ANNUAL REVIEWS

Annual Review of Neuroscience

Mechanosensitive Ion Channels: Structural Features Relevant to Mechanotransduction Mechanisms

Peng Jin,[1] Lily Yeh Jan,[1,2] and Yuh-Nung Jan[1,2]

[1]Department of Physiology, University of California, San Francisco, California 94158, USA; email: yuhnung.jan@ucsf.edu

[2]Department of Biochemistry and Biophysics and Howard Hughes Medical Institute, University of California, San Francisco, California 94158, USA

Annu. Rev. Neurosci. 2020. 43:207–29

First published as a Review in Advance on February 21, 2020

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

https://doi.org/10.1146/annurev-neuro-070918-050509

Keywords

mechanosensitive channel, ENaC/DEG, K2P, PIEZO, NompC, TMEM16 superfamily

Abstract

Activation of mechanosensitive ion channels underlies a variety of fundamental physiological processes that require sensation of mechanical force. Different mechanosensitive channels adapt distinctive structures and mechanotransduction mechanisms to fit their biological roles. How mechanosensitive channels work, especially in animals, has been extensively studied in the past decade. Here we review key findings in the functional and structural characterizations of these channels and highlight the structural features relevant to the mechanotransduction mechanism of each specific channel.

Contents

INTRODUCTION

Physiological Roles of Mechanosensitive Channels

Living organisms depend on their ability to receive and respond to chemical and physical signals from the environment. Mechanosensation is required to feel skin contact, gravity, proprioception, sound waves, food texture, muscle stretch, and air flow. At the cellular level, volume regulation, migration, and differentiation all require mechanosensation. A variety of proteins, including cytoskeletons, focal adhesion–associated molecules, G protein–coupled receptors, and ion channels, mediate the sensation of and response to mechanical forces (Chalfie 2009, Martino et al. 2018, Scholz et al. 2016, Xu et al. 2018). Among those proteins, mechanosensitive (MS) ion channels are directly activated by stresses applied to the lipid bilayer or its associated nonmembrane components. MS channels convert physical stimuli to electrical signals, allowing sensory cells to respond within a millisecond. Studying MS channels is important for understanding this type of signal transduction.

Criteria to Define a Mechanosensitive Channel

What is a MS channel? Árnadóttir & Chalfie (2010) proposed several criteria: First, MS channels must be expressed in a mechanosensory organ. Second, removal of MS channels from the sensory

cell eliminates mechanical response as a direct rather than indirect consequence (such as from developmental defects). Third, alteration of the physical properties of these channels through mutagenesis should be reflected by alteration of mechanical response. And fourth, the heterologously expressed channel could be activated by force. As new evidence has been collected to support the designation of MS channels, and as new MS channels have been identified, we go through the criteria for each MS channel discussed in this review.

The Force Transduction and Gating Mechanisms

One central question concerns how mechanical stimuli are transduced from the environment to switch the MS channel from a closed to an open conformation. Two force transduction and gating models have been proposed: For the membrane tension model, force applied to the lipid bilayer generates membrane tension to gate the channel (**Supplemental Figure 1*a***). For the tether model, force applied to the cell or extracellular matrix is transmitted through a tether connecting the channel with ectomembrane components to gate the channel (**Supplemental Figure 1*b***). The major difference between the two mechanisms is not whether the membrane is involved but whether additional components are required for force transduction.

Supplemental Material >

To understand the force transduction and gating mechanisms of MS channels, it is important to know the physical property of the channel and the channel conformations at different states. The ion channel property is usually characterized by measuring channel activity using patch-clamp electrophysiology. Various configurations are used to deliver mechanical stimuli while recording MS channels (**Supplemental Figure 1*c***). Structural information on MS channels, on the other hand, historically relied on X-ray crystallography. Recent developments in single-particle cryogenic electron microscopy (cryo-EM) technology have enabled structural studies of many more membrane proteins for which crystallization is often challenging and sometimes even unachievable (Cheng 2015). This technique allows the visualization of a 3-D protein structure at near-atomic resolution from thousands of 2-D images of randomly oriented protein particles frozen in vitrified ice (Cheng et al. 2015). By this means, the structures of membrane proteins could even be solved in a membrane-mimetic environment (Autzen et al. 2019). Ever since publication of the first near-atomic-resolution cryo-EM structure of a membrane protein (Cao et al. 2013, Liao et al. 2013), the convenience of obtaining high-resolution channel structures has substantially accelerated the pace of structure-based analysis of ion channels.

The bacterial mechanosensitive channel of large conductance (MscL) and mechanosensitive channel of small conductance (MscS), which meet all the criteria for MS channels (Árnadóttir & Chalfie 2010), are the best-characterized MS channels. Structures, electrophysiological data, and indirect measurements of conformational changes demonstrate that both MscL and MscS follow the tension-driven gating mechanism, which has been recently reviewed in detail (Cox et al. 2018). The MscS-like channels from other cell wall–bearing species like plants and algae probably utilize a similar gating machinery (Hamilton et al. 2014, Wilson et al. 2013). Here we focus on structure-function studies of metazoan MS channels.

DEGENERINS/ACID-SENSITIVE CHANNELS

Evidence for Epithelial Sodium Channel–Family Proteins as Mechanosensitive Channels

The epithelial sodium channel/degenerin (ENaC/DEG)-superfamily proteins are voltage-insensitive, sodium-selective channels, including vertebrate ENaC and acid-sensitive channels (ASICs), nematode DEGs, *Drosophila* pickpocket (PPK) and ripped pocket (RPK), and peptide-gated *Hydra* sodium channels (HyNaCs). The concept that ENaC superfamily members could be

Side view: view of the channel parallel to the membrane plane with the extracellular domain on top

MS channels originated from the finding that two *Caenorhabditis elegans* DEG proteins, MEC-10 and MEC-4, are required for touch sensation (Chalfie & Au 1989, Geffeney et al. 2011, Huang & Chalfie 1994, O'Hagan et al. 2005). Point mutations found in *mec-4* and *mec-10* missense alleles alter channel properties such as ion selectivity and kinetics based on electrophysiological recordings from touch neurons in vivo (Árnadóttir et al. 2011, O'Hagan et al. 2005). MEC-4 could function as a homotrimer, or as a heterotrimer with MEC-10 (Chen et al. 2015, Goodman et al. 2002). Likewise, ENaC proteins from other species also function as either homo- or heterotrimers (Hanukoglu & Hanukoglu 2016). Mechanoactivated (MA) current in response to laminar shear stress was detected in *Xenopus* oocytes with heterologous coexpression of MEC-4 and MEC-10 (Shi et al. 2016). However, the response occurs with a latency of several seconds, much slower than the in vivo MA current, probably reflecting the absence of other components essential for the proper function of this channel. Nevertheless, accumulated evidence supports the notion that the *C. elegans* DEG channel composed of MEC-4 and MEC-10 subunits is a MS channel.

A number of other ENaC proteins, especially ASICs, have been implicated in mechanosensation. Mouse ASIC orthologs are expressed in different subtypes of mechanoreceptors in both the central and peripheral nervous systems; but in the absence of substantiated mechanosensory phenotypes of ASIC-knockout mice (Cheng et al. 2018), it is still uncertain whether ASICs could be direct mechanotransducers.

