In touch - the molecular basis of mechanosensory transduction

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Abstract

Nearly all species employ mechanosensitive channels to detect mechanical cues, such as touch and sound waves, and convert these mechanical forces into electrochemical signals. Genetic, biochemical and electrophysiological studies of touch-insensitive mutants in model organisms such as Caenorhabditis elegans and Drosophila melanogaster provide insights into the molecular basis of mechanosensory transduction.

Introduction

Neurons transduce mechanical stimuli into electrical impulses through mechanosensitive channels within their plasma membranes. Mechanical stimuli directly activate these proteins, inducing a conformational change in the protein, which allows ions to move along their electrochemical gradients across the cellular membrane. The ability to encode these mechanical stimuli is necessary for both survival and reproduction. For example, in the nematode C. elegans and fruit fly Drosophila melanogaster, loss of function mutations in mechanosensitive channels result in touch and proprioception deficits, while, in mammals, disruption of mechanosensitive channels results in chronic pain and hyperalgesia. Candidates for these channels exist in several protein families, including epithelial Na+ channels (ENaC), transient receptor potential (TRP) channels, two-pore region K+ channels, and the newly identified Piezos.

Whether these candidate proteins work as the primary mechanoreceptor activated directly by a mechanical stimulus or, indirectly, as a target of a second messenger system in response to a mechanical stimulus is a central question in unmasking these elusive mechanoreceptors. It is, however, difficult to answer this question in mammals because mammalian mechanosensory cells are sparsely abundant and have low expression levels of mechanosensitive channel proteins. In addition, the mechanotransduction apparatus is typically formed as a protein complex that is difficult to reconstitute functionally in heterologous systems. Finally, in mammals, the difficulty in making whole-cell electrophysiological recordings in vivo also hinders the resolution of this question. However, research utilizing Escherichia coli as well as genetic, biochemical and electrophysiological studies in Drosophila and C. elegans, provide insight into the molecular basis of mechanosensory transduction, making these model organisms ideal for further investigation of this question.
Bacterial mechanosensitive channels sense osmolarity via direct lipid-protein interaction

The best understood mechanosensitive channels are the bacterial Msc proteins which allow us to illuminate the molecular basis of mechanosensory transduction. In *E. coli*, Msc proteins work redundantly as osmosensors for turgor control. In a hypotonic environment, these channels allow for the movement of small, soluble components, such as ions, small sugars or nucleotides across the plasma membrane, which enables the bacterium to regulate its intracellular osmotic pressure and avoid osmotic lysis. *E. coli* with loss of function mutations in both the large and small conductance Msc rapidly lyse under these conditions. Molecular, crystallographic and electrophysiological analyses suggest a ‘tension-gating’ model in which increases in membrane tension directly open these channels through a lipid-protein interaction.

DEG/ENaC channels regulate mechanosensory transduction in metazoans

Most animals can detect at least two distinct types of touch stimuli, innocuous, or gentle, touch and noxious, or harsh, touch. Chalfie and colleagues, using forward genetics to screen for mutants insensitive to gentle touch, identified two ENaC Na\(^+\) channel proteins, MEC-4 and MEC-10. Both MEC-4 and MEC-10 possess two transmembrane domains and intracellular N- and C- termini (figure 1a). They form a heteromeric amiloride-sensitive channel in six touch receptor neurons (ALML/R, PLML/R, AVM and PVM) capable of detecting gentle touch in the force range of 1-10 μN. These channel subunits are associated with a set of accessory subunits such as paraxonase-domain protein MEC-6 and stomatin-like protein MEC-2. In this mechanotransduction apparatus, MEC-4/MEC-10 form the channel pore, and MEC-6/MEC-2 tether the channel to the extracellular matrix and intracellular cytoskeleton. This tether monitors the force applied to the membrane, which, as the force on the plasma membrane increases, induces a conformational change that opens the channel to depolarize these neurons. Genetic and electrophysiological assays make the MEC-4/MEC-10 complex the first identified metazoan channel directly gated by force.

As is the case with mammals, *C. elegans* exhibit differential behavioral responses to gentle and harsh touch. We have shown that these animals recruit a set of specialized mechanosensory neurons distinct from the gentle touch receptor neurons to detect harsh touch, with a force of >100 μN. Interestingly, ENaC channels, as well as TRP channels, participate in harsh touch sensation in *C. elegans*. A role for these channels in touch sensation may be conserved. For example, knockdown or knockout of mammalian ENaC family acid-sensing ion channels show reduced mechanosensitivity in skin sensory neurons. Currently, however, evidence to support the existence of force-gated mammalian ENaC channels is still lacking.

