes and insects, and at least two chordates; however, TRPN1 orthologs have not yet been detected in mammalian genomes. As genome sequencing continues, we will learn whether TRPN1 orthologs, or perhaps related channels, are viable candi-

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dates for mechanotransduction channels in mammalian, amphibian, and reptilian hair cells.

Establishing the correspondence between candidate mechanotransduction channels such as TRPN1 and native channels is challenging. This problem is compounded if some properties of mechanotransduction channels are imparted by accessory proteins. A biophysical and pharmacological characterization of native channels is critical for creating a framework for molecular identification of the channel. Anthony Ricci presented recent work directed toward this goal. In turtle cochlear hair cells, several aspects of the native mechanotransduction channel vary with tuning frequency. For example, channels activate and adapt faster in cells tuned to higher sound frequencies and have larger single-channel conductances (Ricci and Fettiplace, 1997; Fettiplace and Ricci, 2003; Ricci et al., 2003). Increases in single-channel conductance could imply increases in the diameter of the pore of the channel; however, recent estimates revealed a minimum pore diameter of ~ 13 Å that did not vary as a function of frequency (Farris et al., 2004).

Ricci and colleagues further probed the hair-cell transduction channel with antagonists of both TRP channels and cyclic nucleotide-gated channels. Results were mixed: antagonists to both channel subtypes were effective blockers of native transduction channels. Native transduction channels are also blocked by antagonists of acetylcholine receptors and DEG/ENaC channels (Farris et al., 2004). Interestingly, all compounds that are effective antagonists of native transduction channels bear charged amine groups and are large enough to block permeation through the ion pore. At least 10 chemically distinct compounds meet these requirements, underscoring the difficulty of identifying antagonists specific for hair-cell transduction channels. Additional experimental work is essential to determine whether channels formed by TRPN1 or other candidate hair-cell transduction channels share the pharmacological profile of the native channel.

In hair cells, transduction channels are thought to couple to accessory structures that transmit force to the gates of the channels (Howard and Hudspeth, 1988). The most striking of these is the extracellular tip link, which appears to be required for force-dependent channel gating (Pickles et al., 1984; Assad et al., 1991). Because mutations in *CDH23* (*cadherin 23*) cause deafness in humans, mice, and zebrafish (Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001; Sollner et al., 2004) and because of its exceptionally long extracellular domain (composed of 27 cadherin repeats), *CDH23* is an excellent candidate to form tip links (Nicolson, 2004). Consistent with this idea, mutant *CDH23/sputnik* zebrafish lack tip links altogether (Sollner et al., 2004). Additionally, *CDH23* immunoreactivity is concentrated at the tips of stereocilia in mice, frogs, and zebrafish (Siemens et al., 2004; Sollner et al., 2004). Whether *CDH23* interacts (either directly or indirectly) with TRPN1 or other candidate transduction channels remains to be determined.

In addition to their proposed role in ciliated mechanosensors, TRP channels contribute to sensory transduction in nonciliated sensory neurons innervating the body surface of several animals. For example, the capsaicin receptor TRPV1 is a heat- and proton-activated ion channel that is expressed in a subset of mammalian polymodal nociceptors and that mediates nociceptive responses *in vivo* (Caterina et al., 1997, 2000). In genetic screens for mutations that alter responses to noxious stimuli in *Drosophila* larvae, Daniel Tracey and his colleagues uncovered *Painless*, a TRPA (ankyrin-repeat TRP) subfamily member. *Painless* mutant larvae fail to avoid strong mechanical stimuli and heat. Although *Painless* is required for behavioral responses to robust prodding, re-