Structures of Acid-Sensitive and Epithelial Sodium Channels

Crystal and cryo-EM structures of truncated but functional chicken ASIC1 (cASIC1) encompass different ASIC1 conformations at low-pH desensitized, low- and neutral-pH open, and high-pH resting states (Baconguis & Gouaux 2012, Baconguis et al. 2014, Jasti et al. 2007, Yoder & Gouaux 2018, Yoder et al. 2018). The architecture of each cASIC1 subunit resembles a side view of a hand holding a ball (features annotated in **Figure 1*a*,*b***), and the trimer has a chalice-like shape, also from a side view (Jasti et al. 2007). The ion-permeable pathway of cASIC1 spans the entire central axis. Ions could enter a vestibule from the very top of the extracellular region or from

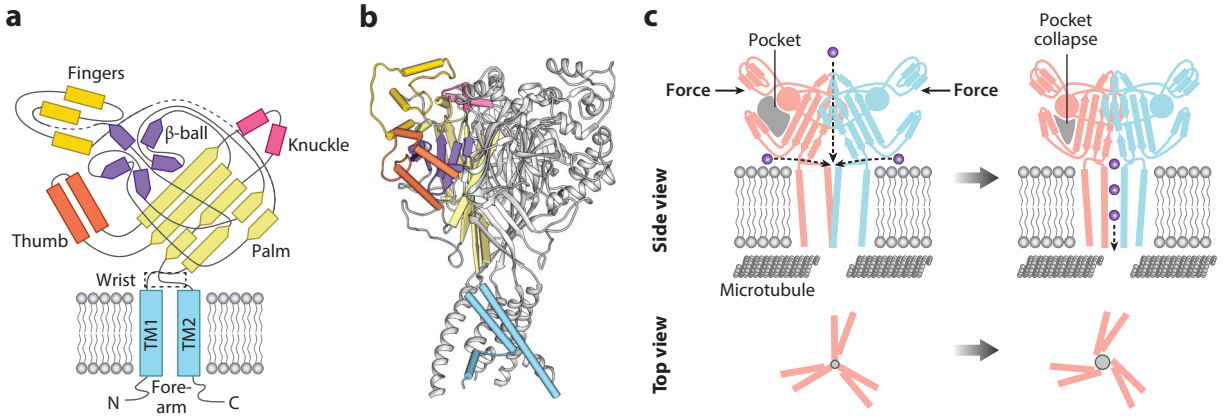

Figure 1

Structure of ASIC1 and gating model of DEG. (*a*) Domain structure of ASIC1 (*rectangle*, helix; *rectangle arrow*, β-sheet; *solid line*, loop region that is well resolved; *dashed line*, loop region that may not be well structured). (*b*) An atomic model of chicken ASIC1 (PDB ID: 4NYK; **https://doi.org/10.2210/pdb4nyk/pdb**). One subunit is shown in pipes and planks and colored with the same color codes used in panel *a*. The other two subunits are shown in ribbon diagrams. (*c*) A hypothetical model of DEG mechanogating by extracellular tether.

portals at the wrist region, as indicated from Cs^+-binding sites (Gonzales et al. 2009). A highly negatively charged cavity for proton binding (the acidic pocket) is formed by acidic residues on the thumb, fingers, and β-ball from one subunit and the palm from an adjacent subunit. Upon proton activation, the acidic pocket collapses and the thumb comes closer to the β-ball. These motions in turn cause a slight rotation of the palm region, pulling the lower palm away from the central axis, and eventually lead to a counterclockwise rotation of each transmembrane (TM) segment from the top view and an iris-like opening (Baconguis et al. 2014, Yoder et al. 2018). Continuous exposure to low pH results in rapid desensitization through substantial reorganization of the palm, which brings the TM region back to a resting-like conformation (Jasti et al. 2007, Yoder et al. 2018). All these structural components are important because mutations throughout the whole hand affect channel function (Eastwood & Goodman 2012).

Top view: view of the channel vertically down to the membrane plane from the extracellular area

The cryo-EM structure of truncated heterotrimeric human ENaC (ΔENaC) is similar to that of cASIC1 in its overall architecture (Noreng et al. 2018). Although ΔENaC is not trafficked to the plasma membrane, amiloride-sensitive current from chimeric channels composed of full-length and truncated subunits and proteolysis results indicate that ΔENaC represents a biologically relevant channel (Noreng et al. 2018). The three different subunits, α:β:γ, assemble in a counterclockwise manner from the top view. Unlike ASIC, all three subunits of ENaC possess a unique gating relief of inhibition by proteolysis (GRIP) domain that connects the finger and the thumb. The channel enters a state of higher open probability once the GRIP domain of the α and γ subunits is cleaved (Kleyman et al. 2018), and processed ENaC shows constitutive channel activity in vivo (Canessa et al. 1994). Moreover, an aromatic pocket in ENaC substitutes the acidic pocket in ASIC1 (Noreng et al. 2018), consistent with their distinct activation mechanisms.

Because of the homology of ENaC proteins (Hanukoglu & Hanukoglu 2016), these structures provide an entry to understanding the gating machinery of ENaC/DEG proteins.

Model of Degenerin Mechanogating

As *C. elegans* DEG lacks the acidic residues forming the acidic pocket of ASIC1 (Eastwood & Goodman 2012), the collapse of the equivalent pocket area in DEG is hypothetically triggered by mechanical force if it undergoes a conformational change similar to that of ASIC1 (**Figure 1*c***). Force transduction might require additional extracellular proteins such as collagen MEC-5 and the Kunitz and EGF domain–containing proteins MEC-1 and MEC-9 (Du et al. 1996, Emtage et al. 2004). These extracellular components may directly associate with the DEG hand and push or pull the channel open through a tether mechanism (Chalfie 2009). However, there was no MEC-4 and MEC-5 colocalization in vivo when examined with immuno-EM (Cueva et al. 2007). Notably, the microtubule composed of special tubulins, MEC-7 and MEC-12, also affects *C. elegans* touch sensitivity (Fukushige et al. 1999, Savage et al. 1989), conceivably through indirect processes as a consequence of ubiquitous gene expression regulation in sensory neurons (Bounoutas et al. 2011).

PIEZO

The Role of Piezo as Mechanosensitive Channels

The discovery of the Piezo channel family was a major breakthrough during the past decade. Most vertebrates have two Piezo paralogs. Piezo1 was first identified as a MS channel responsible for MA current in mouse N2A neuroblastoma cells, and mouse Piezo2 generates force-evoked current with distinct kinetics of inactivation (Coste et al. 2010). The requirement of Piezo for *Drosophila* nociception and zebrafish light-touch response provided the earliest evidence for its involvement

in mechanosensory-related behaviors (Faucherre et al. 2013, Kim et al. 2012). In HEK293 cells overexpressing Piezo1/2, MA current could be induced by external forces applied through multiple ways: pipet pressure, laminar shear stress, blunt-probe poking, optic tweezers, and magnetic nanoparticles (Coste et al. 2010, 2012; Falleroni et al. 2018; Wu et al. 2016). Piezo1 also responds to Myosin-II-mediated internal force (Ellefsen et al. 2019). Multiple point mutations influence the conductance or kinetics of Piezo channels (Bae et al. 2013a,b; Coste et al. 2015). Purified Piezo reconstituted into lipid bilayers generates spontaneous current (Coste et al. 2012, Jaggers et al. 2019) and osmolality-sensitive MS current (Syeda et al. 2016). Piezo2 is primarily expressed in mechanosensory cells such as Merkel cells of hair follicles and hair cells of the auditory system (Woo et al. 2014, Wu et al. 2017). In contrast, Piezo1 is predominantly found in nonneuronal cell types. Hence, Piezo1 plays a role in a wide range of physiological processes such as cardiovascular homeostasis (Li et al. 2014), cell-fate determination (He et al. 2018, Pathak et al. 2014, Sugimoto et al. 2017), axon growth and regeneration (Koser et al. 2016, Song et al. 2019), stem cell aging (Segel et al. 2019), and innate immunity (Solis et al. 2019). Regulation of Piezo1 expression also correlates with the metastasis of certain cancers (Chen et al. 2018, Li et al. 2015). Therefore, Piezo proteins not only play important roles in mechanosensory transduction but also provide functions of general relevance to diseases and health (Murthy et al. 2017). These studies underscore the possibility that MS channels are functionally important in nonsensory organs.

The Architecture of Piezo Proteins

Piezo is the largest ion channel subunit identified to date, composed of over 2,500 amino acid residues, and it bears no resemblance to other proteins. Piezo1 adopts a unique architecture (Ge et al. 2015); higher-resolution structures of Piezo1 and Piezo2 have revealed features that are likely relevant to the mechanical gating machinery (Guo & MacKinnon 2017, Saotome et al. 2018, Wang et al. 2019, Zhao et al. 2018).