TRP proteins are leading candidates for mechanosensitive channels

In 1969, Cosens and Manning identified a loss-of-function mutation in *Drosophila* that results in photoreception deficits. Twenty years later, Montell and Rubin cloned this gene, the first TRP channel gene. Since that time, researchers have identified a vast number of...
TRP channels: 17 in *C. elegans*, 13 in *Drosophila*, 28 in mice and 27 in human. These genes encode seven TRP subfamilies: TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPN (NompC), TRPA (Ankyrin), TRPP (Polycystin) and TRPML (MucoLipin)\(^{11,12}\). All known TRP proteins contain intracellular N- and C- termini and six transmembrane domains (figure 1b). Four TRP subunits form a homomeric or heteromeric channel, which is usually non-selectively permeable to cations, although Ca\(^{2+}\)-selective TRP channels do exist\(^{11,12}\).

TRP channels are widely involved in sensory transduction, including thermo-, chemo- and mechanosensation. Members from nearly every TRP subfamily are implicated in mechanosensation\(^{13}\). However, whether any TRP channel serves directly as a force-gated mechanoreceptor or secondary modulator of a mechanoreceptor remained a mystery\(^{13}\). The TRPN subfamily of proteins, such as TRP-4 and NompC, are leading mechanoreceptor candidates. These proteins consistently play a role in mechanosensation. For example, *C. elegans* can sense a bacterial food source via mechanosensation. When the nose tip touches the bacterial lawn, the worm slows movement to stay in a food-rich region\(^{14}\). The four mechanosensory CEP neurons, along with the ADE and PDE neurons, mediate this basal slowing response\(^{14}\). The *C. elegans* TRPN homolog TRP-4 is expressed in these dopaminergic CEP neurons\(^{3}\). We have characterized the function of this TRPN channel in mechanosensation in *C. elegans* and found that *trp-4* mutant worms do not show the basal slowing response\(^{3}\). Interestingly, TRP-4 also controls proprioception (body posture) in *C. elegans* via a single proprioceptor neuron DVA\(^{3}\). Unlike touch and hearing, proprioception is a type of mechanosensation that senses forces arising within the animal body and controls body posture/movement and balance. In *Drosophila*, NompC, a TRPN homologue identified by Zuker and colleagues, regulates touch, hearing and proprioception\(^{4,15,16}\). However, these studies did not provide direct evidence that TRPN is a force-gated channel.

Using *in vivo* electrophysiology combined with molecular genetic manipulation, we have recently demonstrated that TRP channels can form force-gated mechanosensitive channels *in vivo*\(^{17}\). We used patch-clamp recording to directly monitor the electrical response of CEP neuron to touch\(^{17}\). Touch evokes rapid mechanoreceptor currents with latency in the range of microseconds, thereby excluding the involvement of a second messenger cascade\(^{17}\). This suggests that the underlying channel is mechanically activated. Importantly, point mutations in the predicted pore region of TRP-4 alter the ion selectivity of these receptor currents, which demonstrates that TRP-4 is a pore-forming subunit of a native force-gated channel in CEP neuron\(^{17}\).

How does TRP-4 transduce force into an electrical signal? TRP-4 has an unusually large N-terminus with 29 ankyrin repeats, which may anchor TRP-4 to the cytoskeleton (figure 1b). Force may induce a conformational change in the channel through ankyrin repeats to pull open the channel. Little, however, is known as to how TRP-4 might be anchored to extracellular matrix. Nevertheless, TRPN homologues are absent in mammals, which necessitates future work to determine whether any mammalian TRP family proteins are force-gated, mechanosensitive channels.
Conclusion and perspective

Mechanosensory transduction is evolutionarily ubiquitous and essential for both survival and reproduction. Though diverse candidates exist, to date, electrophysiological and genetic studies have identified only two distinct metazoan channels that can act as force-gated mechanosensitive channels in C. elegans – the ENaC family MEC-4/MEC-10 complex and TRP family channel TRP-4. However, we have yet to identify any mammalian ENaC or TRP proteins that act as force-gated channels, owing largely to technical obstacles. Mammalian two-pore-domain K\(_{2p}\) channel family proteins TREK1 and TRAAK are also mechanically gated in vitro, but in vivo evidence is lacking\(^{18}\). These K\(_{2p}\) channels, however, are unlikely to be the primary mechanosensitive channels in excitable cells, since they only open to hyperpolarize and inhibit the cells. Interestingly, Patapoutian and colleagues have recently identified a novel class of channel-like membrane proteins, Piezo-1 and Piezo-2, with a role in mammalian mechanosensation\(^6\). It will be interesting to identify the exact role of these appealing candidates in mechanosensation.

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Figure 1. Schematics of ENaC and TRP channels

a. schematic showing of the DEG/ENaC family channel subunit MEC-4, one of the core subunits forming the MEC-4/MEC-10 channel complex. Cysteine-rich domains are marked. TM: transmembrane domain.
b. The predicted topology of the TRP family channel subunit TRP-4/TRPN1. 29 ankyrin repeats shown in the N-terminus may anchor TRP-4 to the cytoskeleton.