sponses to light touch are present in mutant larvae. Moreover, sensory nerves in wild-type larvae contain neurons that spike vigorously in response to temperatures $>38^{\circ}\text{C}$; such activity is absent in *painless* mutants (Tracey et al., 2003). The *painless* gene is expressed in sensory neurons that extend multiple branched dendrites beneath the epidermis, similar to vertebrate nociceptors. A single TRPA gene (*TRPA1/ANKTM1*) is present in mammalian genomes and is expressed in a subset of neurons that are likely to be nociceptive. In heterologous cells, TRPA1 channels have been shown to be activated by cold, irritants such as wasabi, and receptors that couple to phospholipase C (Story et al., 2003; Bandell et al., 2004; Jordt et al., 2004). A major question for the future is to understand how *Painless* and TRPA1 contribute to signaling in polymodal or nociceptive sensory neurons. Additional *Drosophila* mutants discussed by Tracey are beginning to shed light on this problem.

For putative mechanotransduction channels of the TRP family, the evidence linking application of mechanical energy to channel activation *in vivo* is incomplete. To establish this link for putative mechanotransduction channels of the DEG/ENaC family, Miriam Goodman and her collaborators, Robert O'Hagan, and Martin Chalfie from the Department of Biological Sciences at Columbia University (New York, NY) (work presented by R. O'Hagan) are deconstructing the protein machinery responsible for transduction in *C. elegans* touch receptor neurons by asking how mutations that abolish touch sensation modify native mechanotransduction currents. The first mutations analyzed eliminate or alter four membrane proteins proposed to form mechanotransduction channels. Two of these proteins, MEC-4 and MEC-10 (mechanotransducing channel subunits), are DEG/ENaC family members. When coexpressed in heterologous cells, they form voltage-insensitive, amiloride-sensitive Na^+ channels (Goodman et al., 2002). Two are accessory proteins, MEC-2 and MEC-6, which enhance macroscopic current amplitude in heterologous cells (Chelur et al., 2002; Goodman et al., 2002). Consistent with the idea that these proteins form mechanotransduction channels *in vivo*, null mutations in *mec-4*, *mec-2*, and *mec-6* abolish mechanotransduction currents without affecting other ionic currents. Additionally, a missense mutation in *mec-10* alters the ion selectivity of transduction currents and reduces their amplitude. In short, mutations in *mec-4*, *mec-10*, *mec-2*, and *mec-6* that abolish behavioral responses to light touch dramatically alter native transduction currents in touch-receptive neurons.

As in nematodes, the perception of gentle touch in mammals is initiated by specialized somatosensory receptors, such as Merkel cell–neurite complexes. Similarities between cutaneous Merkel cells and mechanosensitive hair cells have fueled the speculation that Merkel cells are sensory receptor cells. For example, Merkel cells express the transcription factors Math1 (mouse atonal homolog) and Gfi1 (growth factor-independent), which are required for proper hair-cell differentiation (Ben-Arie et al., 2000; Wallis et al., 2003). To explore the hypothesis that Merkel cells mediate mechanotransduction and activate sensory neurons via synaptic transmission, Ellen Lumpkin and her colleagues purified green fluorescent protein-labeled Merkel cells from transgenic mice (Lumpkin et al., 2003) and characterized these cells *in vitro* (Haeberle et al., 2004). Expression profiling revealed that Merkel cells express many neuronal molecules, including a number that are essential for synaptic vesicle release. Furthermore, live-cell imaging showed that Merkel cells are excitable cells that express voltage-gated Ca^{2+} channels. An important future direction is to determine whether touch, or perhaps another stimulus,

initiates neurotransmission between Merkel cells and sensory neurons.

With the molecular identification of candidate sensory mechanotransduction channels in a number of systems, we are poised to address several outstanding questions. How are mechanotransduction channels activated? If both TRPs and DEG/ENaCs function as mechanotransduction channels, are they activated by similar mechanisms? How are transduction channels optimized to detect different forms of mechanical energy? To what extent do accessory structures fine-tune the response characteristics of mechanoreceptive cell types? Answers are likely to come from the model systems discussed in this mini-symposium and from a multidisciplinary approach that combines classical genetics, molecular biology, and electrophysiology.

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