Although Piezo1 and Piezo2 only share ∼42% sequence homology, their structures are similar (Wang et al. 2019). Both Piezo1 and Piezo2 are homotrimeric channels. The trimer looks like a propeller or triskelion from the top view: The large TM regions resemble three blades surrounding the central pore beneath a cap region (**Figure 2*b***). Each blade consists of nine structurally repeated arrays, with each repeat composed of four TM segments (**Figure 2*a*,*b***). All nine repeats are solved in the Piezo2 structure (Wang et al. 2019), but only three or six repeats closer to the central axis are assigned in the Piezo1 structures, with three more unresolved repeats at the peripheral region based on topology predictions (Guo & MacKinnon 2017, Saotome et al. 2018, Zhao et al. 2018). Removal of extracellular loops in two repeats in the middle of the blade in Piezo1 reduced the poking- but not the stretching-evoked current, suggesting Piezo1 may recruit separate structural elements upon responding to different types of mechanical stimuli (Zhao et al. 2018). The blade could be further divided into two parts: Repeat7 and repeat8 are connected through a large cytosolic domain characterized by one long α-helix (the beam, ∼9 nm long), followed by a short coiled peptide (the latch) that contacts the C-terminal domain (CTD) and several helices (the clasp) that are spatially close to the TM region (**Figure 2*a*,*b***). Notably, each blade of Piezo1/2 is profoundly curved, and three blades together form a nanodome configuration (**Figure 2*b***).

The whole blade is connected to the pore through a wedge-like anchor domain composed of three semitransmembrane helices. The central pore is formed by two TM α-helices (TM37–38) immediately adjacent to the CTD. Notably, the ion-accessible pathway is covered by the dome-like C-terminal extracellular domain (CED). The architecture of the pore-lining helices and CED together resembles that of ASIC1 or ENaC (Zhao et al. 2017) (**Figure 1*a*,*b*** and **Figure 2*a*,*b***). The CTD also contains an open cavity at the center, so ions could possibly enter the pore either along

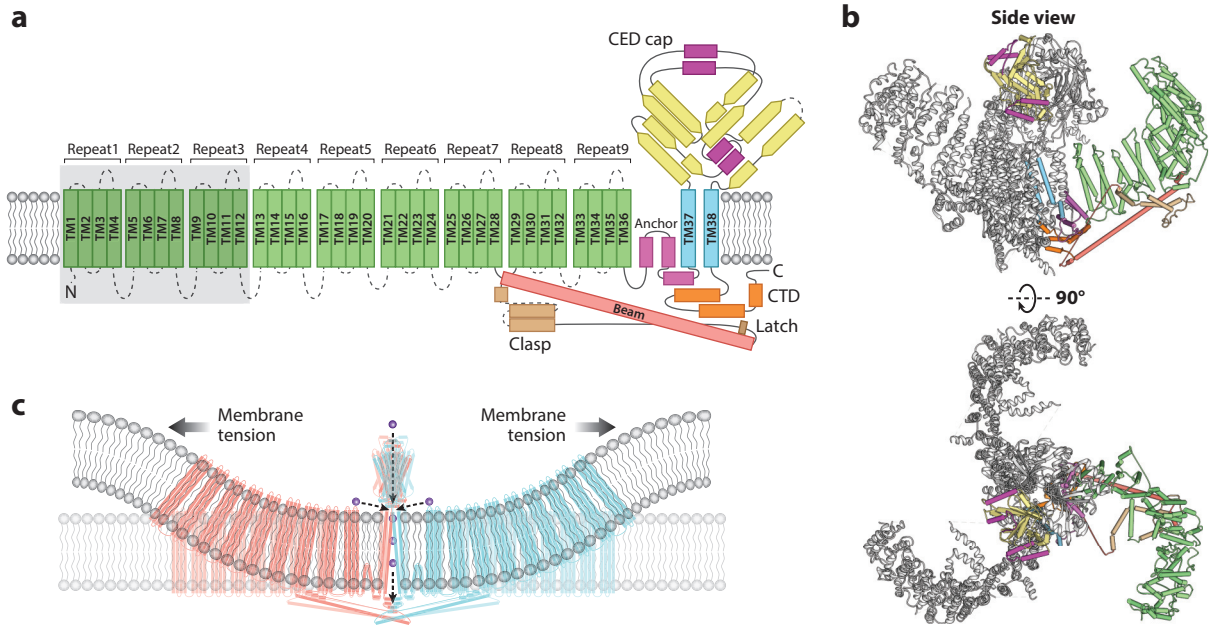

Figure 2

Structure and gating model of Piezo. (*a*) Domain structure of Piezo proteins (*rectangle*, helix; *rectangle arrow*, β-sheet; *solid line*, loop region that is well resolved; *dashed line*, loop region that may not be well structured). The regions masked by a gray block are not solved in the Piezo1 structure but are solved in the Piezo2 structure. (*b*) Two views of an atomic model of mouse Piezo1 (PDB ID: 5Z10; **https://doi.org/10.2210/pdb5z10/pdb**). One subunit is shown in pipes and planks and colored with the same color codes used in panel *a*. The other two subunits are shown in ribbon diagrams. (*c*) A schematic of the Piezo channel gating mechanism. The transmembrane region of Piezo could induce a membrane curvature, which might be flattened under force. Membrane flattening might associate with Piezo activation, but the detailed mechanism is unclear.

the symmetry axis of the CTD or laterally through the gap between the CTD and the membrane (**Figure 2*c***). The smallest constriction of the pore measures less than 1 Å in all structures, indicating a closed conformation. Jointly, the blade, the cytosolic regions (the beam, latch, and clasp), the anchor, and the CTD may move in a coordinated manner to mediate force transduction and control channel activation, which is supported by a series of structure-guided mutagenesis studies in both proteins (Saotome et al. 2018, Taberner et al. 2019, Zhao et al. 2018) and the results from magnetic nanoparticle–based MS domain identifications in Piezo1 (Wu et al. 2016).

Membrane Curvature and Piezo Activation

The nanodome configuration at the TM region suggests Piezo1/2 are probably capable of inducing local distortion of the lipid bilayer. Indeed, the curvature is evident not only for detergent-embedded Piezo1/2 but also for Piezo1 in small unilamellar vesicles (Guo & MacKinnon 2017, Lin et al. 2019). Interestingly, the dome becomes more flattened with increased vesicle size, indicating an interaction between protein and membrane (Lin et al. 2019). Force exerted perpendicularly to the membrane through atomic force microscopy (AFM) could induce a reversible flattening deformation of the dome (Lin et al. 2019). This conformational change may associate with channel activation, as the calculated half-activation tension under this situation is consistent with earlier reports (Cox et al. 2016, Lewis & Grandl 2015, Lin et al. 2019). Symmetry-free 3-D classification of Piezo1 protein particles also showed that the blades from certain subclasses could be slightly

flattened and twisted (Ge et al. 2015). Analyses using structure-based modeling approaches suggest that the free energy generated by membrane distortion tends to enhance the channel sensitivity by canceling the free energy needed for channel activation if the curvature lessens upon opening (Guo & MacKinnon 2017, Haselwandter & MacKinnon 2018). This concept is consistent with the finding that the mechanical sensitivity of Piezo1 is modulated by the resting membrane tension (Lewis & Grandl 2015). It will be interesting to examine whether Piezo1/2-promoted curvature also exists in native cells and how the shape of the dome changes in response to mechanical stimuli.

