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New TRP Channels in Hearing and Mechanosensation

Minireview

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Despite extensive biophysical characterization and the superb example of the bacterial MscL channel, molecular identification of eukaryotic mechanosensitive channels has been slow. New members of the TRP superfamily have emerged as candidate channels to mediate touch, hearing, fluid flow, and osmosensation in sensory and nonsensory cells. Distinguishing between direct mechanical activation and indirect second messenger activation is still a challenge.

The idea of a mechanically gated ion channel is old: studies in the 1950s and 60s by Lowenstein on Pacinian corpuscles, by Ottoson and Shepherd on muscle spindles, and by Davis on cochlea all discussed the possibility that channels could be regulated by mechanical stimuli. Experiments in the 1980s by Corey and Hudspeth in hair cells and by Sachs in muscle showed that mechanical stimuli could directly activate ion channels by affecting transition rates between conformational states. Yet the molecular identification of these ion channels has been almost painfully slow, in part because relatively few channels are needed to produce an adequate receptor potential and channel abundance is consequently low.

The first cloned channel shown to be directly activated by membrane tension was the MscL channel of bacteria (Sukharev et al., 1994). While crystal structure and mutagenesis studies have provided an elegant model for gating, this channel has no homolog in eukaryotes. In the past 5 years, mutagenesis studies in flies and worms have revealed some new channels, and subsequent homology cloning has produced more that apparently play a role in mechanosensation. Many of these are in the DEG/ENaC and TRP ion channel superfamilies. Some have been localized to sensory endings, and others have been shown to be at least necessary for function, but in contrast to MscL, none have met all the desired criteria for bona fide mechanically gated channels.

Some of the most intriguing new candidates are members of the TRP superfamily, a group numbering about 30 channels in mammalian genomes (Figure 1). While serving a variety of functions in neurons, TRPs are conspicuously favored by sensory cells: they mediate invertebrate vision, vertebrate pheromone transduction, temperature sensation, osmosensation, and probably mechanosensation (Minke and Cook, 2002). Here I will focus on three new TRP channels involved in hearing and on a new role for old TRP channels in mechanosensation.

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OSM-9 and OCR Channels

The first TRP channel implicated in mechanosensation was the *C. elegans* OSM-9. Mutant alleles were identified by several groups as defective in osmotic avoidance and in sensitivity to nose touch. The *osm-9* gene was then found through positional cloning (Colbert et al., 1997) and was shown to encode a protein with six putative transmembrane domains. Phylogenetically, it falls in the TRPV branch of the TRP superfamily. (Within the TRPVs, invertebrate and vertebrate channels form two distinct groups, sufficiently dissimilar that the OSM-9-like invertebrate group can be considered to form its own branch.) OSM-9 is expressed in a variety of nematode neurons but is conspicuously present in most of the chemosensory and mechanosensory amphid neurons. Mutants are also defective in attraction to volatile odorants and in forms of olfactory adaptation (Colbert et al., 1997).

More recently, a closely related set of four genes, *ocr-1* to *ocr-4*, were identified in the *C. elegans* genome as homologs of *osm-9* (Tobin et al., 2002). These are expressed in many of the same neurons as *osm-9* but in a more limited set. For instance, *ocr-2* is expressed in the odor-sensing AWA and ADL neurons and in the multimodal nociceptive ASH neurons, and the protein colocalizes with OSM-9 in sensory cilia. *ocr-2* mutants have reduced osmotic sensitivity and nose-touch response. Similarly, *ocr-4* is expressed in the touch-sensing OLQ neurons. Together, these suggest that OSM-9 and one of several OCR proteins may form heteromultimeric channels, sensing different stimuli in different cells depending on the OCR subunit. More direct recording of physiological responses will be needed to test this idea.

TRPV4

If OSM-9 is a mechanosensitive channel sensing osmolarity and touch, there may be vertebrate homologs that sense osmolarity or other mechanical stimuli. This reasoning, along with screening of inner ear libraries and cloning based on expressed sequence tags, led four groups to the vertebrate TRPV4 channel (Strotmann et al., 2000, Liedtke et al., 2000, Wissenbach et al., 2000; Delany et al., 2001). TRPV4 is a homolog of both OSM-9 and the capsaicin receptor TRPV1.