Role of the Cytoskeleton in Piezo Gating

The cytoskeleton is an important energy contributor in channel gating in the curvature model (Haselwandter & MacKinnon 2018). Disassembly of the actin cytoskeleton by cytochalasin D can dramatically reduce the amplitude of whole-cell current and the steady-state current during inactivation of Piezo1 (Gottlieb et al. 2012). However, the reduction in current can be mostly reverted after prestressing the channel by swelling the cell. A systematic study on the role of the cytoskeleton on Piezo1 activation (Cox et al. 2016) as well as a study on a filamin-knockout mouse (Retailleau et al. 2015) suggested that the cytoskeleton plays a mechanoprotective role. Loss of the actin cytoskeleton causes the channel to activate more easily. Hence, the cytoskeleton may affect Piezo activity as a membrane-tension regulator instead of as a direct stimulus transducer.

TREK/TRAAK

Mechanoactivated Two Pore–Domain Potassium Channel Subfamily

The two pore–domain potassium channels (K2P/KCNK) form a family of potassium-selective channels. Each channel subunit consists of four TM segments and two reentrant pore-forming (P) loops (Enyedi & Czirják 2010). The TREK subfamily proteins, including TREK-1 (K2P2.1/KCNK2), TREK-2 (K2P10.1/KCNK10), and TRAAK (K2P4.1/KCNK4), are mechanosensitive and thermosensitive (Bang et al. 2000, Maingret et al. 1999, Noël et al. 2009, Patel et al. 1998, Pereira et al. 2014). The proteins can be mechanoactivated through membrane stretch in a heterologous expression system or proteoliposome, indicating a membrane tension–gated mechanism (Brohawn et al. 2012, 2014a,b). TREK/TRAAK proteins are expressed in both central and peripheral nervous systems, including sensory neurons of the dorsal root ganglia (DRG) (Enyedi & Czirják 2010). Whereas no obvious impairment of mechanosensory-relevant behavior was detected in TREK-1-, TREK-2-, or TRAAK-knockout mice, the knockout mice exhibited hypersensitivity to gentle touch (Alloui et al. 2006, Noël et al. 2009, Pereira et al. 2014), raising the possibility that hyperpolarization mediated by TREK channels may compensate for depolarization caused by an unknown MS channel in DRG neurons (Alloui et al. 2006). Moreover, hyperpolarization via TRAAK activity can suppress action potential firing by counteracting the activation of Piezo1 that causes depolarization in cultured N2A cells (Brohawn et al. 2014b). These findings may represent a mode of fine-tuning the sensitivity of a mechanosensory organ through counterinteraction between two or more MS channels.

TREK/TRAAK Structures and the Mechanogating Model

The TM domain organization of the dimeric TREK-1/2 or TRAAK recapitulates that of tetrameric potassium channels (Brohawn et al. 2012, Dong et al. 2015, Lolicato et al. 2017). The twofold symmetric channel is rhomboid shaped from the top view, with pore helices and

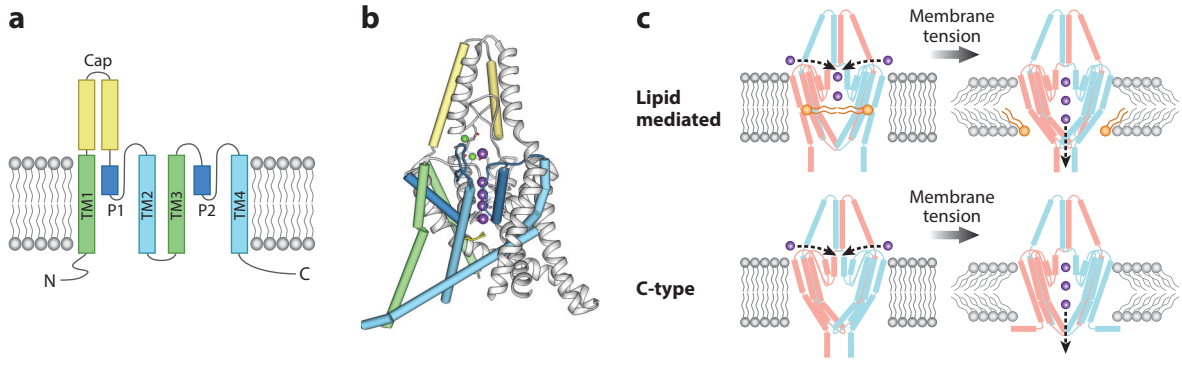

Figure 3

Structure and gating model of TRAAK. (*a*) Domain structure of TRAAK (*rectangle*, helix). (*b*) An atomic model of TRAAK (PDB ID: 4WFF; **https://doi.org/10.2210/pdb4wff/pdb**). One subunit is shown in pipes and planks and colored with the same color codes used in panel *a*. The other subunit is shown in a ribbon diagram. (*c*) Two models of TREK/TRAAK gating: a lipid-mediated gating model (*top*) and a C-type gating model (*bottom*).

selectivity filter arranged in a pseudo-fourfold symmetry. The extracellular loop connecting TM1 and P1 forms a helical cap that blocks the ion-permeable path, only allowing ions to enter the pore through side portals at the TM region–cap junction (**Figure 3*a*,*b***). TREK-2 and TRAAK were crystalized in two conformations (Brohawn et al. 2014a, Dong et al. 2015, Lolicato et al. 2014). The TM2 and TM4 inner helices could be kinked by ∼20° up toward the outer leaflet near the middle of the bilayer around a glycine hinge, thus termed the up conformation. At the down conformation, TM4 is straighter and projects toward the inner leaflet.

When TM4 is down, electron density consistent with a lipid acyl chain accesses the central cavity of TRAAK and TREK-2 from an intramembrane fenestration and impedes the conductance path (Brohawn et al. 2014a, Dong et al. 2015). A sensitive measure of ion occupancy showed no ion at the site occupied by lipid. In contrast, the fenestration is sealed in the up conformation, and additional ion density was observed in the cavity, with the lipid density nonexistent (Brohawn et al. 2014a). Moreover, TRAAK activity is elevated when the protein is reconstituted into lipids with a bulky branched acyl chain that is less capable of penetrating the fenestration (Brohawn et al. 2014a). Based on the TRAAK studies as well as the similar conformational changes that TRAAK and TREK-2 undergo at the two states, Brohawn (2015) proposed a mechanogating mechanism for TRAAK/TREK proteins: Membrane tension induces concerted movement of the TM helices and expansion of TRAAK/TREK along the membrane surface, which in turn seals the entrance for ion path–blocking lipid and opens up the channels (**Figure 3*c***). Alternatively, it might be the lipid that obstructs conformational change, while membrane tension causes lipid rearrangement to withdraw the acyl chain and promote fenestration closure, as suggested by molecular-dynamic simulation (Aryal et al. 2017).

An alternative gating model has been proposed based on structures of TRAAK carrying a G124I or W262S mutation that causes constitutive activation (Lolicato et al. 2014). The TM4 of the two mutant forms is straight, resembling the down conformation of wild-type TRAAK. In contrast, TM4 in TREK-1, with or without activators, stays at the up conformation (Lolicato et al. 2017). Thus, Lolicato et al. (2017) proposed that the up and down conformations do not necessarily correspond to channel opening and closure, respectively. Instead, the finding that activator binding eliminated flux-dependent outward rectification supports the idea that channel activation is controlled by a C-type-like gate composing the selectivity filter (**Figure 3*c***). As both models

are corroborated by structures and electrophysiological data, further investigation may help to elucidate how mechanical force gates K2P channels.

Johnston's organ: a collection of sensory cells in the second segment of the antennae in insects

Campaniform sensilla: a class of mechanoreceptors that respond to stress and strain within insect exoskeleton

Chordotonal organ: stretch receptor organ in insects and other arthropods

TRANSIENT RECEPTOR POTENTIAL CHANNELS

Transient Receptor Potential–Family Proteins as Mechanosensors

The transient receptor potential (TRP) superfamily of nonselective cation channels is divided into seven subfamilies: TRPV, TRPC, TRPA, TRPM, TRPML, TRPP/PKD, and TRPN/NompC (Montell 2001). TRP channels are involved in sensing a wide range of stimuli, including light, heat, smell, pain, and mechanical forces (Clapham 2003). Members from almost every TRP subfamily show MS characteristics (Árnadóttir & Chalfie 2010, Christensen & Corey 2007, Eijkelkamp et al. 2013), probably because these channels can be activated upon architectural modifications of the cell membrane (Liu & Montell 2015). However, whether they fulfill the criteria for a MS channel has been under debate (Árnadóttir & Chalfie 2010, Christensen & Corey 2007). For example, the suggestion that TRPC1 and TRPC6 are force sensors in the mammalian vesicular system has not been substantiated by phenotypes of TRPC1- or TRPC6-knockout mice (Dietrich et al. 2005, 2007). MA current from heterologously expressed TRPC1 or TRPC6 was reported in some studies (Maroto et al. 2005, Spassova et al. 2006) but not in others (Gottlieb et al. 2008). Given these complications, we focus on the MS features of NompC/TRPN, which is a bona fide MS channel (Walker et al. 2000, Yan et al. 2013).

NompC/TRPN Is a Mechanosensitive Channel

NompC emerged as a MS channel candidate in *Drosophila* through a genetic screen for mutants with altered or abolished mechanoreceptor current in sensory bristle (Kernan et al. 1994, Walker et al. 2000). NompC is expressed in *Drosophila* peripheral sensory organs, including the Johnston's organ, dendritic arborization neurons, campaniform sensilla, and the chordotonal organ (Cheng et al. 2010, Liang et al. 2011). *Drosophila* NompC is involved in mechanosensory-related behaviors, including locomotion (Cheng et al. 2010), gentle-touch sensation (Yan et al. 2013), sound detection (Effertz et al. 2011, 2012; Lehnert et al. 2013), collective behavior (Ramdya et al. 2015), feeding (Zhou et al. 2019), and defecation (Zhang et al. 2014). Heterologous expression of NompC in both *Drosophila* S2 cells and mammalian HEK cells gives rise to mechanogated-channel activity (Jin et al. 2017, Yan et al. 2013, Zhang et al. 2015). NompC is a pore-lining MS channel subunit, because physical properties such as adaptation and ion selectivity of the MA current could be altered by point mutations (Walker et al. 2000, Yan et al. 2013). NompC homologs exist in species ranging from *Hydra* to *Xenopus* (Schüler et al. 2015). The *C. elegans* homolog of NompC, TRP-4, is a pore-forming subunit of MS channels (Kang et al. 2010) involved in proprioception (Li et al. 2006) and ultrasound detection (Ibsen et al. 2015). Zebrafish and *Xenopus* TRPN is expressed in hair cells of the auditory system; zebrafish TRPN is required for hair cell mechanotransduction (Shin et al. 2005, Sidi et al. 2003).

NompC Is Tether-Gated Through the Microtubule Cytoskeleton

Transmission EM of *Drosophila* campaniform mechanoreceptors revealed a tether connecting the microtubule cytoskeleton and plasma membrane, thus named the membrane-microtubule connector (MMC) (Liang et al. 2013, Sun et al. 2019, Zhang et al. 2015). MMC is very likely the cytosolic part of NompC, as it is absent in *nompc*-null mutant flies and elongated in flies expressing NompC with a duplicated cytosolic domain (Sun et al. 2019, Zhang et al. 2015). The

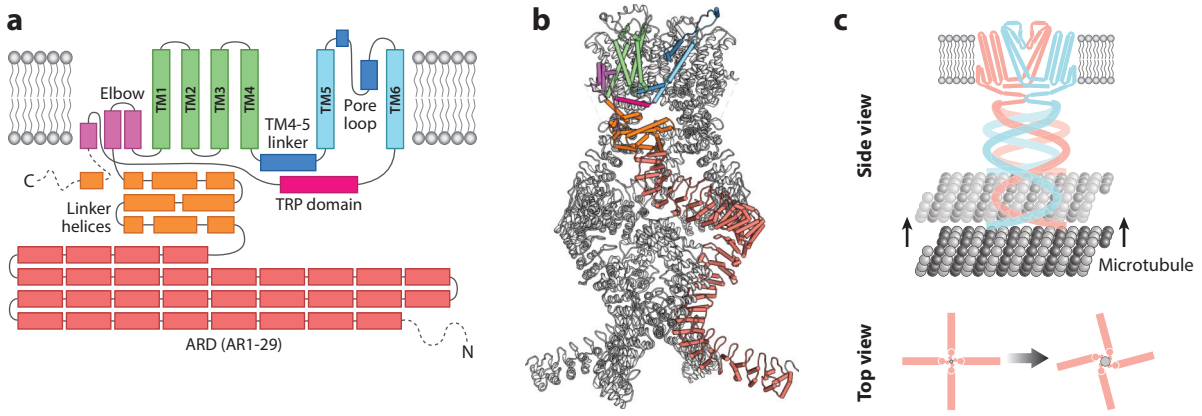

Figure 4

Structure and gating model of NompC (*rectangle*, helix; *solid line*, loop region that is well resolved; *dashed line*, loop region that may not be well structured). (*a*) Domain structure of NompC. (*b*) An atomic model of *Drosophila* NompC (PDB ID: 5VKQ; **https://doi.org/10.2210/pdb5vkq/pdb**). One subunit is shown in pipes and planks and colored with the same color codes used in panel *a*. The other three subunits are shown in ribbon diagrams. (*c*) A schematic of the NompC gating mechanism. Note that it is still unclear whether compression or expansion of the ankyrin repeats domain (ARD) causes channel activation. Here, compression of ARD is only shown as an example.

assessment of the length of MMC, 20 nm (Liang et al. 2013, Zhang et al. 2015) or 40 nm (Sun et al. 2019), likely varies with the fixation method. NompC colocalization with microtubules in the heterologous expression system was detected by confocal microscopy (Cheng et al. 2010, Yan et al. 2018, Zhang et al. 2015). Purified NompC can decorate or cosediment with in vitro–assembled microtubules (Jin et al. 2017, Zhang et al. 2015). NompC mechanosensing activity is impaired by pharmacologically disassembling the microtubule in cultured cells (Zhang et al. 2015) or destabilization of the microtubule by reducing tubulin acetylation in the *Drosophila* sensory organ (Yan et al. 2018). These observations suggest that NompC physically interacts with microtubules and that the microtubule cytoskeleton is required for NompC activation. Notably, there are additional, regularly spaced NompC-independent linkers in *nompc*-null mutant flies (Sun et al. 2019). Moreover, NompA, a zona pellucida domain containing TM protein from support cells ensheathing the neuronal sensory process, may act as an extracellular anchor of the sensory neurons (Chung et al. 2001, Göpfert & Robert 2003). Thus, whether NompC activation needs additional internal and external structures remains an open question.

The Structure of NompC

NompC shares a common topological structure of the TM domain with most TRP channels: The four subunits form a pinwheel-like tetrameric structure from the top view through domain-swapped chain connectivity, with the pore-lining TM5 and TM6 protruding to the adjacent subunit (Jin et al. 2017) (**Figure 4*a*,*b***). The central pore of NompC has two major constrictions, with the lower constriction sealed by an isoleucine residue (I1554), indicating a closed conformation. A semitransmembrane domain (pre-S1 elbow) was found between the core TM domain and the cytosolic domains. The elbow later turned out to be a common structural feature in several TRP proteins, including TRPM2/4/7/8 and TRPC3/4/6 (see **Supplemental References**). This elbow resembles the wedge-like anchor domain found in Piezo1 in that both reside at the junction between the hypothetical force-sensing apparatus and the pore. However, the exact role of the elbow remains elusive.