Channels composed of TRPV4 alone are mechanosensitive, in that they are activated by osmotic stress when expressed in cultured cells. TRPV4 was found in a wide variety of tissues: some are transporting epithelia where osmotic regulation would be important, such as kidney distal tubules, airway epithelial linings, and serous cells of submucosal glands; others are known to have a mechanical sensitivity, such as heart and vascular epithelial cells. But still others, such as fat tissue and spleen, have little relation to mechanical sensing. TRPV4 is expressed by the mechanically sensitive hair cells of the inner ear (Liedtke et al., 2000) but is expressed much more by the stria vascularis of the ear, a transporting epithelium. Surprisingly, perhaps, TRPV4 is also activated by warm temperatures (25°C–30°C), and osmotic stimuli alter the response to warmth (Nilius et al., 2003).

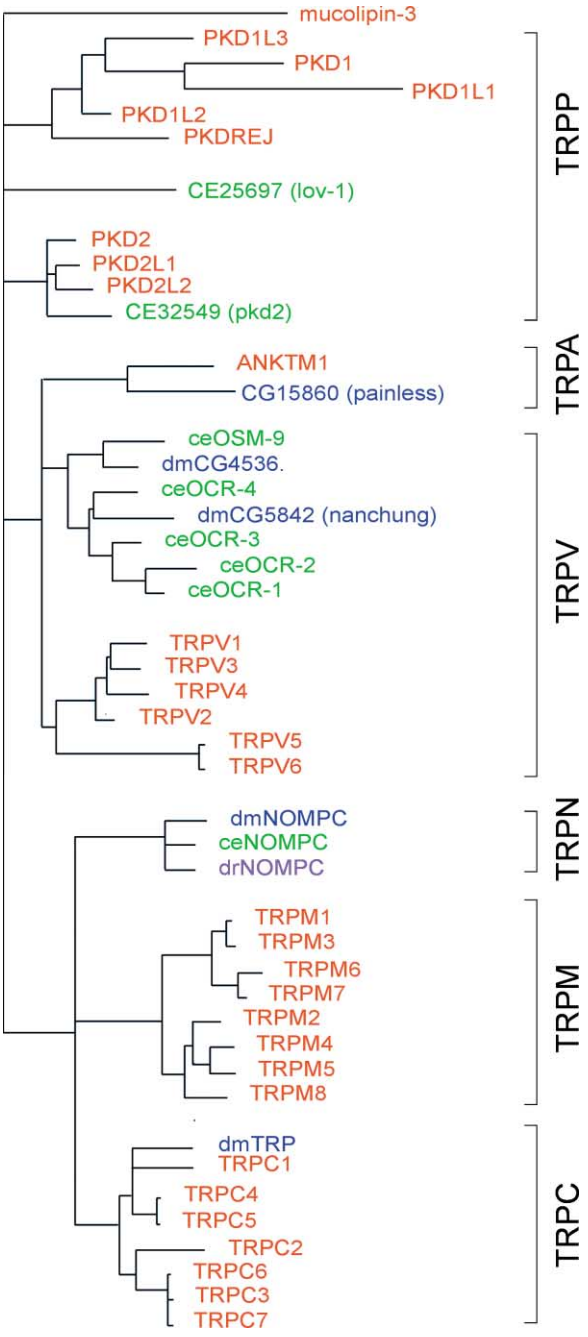

Figure 1. Phylogenetic Relationships among the TRP Channel Superfamily

Shown are all 30 of the mouse TRP channels and mucolipin-3 (red), with selected channels from *Drosophila* (blue), *C. elegans* (green), and zebrafish (violet) genomes. Subfamilies as currently named are shown to the right.