Supplemental Material >

The cytosolic part of NompC is mainly composed of a gigantic ankyrin repeats domain (ARD), comprising 29 ankyrin repeats (ARs) at the N terminus (most other TRP proteins have ~5 ARs, except TRPA and TRPP), and a linker region that connects the ARD with the TM domain (**Figure 4*a*,*b***). The linker, the TRP domain (a domain shared by TRP channels) following TM6, and the TM4–5 linker stack together, with the TRP domain sandwiched in between. This helical stack may move coordinately during activation (Jin et al. 2017). The entire ARD of NompC extends around 20 nm, which is consistent with the length of MMC in chemically fixed campaniform sensilla (Liang et al. 2013, Zhang et al. 2015), but the unsolved N-terminal unstructured region may account for the extra length assessed in samples fixed by high-pressure freezing (Sun et al. 2019). Each single ARD is organized into a structure that mimics the shape of a helical spring (**Figure 4*b***). This finding, as well as studies on the elasticity of the individual stack of 24 ankyrin-B repeats using AFM (Lee et al. 2006), lends support to the prediction that the ARD may function as a gating spring (Howard & Bechstedt 2004). Notably, adjacent ARDs interact at two contact sites, and together the four ARDs form a quadruple helical structure. Analyses using structure-based modeling approaches suggest that force exerted on the ARD along the symmetry axis could promote rotation of the TRP domain (Argudo et al. 2019, Wang et al. 2019). Compression of the ARD induces a counterclockwise rotation of the TRP domain from the top view for deflections of <15 Å. When further compressed, the direction of the TRP domain rotation depends on the tightness of the intersubunit interaction at the upper contact site (Argudo et al. 2019). The TRP torque could in turn promote rotation of the pore-sealing side chain of I1554 away from the central canal through the helical stack and lead to an iris-like opening, as suggested for TRPV1 and TRPV6 (Cao et al. 2013, Saotome et al. 2016) (**Figure 4*c***). Interestingly, simulations indicate that stretching the ARD of NompC causes a clockwise rotation of the TRP domain from the top view (Argudo et al. 2019), which may lead to channel closure (Wang et al. 2019). However, how the microtubule interacts with the ARD and transduces force to the protein remains an open question.

MECHANOSENSITIVE CHANNELS OF THE TMEM16 SUPERFAMILY

The TMEM16 Superfamily

The transmembrane protein 16/Anoctamin (TMEM16/Ano) family comprises members with different functions (Whitlock & Hartzell 2016). For instance, the mammalian TMEM16A and TMEM16B function as calcium-activated Cl^- channels (Caputo et al. 2008, Schroeder et al. 2008, Yang et al. 2008), while TMEM16F functions as a calcium-activated nonselective cation channel and lipid scramblase (Yang et al. 2012). Both TMEM16A and TMEM16F are dimeric channels. Each subunit includes 10 TM segments, with TM3–8 lining one ion-conductive path as revealed by cryo-EM (Alvadia et al. 2019; Dang et al. 2017; Feng et al. 2019; Paulino et al. 2017a,b). Bioinformatics analyses using strategies for identifying distant phylogenetic relationships suggest several families are evolutionarily related to TMEM16, including the Ca^{2+}-permeable stress-gated cation channel (CSC); transmembrane channel (TMC); and ANO-like, TMC-like, and CSC-like families (Medrano-Soto et al. 2018). Among those, OSCA of the CSC family and TMC are candidate MS channels.

TMC1/2 as Candidate Mechanotransducers in Hair Cells

TMC1 and TMC2 are candidates for the long-sought pore-forming subunits of MS channels in hair cells of the inner ear (Corey & Holt 2016, Qiu & Müller 2018). Both fluorescent-tagged TMC1 and TMC2 are at the tips of hair cell stereocilia (Kawashima et al. 2011, Kurima et al. 2015),

a structure where MS channels reside (Jaramillo & Hudspeth 1991). Genetic studies in mice showed that both TMC1 and TMC2 contribute to MA current in cochlear hair cells at postnatal stages. The current could be restored in *Tmc1/2* mutants through expression of either TMC1 or TMC2. One point mutation found in a mouse *Tmc1*-dominant mutant allele causes a reduction of the single-channel conductance, calcium permeability, and sensitivity to the MS channel antagonist dihydrostreptomycin (Corns et al. 2016, Pan et al. 2013). Approximately 3–7% of human deafness mutations reside in the *TMC1* gene (Imtiaz et al. 2016, Sloan-Heggen et al. 2016), while the loss of *TMC2* does not directly cause hearing loss. Targeted genome editing of the mouse *Tmc1* mutant allele *Beethoven* prevents progressive hearing loss (Gao et al. 2018, György et al. 2019). Beyond a role in the mammalian auditory system, TMC family members are also involved in mechanosensory-related behavior such as locomotion, proprioception, and direction selectivity in *Drosophila* (Guo et al. 2016, He et al. 2019, Zhang et al. 2016).

TMIE: transmembrane inner ear

TMHS: tetraspan membrane protein of hair cell stereocilia

Whether TMC1/2 is a pore-forming MS channel subunit and the primary signal transducer in hair cells has been under debate (Corey & Holt 2016, Fettiplace 2016, Qiu & Müller 2018, Wu & Müller 2016). TMC might function as part of a large protein complex that requires other membrane components, including TMIE and TMHS/LHFPL5 at the tip link (Xiong et al. 2012, Zhao et al. 2014). Loss of those components also causes deafness; similar changes in conductance and selectivity as observed in the *Beethoven* mutant could be recapitulated by mutations in other proteins at the tip link (Beurg et al. 2015, Xiong et al. 2012). MA current disappears in *Tmie*-deficient mouse cochlear hair cells with tip links remaining intact (Zhao et al. 2014), although TMIE in zebrafish might be involved in recruiting TMC to the right site in hair cells instead of direct ion conductance (Pacentine & Nicolson 2019). Moreover, reconstituting the channel activity of TMC in vitro remains a major challenge. Except for one study with *C. elegans* TMC that functions as a chemosensor instead of a force sensor (Chatzigeorgiou et al. 2013, Wang et al. 2016), overexpressed TMC1/2 from different species failed to reach the plasma membrane (Guo et al. 2016, Labay et al. 2010, Zhang et al. 2016, Zhao et al. 2014). Although coexpression of TMC1 with the KCNQ1 K^+ channel may promote plasma membrane localization of TMC1, no MA current was detected from the presumed hybrid complex (Harkcom et al. 2019). To circumvent the failure of membrane localization as an obstacle for studying the function of TMC, Jia et al. (2020) purified TMC1 from the green sea turtle and TMC2 from the budgerigar and reconstituted MA channel activity in proteoliposome, which thus provided direct evidence that TMC1/2 can be pore-forming subunits of a MS channel.

In the absence of TMC structures, bioinformatics analyses predict 10 TM segments in TMC1. Molecular mass estimation of TMCs by size exclusion chromatography suggests that TMC is a dimer (Jia et al. 2020, Pan et al. 2018), which was confirmed by two-dimensional images of TMC1 by cryo-EM (Pan et al. 2018). This topology is similar to that of TMEM16, prompting the generation of homology models of TMC1 (Ballesteros et al. 2018, Pan et al. 2018). TM6–8, the most conserved region among TMC homologs, are part of the hypothetical pore-lining helices. Unlike TMEM16, the surface around the cavity of TMC1 is negatively charged overall, consistent with the cation selectivity of mechanoreceptors in hair cells (Corey & Hudspeth 1979). Interestingly, the model-based molecular dynamics simulation revealed the existence of potassium ions and water molecules in the TM4–7 groove, indicating an ion-permeation pathway. Substituting the predicted pore-lining residues with cysteines and covalently linking a methanethiosulfonate reagent to the cysteine resulted in either decreased transduction current or reduced cation selectivity when these cysteine substitution constructs were expressed in *Tmc1/Tmc2* double-knockout mice (Pan et al. 2018). These findings also support the role of TMC1/2 as pore-forming subunits of a MS channel. Although TMC1 and TMEM16A could be structurally similar, the attempt to

reconstitute channel activity of two chimeric TMEM16A-TMC1 proteins in HEK293 cells was unsuccessful due to persistent intracellular localization (Ballesteros et al. 2018).

OSCA/TMEM63

OSCA, a channel conserved across eukaryotes, was initially identified as an osmolality sensor in plants (Hou et al. 2014, Yuan et al. 2014). When expressed in mammalian HEK293 cells, *Arabidopsis thaliana* (*At*) OSCA1.1 and OSCA1.2 could generate pressure-evoked current (Murthy et al. 2018, Zhang et al. 2018b) but without obvious osmolality-sensitive current (Maity et al. 2019, Murthy et al. 2018). This current could be enhanced by lysophosphatidylcholine (Zhang et al. 2018b). Proteoliposome-reconstituted *At* OSCA1.2 could be directly activated by stretch (Murthy et al. 2018).

Cryo-EM structures of OSCA proteins reveal that OSCA forms a symmetric homodimer with each subunit containing 11 TM segments (Jojoa-Cruz et al. 2018, Liu et al. 2018, Maity et al. 2019, Zhang et al. 2018b). OSCA and TMEM16 share a similar architecture, notwithstanding slight differences in dimer assembly (**Supplemental Figure 2*c***), even though their primary sequences are only remotely related. Ten of the 11 TM segments of OSCA align closely with the topology of the 10 TM segments of mTMEM16A (Dang et al. 2017; Paulino et al. 2017a,b). One extra N-terminal TM helix positioned at the periphery of the dimer is assigned TM0 for the convenience of comparison with TMEM16 (Jojoa-Cruz et al. 2018, Maity et al. 2019, Zhang et al. 2018b) (**Supplemental Figure 2*a***). Each OSCA subunit contains one ion-permeable pore lined with TM3–7, the most conserved region of the protein. The narrowest region of the pore measures less than 1 Å, indicating a closed state. Single-channel conductance is reduced by structure-guided mutation of a negatively charged glutamate residue (E531A) facing the pore or a glutamate residue (E462K) protruding to a vestibule at the cytosolic end of the pore in *At* OSCA1.2 (Jojoa-Cruz et al. 2018, Zhang et al. 2018b). Notably, a glycine residue (G530) may establish a hinge in the TM6 of *At* OSCA1.2 (Liu et al. 2018). Similarly, TM6 is kinked at a glycine residue in the Ca^{2+}-bound conformation of TMEM16A (Dang et al. 2017, Paulino et al. 2017a) and at a proline residue in the PIP_2-bound conformation of TMEM16F (Feng et al. 2019). The kinks could be functionally relevant: For TMEM16A, the distortion might be related to Ca^{2+}-dependent activation; for TMEM16F and its fungal homolog with scramblase activity, the kink induces a remarkable membrane distortion, which could be an intermediate step during lipid scrambling (Falzone et al. 2019, Feng et al. 2019). It will be interesting to check whether TM6 plays a similar role during OSCA activation. Two of the linkers connecting TMs might be functionally important: A wedge-like semitransmembrane anchor links TM0 and TM1 of OSCA and the cytosolic part of OSCA, mainly composed of a series of α-helices and β-sheets that link TM2 and TM3. The cytosolic linkers of OSCA homologs vary in structures, possibly related to different channel properties (Liu et al. 2018, Murthy et al. 2018). Both the anchor and the cytosolic linker may be part of the mechanotransduction machinery during the concerted movement of the mechanotransducing modules (Jojoa-Cruz et al. 2018, Liu et al. 2018), whereas the presence of a relatively small ectomembrane domain and the direct channel activation in proteoliposome indicate that OSCA gating may rely on a tension-based mechanism similar to that of TRAAK or bacterial MscS (Jojoa-Cruz et al. 2018, Liu et al. 2018, Murthy et al. 2018, Zhang et al. 2018b).

Supplemental Material >

A systematic examination of TMEM63, the animal homolog of OSCA, demonstrated that heterologously expressed TMEM63 from *Drosophila*, mouse, and human can be activated by negative pressure applied to an excision patch (Murthy et al. 2018). These results indicate that the MS characteristics of OSCA family proteins are conserved, even though TMEM63 family members only share less than 20% identity with OSCAs in primary amino acid sequences. Determining the physiological function of TMEM63 family members in animals will be of much interest.

CONCLUSIONS AND PERSPECTIVES

When the criteria for defining a MS channel were developed, DEG was the most convincing case of metazoan MS channels (Árnadóttir & Chalfie 2010). Over the past decade, more MS channels have been identified or confirmed, with structures available in most cases for deciphering the molecular principles of mechanotransduction. In addition, there are more candidates that remain to be validated as MS channels, including TMEM150, Brv1, and CFTR (Anderson et al. 2018; Hong et al. 2016; Zhang et al. 2010, 2018a). Meanwhile, the search for new MS channels continues. For example, a culture-based high-throughput assay has been developed to screen for receptors that could respond to laminar shear stress (Xu et al. 2018).

Besides identifying new MS channels, it will be important to ask whether there is a general mechanism underlying channel gating. Could membrane tension underlie both types of mechanogating, including the tether-mediated gating, as was previously proposed (Kung 2005, Teng et al. 2014)? It was reported that the channel sensitivity of Piezo1/2 could be tuned by stomatin-like protein-3 (STOML3), a membrane-stiffness regulator, through recruiting cholesterol to the lipid rafts surrounding the channel (Poole et al. 2014, Qi et al. 2015). Interestingly, MEC-2, a *C. elegans* protein evolutionarily related to STOML3, was shown to modulate the activity of the extracellular tether–gated DEG channel. The intracellular tether–gated channel NompC may also react to temperature-induced architectural rearrangement of the cell membrane, since NompC is needed to mediate an acute cold-evoked behavior in *Drosophila* larvae (Turner et al. 2016). Notably, a few lipid molecules reside in a hydrophobic bay within the TM region of NompC. Mutation of a hypothetical lipid-interacting histidine residue (H1423) abolished the mechanogated current (Jin et al. 2017). However, whether H1423 and the lipids are directly involved in tension sensing is unclear. In addition, it appears that a semitransmembrane anchor exists in multiple MS channels or MS channel candidates, including MscL, Piezo1, TRPCs, TRPMs, NompC, OSCA, and CFTR (named lasso motif; Zhang & Chen 2016), which might function as a tension sensor. Such questions regarding mechanotransduction may be resolved by characterizing more MS channels and their important structural features.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank David Bulkley and Shengjie Feng for their critical reading of the manuscript. This work is supported by NIH grant R35NS097227 to Y.-N.J. L.Y.J. and Y.-N.J. are HHMI investigators.

LITERATURE CITED

Alloui A, Zimmermann K, Mamet J, Duprat F, Noël J, et al. 2006. TREK-1, a K^+ channel involved in polymodal pain perception. *EMBO J.* 25(11):2368–76

Alvadia C, Lim NK, Mosina VC, Oostergetel GT, Dutzler R, Paulino C. 2019. Cryo-EM structures and functional characterization of the murine lipid scramblase TMEM16F. *eLife* 8:e44365

Anderson EO, Schneider ER, Matson JD, Gracheva EO, Bagriantsev SN. 2018. TMEM150C/Tentonin3 is a regulator of mechano-gated ion channels. *Cell Rep.* 23(3):701–8

Argudo D, Capponi S, Bethel NP, Grabe M. 2019. A multiscale model of mechanotransduction by the ankyrin chains of the NOMPC channel. *J. Gen. Physiol.* 151(3):316–27

Árnadóttir J, Chalfie M. 2010. Eukaryotic mechanosensitive channels. *Annu. Rev. Biophys.* 39:111–37

Árnadóttir J, O'Hagan R, Chen Y, Goodman MB, Chalfie M. 2011. The DEG/ENaC protein MEC-10 regulates the transduction channel complex in *Caenorhabditis elegans* touch receptor neurons. *J. Neurosci.* 31(35):12695–704

Aryal P, Jarerattanachat V, Clausen MV, Schewe M, McClenaghan C, et al. 2017. Bilayer-mediated structural transitions control mechanosensitivity of the TREK-2 K2P channel. *Structure* 25(5):708–18.e2