A new paper (Alessandri-Haber et al., 2003) confirms the osmosensitivity of TRPV4 and shows, moreover, that TRPV4 mediates sensitivity of nociceptive neurons of the dorsal root ganglia to hypoosmotic challenges. In cultured neurons, the activation by hypoosmotic stimuli is slow (tens of seconds; slower than the perfusion system), and hypoosmotic activation can interact with activation by a phorbol ester (4αPDD). Thus, TRPV4 may not be directly mechanosensitive; instead, a variety of stimuli may converge to activate it by a second messenger.

What is TRPV4's real purpose? It may be that some modes of activation are epiphenomena, not related to the normal function of the channel, or perhaps TRPV4, like Osm-9, will turn out to be a multifunctional subunit that combines with others to mediate a variety of sensitivities.

PKD1 and PKD2

Polycystic kidney disease is an autosomal-dominantly inherited disease causing progressive development of cysts in the kidney and liver. Nearly all cases result from mutations in either PKD1 or PKD2 genes, both of which encode proteins of the TRP superfamily (Arnaout, 2001). PKD1 and PKD2 and their close homologs are usually lumped together in the TRPP branch of the TRP superfamily, but the two groups have markedly different molecular architecture and represent distinct phylogenetic branches of the superfamily. The PKD1 group are very large proteins with 10 to 12 transmembrane domains and long N-terminal extensions and may or may not contribute to a channel pore, whereas the PKD2 group are smaller, have 6 transmembrane domains, and clearly conduct ions.

Recent papers have elucidated a likely role for PKD1 and PKD2 in sensing fluid flow in ciliated epithelial cells. Although widely expressed in various organs, PKD1 and PKD2 were found to be colocalized in the apical cilium of kidney tubule epithelia (Pazour et al., 2002; Yoder et al., 2002). Bending of the cilia in cultured kidney cells by fluid flow causes calcium influx (Praetorius and Spring, 2001). When expressed in CHO cells together, PKD1 and PKD2 form a Ca^{2+}-permeable ion channel (Hanaoka et al., 2000). Most recently, Nauli et al. (2003) found that the response to bending is abolished in cells lacking cilia, in cells lacking PKD1, or in cells treated with a blocking antibody to PKD2.

These experiments suggest that PKD1 and PKD2 form an ion channel that is activated by bending of the apical cilium in certain epithelia and that senses fluid flow parallel to the epithelial surface. There are intriguing structural parallels, and now perhaps molecular parallels, both to ciliated mechanoreceptors in flies and nematodes and to hair bundles on receptor cells in cephalopod and vertebrate inner ears.

Nematode LOV-1 and PKD-2

The TRPP family also includes two members in the *C. elegans* genome. A homolog of PKD1, LOV-1 (for location of vulva), was identified by a genetic screen for mating mutants, while the *C. elegans* PKD-2 was identified as a homolog of mammalian PKD2 (Barr and Sternberg, 1999). Expression of GFP-tagged proteins showed that both LOV-1 and PKD-2 are located in ciliated mechanosensory neurons of male copulatory organs and in other ciliated mechanoreceptors. Antibodies to both LOV-1 and PKD-2 labeled the male-specific sensory neurons, with label appearing in cell bodies as well as sensory cilia (Barr et al., 2001; Kaletta et al., 2003).

Comparisons of single and double mutants showed that LOV-1 and PKD-2 act in the same pathway in nematode mating. A plausible function in mating is mechanosensory recognition of the hermaphrodite and location

of the vulva. However, a mechanosensitivity role can only be inferred from the behavioral defects at present.

Drosophila *NOMPC*

A classical mutant screen, this time in *Drosophila*, produced another TRP channel needed for mechanosensation. Kernan, Cowan, and Zuker (Kernan et al., 1994) screened fly larvae for defects in withdrawal from a touch stimulus and for uncoordination, eventually identifying 27 mutants in 20 complementation groups. Many of these mutations affected the function of mechanosensory bristles. A clever technique for extracellular recording from individual bristles confirmed defects in mechanotransduction in many mutants. Subsequent positional cloning showed that one gene, nompC (for no mechanoreceptor potential) encodes an unusual TRP channel with 29 ankyrin repeats preceding the transmembrane domains (Walker et al., 2000). This channel is sufficiently different that it defines a new branch of the TRP superfamily: the TRPNs. Three of the four nompC alleles contained stop mutations in the N terminus, which would most likely block channel function, and they had only small, nonadapting mechanoreceptor potentials. The fourth allele is a simple missense mutation. Its phenotype—a speeding of adaptation without loss of the initial response—is relatively subtle and is consistent with NOMPC being the mechanosensory channel itself rather than providing general support for the sensory neuron.

The discovery of nompC was particularly influential in focusing the attention of the mechanosensation field on the TRP superfamily. Thus it will be important to confirm that NOMPC does indeed form a mechanically activated channel that mediates the sensory neurons' response to bristle deflection. For instance, the nompC gene is expressed within the bristle complex, in or near the sensory neurons, but it has not been definitively shown to be made by the sensory neuron. Nor has the NOMPC protein been localized in the neuron's cilium, the probable site of mechanotransduction. Similarly, we could have more confidence that NOMPC forms the channel itself, rather than an activating intermediate, if mutation of the putative pore domain could be shown to affect the ionic selectivity of the receptor current.

Nanchung

The discovery of NOMPC in *Drosophila* bristle organs and its role in touch sensation raised the possibility that this channel serves as a general mechanosensor for a variety of senses in *Drosophila*. nompC mutants do in fact show a deficit in auditory response. Hearing in flies is mediated by Johnston's organ, a group of several hundred ciliated neurons that send processes to the joint between the second and third antennal segments. Johnston's organ is one of a variety of chordotonal organs that sense flexion of segmental joints, distinct from the external bristle receptors but like them in possessing a ciliated mechanosensitive ending. Recordings from the auditory nerve in nompC mutants showed a reduction in the amplitude of the compound action potential, indicating that the NOMPC channel plays a role in audition as well (Eberl et al., 2000). On the other hand, the deficit in nompC mutants is rather mild: action potential amplitude was about 50% of normal, compared to less than 10% for a handful of other nomp mutants. Another channel (or channels) must constitute the mechanosensor in Johnston's organ of *Drosophila*.

A new paper (Kim et al., 2003) reports that at least one component is a member of the OSM-9 branch of the TRPV family. The *Drosophila* genome harbors two members of this group: CG4536 and CG5842. CG5842 is most like OCR-4, the channel needed for anterior touch sensation in nematodes. Kim et al. first asked whether the CG5842 channel—named Nanchung or Nan—is osmosensitive. Hypoosmotic solutions elicited a rise in intracellular Ca^{2+} in CHO cells expressing Nan, as well as transient increases in a nonselective conductance. The delayed activation of the current (up to several minutes), the irregular and transient character of the currents, and the unconventional experimental conditions leave some doubt about the osmosensitivity of Nan. However, Kim et al. then used both in situ hybridization and a reporter gene linked to the Nan promoter to show that Nan is expressed almost exclusively in chordotonal neurons, both in the larvae and adults. An antibody to Nan beautifully labeled the ciliated endings of chordotonal neurons in Johnston's organ.

What is Nan's function? Two deletion mutants showed a sedentary and uncoordinated phenotype, consistent with a role in joint chordotonal neurons. They also are completely deaf, as assessed by auditory nerve recordings. Finally, the phenotype was rescued by a cDNA transgene encoding Nan. Together, these experiments nicely demonstrate both specific localization and specific function that are consistent with Nan acting as at least one component of the auditory transduction channel in *Drosophila*. At the same time, an extension to vertebrate hearing or mechanosensation is unlikely because OSM-9-like TRP channels are not expressed in vertebrates.

A Vertebrate TRPN

The *Drosophila* NOMPC has been a paradigm for mechanosensory channels since it was cloned in 2000, but again, the common wisdom was that no direct ortholog existed in vertebrates. Indeed, a variety of screens for homologs in vertebrate inner ear libraries yielded nothing. Thus it was a surprise when Nicolson and colleagues recently reported an ortholog in the zebrafish genome (Sidi et al., 2003). Using the fly NOMPC sequence to search the zebrafish genome, followed by long-range PCR with primers from genomic sequence, Sidi et al. isolated a cDNA that clearly encodes a vertebrate NOMPC. Like fly and nematode nompC, it has 29 ankyrin repeats in a long N-terminal domain, followed by six or seven transmembrane domains with similarity to other TRPs. In situ hybridization showed nompC in all the sensory epithelia of the larval zebrafish inner ear, apparently in the hair cells themselves. Zebrafish also have hair cells in their lateral line organs, which detect water currents, but no in situ signal was detectable there.

Gene function can be disrupted in zebrafish by the injection of morpholino oligonucleotides into embryos. Although morpholinos are often used to target the translation start site of a message to prevent translation, Nicolson's group instead targeted a splice site to allow a PCR assay of message disruption. Injected fish failed several tests of inner ear and lateral line function. First, they failed a dye accumulation assay. FM1-43 is a fluorescent dye normally used to label endocytic vesicles

and to follow their subsequent exocytosis. However FM1-43 can also pass directly into vertebrate hair cells through their transduction channels and thus permanently label the cells, providing a simple test of functional transduction (Meyers et al., 2003). In Nicolson's experiments, normal fish have brightly fluorescent lateral line hair cells after brief exposures to the dye, but the morpholino-injected larvae lacked this labeling. Second, the receptor current through hair cell transduction channels can be recorded as an extracellular microphonic potential when the mechanosensitive hair bundles of lateral line hair cells are mechanically deflected. Injected fish lacked microphonic potentials. Finally, morpholino-injected fish failed to show a startle reflex evoked by a tap to their dish, which presumably tests auditory function, and they often swam in a circular path or with a tilt, evidence of vestibular dysfunction.

Could the morpholinos have produced nonspecific affects? Sidi et al. found that the hair cells' morphology and number were unaffected. Moreover, as the fish grew and the morpholino effect was diluted over several days, the phenotype returned to normal, consistent with disruption of hair cell function but not of development.

These are all strong evidence that nompC is necessary for hair cell transduction in zebrafish. Yet questions remain: is nompC the transduction channel itself, or does it play a supporting role? Antibody localization and single-cell recording will help answer this. And a final mystery: while nompC is clearly in zebrafish (and in the primitive chordate *Ciona intestinalis*), there is as yet no trace of an ortholog in any other vertebrate genome, not even in other fish. If this gene has indeed been lost in higher vertebrates, what might be the transduction channel in mammalian hair cells?

Mucolipin-3

Positional cloning of deafness genes in mice has revealed a large number of proteins—some of them ion channels—that are expressed in the cochlea and are essential for hearing. For the most part, these various channels are expressed in supporting cells and not the mechanosensory hair cells. One such mouse mutant, varitint waddler, is deaf and shows circling behavior indicative of vestibular dysfunction. Its cochlear hair bundles develop improperly and degenerate further in the first few weeks after birth. When identified by positional cloning, the defective gene was found to encode mucolipin-3, a channel related to the TRP superfamily (Di Palma et al., 2002). Mucolipin-3 is expressed by hair cells, and the protein was found in the hair bundle and throughout the cell body—in the right cells but perhaps more broadly localized than expected for a transduction channel. While both alleles of varitint waddler have missense mutations in or near critical transmembrane domains of the channel, their physiological consequence is unknown. Despite these uncertainties, mucolipin-3 is an intriguing candidate.

Summary

Research in the past 5 years has produced many new candidates for mechanically gated ion channels. A surprising number of these, in both vertebrates and invertebrates, are part of the TRP ion channel superfamily. They are needed for mechanosensory cells involved in touch and hearing, and they also mediate nonsensory mechanical responses such as osmosensation. In many cases, though, additional work is needed to identify a specific role and to test whether the channels are themselves mechanically activated or are indirectly activated by a different mechanotransducer. Some exciting recent papers have raised the standards of proof, and largely met them.

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