Autzen HE, Julius D, Cheng Y. 2019. Membrane mimetic systems in cryoEM: keeping membrane proteins in their native environment. *Curr. Opin. Struct. Biol.* 58:259–68

Baconguis I, Bohlen CJ, Goehring A, Julius D, Gouaux E. 2014. X-ray structure of acid-sensing ion channel 1-snake toxin complex reveals open state of a Na^+-selective channel. *Cell* 156(4):717–29

Baconguis I, Gouaux E. 2012. Structural plasticity and dynamic selectivity of acid-sensing ion channel–spider toxin complexes. *Nature* 489(7416):400–5

Bae C, Gnanasambandam R, Nicolai C, Sachs F, Gottlieb PA. 2013a. Xerocytosis is caused by mutations that alter the kinetics of the mechanosensitive channel PIEZO1. *PNAS* 110(12):E1162–68

Bae C, Gottlieb PA, Sachs F. 2013b. Human PIEZO1: removing inactivation. *Biophys. J.* 105(4):880–86

Ballesteros A, Fenollar-Ferrer C, Swartz KJ. 2018. Structural relationship between the putative hair cell mechanotransduction channel TMC1 and TMEM16 proteins. *eLife* 7:e38433

Bang H, Kim Y, Kim D. 2000. TREK-2, a new member of the mechanosensitive tandem-pore K^+ channel family. *J. Biol. Chem.* 275(23):17412–19

Beurg M, Xiong W, Zhao B, Müller U, Fettiplace R. 2015. Subunit determination of the conductance of hair-cell mechanotransducer channels. *PNAS* 112(5):1589–94

Bounoutas A, Kratz J, Emtage L, Ma C, Nguyen KC, Chalfie M. 2011. Microtubule depolymerization in *Caenorhabditis elegans* touch receptor neurons reduces gene expression through a p38 MAPK pathway. *PNAS* 108(10):3982–87

Brohawn SG. 2015. How ion channels sense mechanical force: insights from mechanosensitive K2P channels TRAAK, TREK1, and TREK2. *Ann. N. Y. Acad. Sci.* 1352(1):20–32

Brohawn SG, Campbell EB, MacKinnon R. 2014a. Physical mechanism for gating and mechanosensitivity of the human TRAAK K^+ channel. *Nature* 516(729):126–30

Brohawn SG, del Mármol J, MacKinnon R. 2012. Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K^+ ion channel. *Science* 335(6067):436–41

Brohawn SG, Su Z, MacKinnon R. 2014b. Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K^+ channels. *PNAS* 111(9):3614–19

Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, et al. 1994. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature* 367:463–67

Cao E, Liao M, Cheng Y, Julius D. 2013. TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature* 504(7478):113–18

Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, et al. 2008. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. *Science* 322:590–94

Chalfie M. 2009. Neurosensory mechanotransduction. *Nat. Rev. Mol. Cell Biol.* 10(1):44–52

Chalfie M, Au M. 1989. Genetic control of differentiation of the *Caenorhabditis elegans* touch receptor neurons. *Science* 243(29):1027–33

Chatzigeorgiou M, Bang S, Hwang SW, Schafer WR. 2013. *Tmc-1* encodes a sodium-sensitive channel required for salt chemosensation in *C. elegans*. *Nature* 494(7435):95–99

Chen X, Wanggou S, Bodalia A, Zhu M, Dong W, et al. 2018. A feedforward mechanism mediated by mechanosensitive ion channel PIEZO1 and tissue mechanics promotes glioma aggression. *Neuron* 100(4):799–815.e7

Chen Y, Bharill S, Isacoff EY, Chalfie M. 2015. Subunit composition of a DEG/ENaC mechanosensory channel of *Caenorhabditis elegans*. *PNAS* 112(37):11690–95

Cheng LE, Song W, Looger LL, Jan LY, Jan YN. 2010. The role of the TRP channel NompC in *Drosophila* larval and adult locomotion. *Neuron* 67(3):373–80

Cheng Y. 2015. Single-particle cryo-EM at crystallographic resolution. *Cell* 161(3):450–57

Cheng Y, Grigorieff N, Penczek PA, Walz T. 2015. A primer to single-particle cryo-electron microscopy. *Cell* 161(3):438–49

Cheng Y-R, Jiang B-Y, Chen C-C. 2018. Acid-sensing ion channels: dual function proteins for chemo-sensing and mechano-sensing. *J. Biomed. Sci.* 25(46):46
Christensen AP, Corey DP. 2007. TRP channels in mechanosensation: direct or indirect activation? *Nat. Rev. Neurosci.* 8(7):510–21
Chung YD, Zhu J, Han YG, Kernan MJ. 2001. *nompA* encodes a PNS-specific, ZP domain protein required to connect mechanosensory dendrites to sensory structures. *Neuron* 29(2):415–28
Clapham DE. 2003. TRP channels as cellular sensors. *Nature* 426(6966):517–24
Corey D, Hudspeth J. 1979. Ionic basis of the receptor potential in a vertebrate hair cell. *Nature* 281(5733):675–77
Corey DP, Holt JR. 2016. Are TMCs the mechanotransduction channels of vertebrate hair cells? *J. Neurosci.* 36(43):10921–26
Corns LF, Johnson SL, Kros CJ, Marcotti W. 2016. *Tmc1* point mutation affects Ca^{2+} sensitivity and block by dihydrostreptomycin of the mechanoelectrical transducer current of mouse outer hair cells. *J. Neurosci.* 36(2):336–49
Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, et al. 2010. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* 330(6000):55–60
Coste B, Murthy SE, Mathur J, Schmidt M, Mechioukhi Y, et al. 2015. Piezo1 ion channel pore properties are dictated by C-terminal region. *Nat. Commun.* 6:7223
Coste B, Xiao B, Santos JS, Syeda R, Grandl J, et al. 2012. Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature* 483(7388):176–81
Cox CD, Bae C, Ziegler L, Hartley S, Nikolova-Krstevski V, et al. 2016. Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension. *Nat. Commun.* 7:10366
Cox CD, Bavi N, Martinac B. 2018. Bacterial mechanosensors. *Annu. Rev. Physiol.* 80:71–93
Cueva JG, Mulholland A, Goodman MB. 2007. Nanoscale organization of the MEC-4 DEG/ENaC sensory mechanotransduction channel in *Caenorhabditis elegans* touch receptor neurons. *J. Neurosci.* 27(51):14089–98
Dang S, Feng S, Tien J, Peters CJ, Bulkley D, et al. 2017. Cryo-EM structures of the TMEM16A calcium-activated chloride channel. *Nature* 552(7685):426–29
Dietrich A, Kalwa H, Storch U, Mederos y Schnitzler M, Salanova B, et al. 2007. Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch.* 455(3):465–77
Dietrich A, Mederos y Schnitzler M, Gollasch M, Gross V, Storch U, et al. 2005. Increased vascular smooth muscle contractility in *TRPC6*$^{-/-}$ mice. *Mol. Cell. Biol.* 25(16):6980–89
Dong YY, Pike ACW, Mackenzie A, McClenaghan C, Aryal P, et al. 2015. K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac. *Science* 347(6227):1256–59
Du H, Gu G, William CM, Chalfie M. 1996. Extracellular proteins needed for *C. elegans* mechanosensation. *Neuron* 16(1):183–94
Eastwood AL, Goodman MB. 2012. Insight into DEG/ENaC channel gating from genetics and structure. *Physiology* 27(5):282–90
Effertz T, Nadrowski B, Piepenbrock D, Albert JT, Göpfert MC. 2012. Direct gating and mechanical integrity of *Drosophila* auditory transducers require TRPN1. *Nat. Neurosci.* 15(9):1198–200
Effertz T, Wiek R, Göpfert MC. 2011. NompC TRP channel is essential for *Drosophila* sound receptor function. *Curr. Biol.* 21(7):592–97
Eijkelkamp N, Quick K, Wood JN. 2013. Transient receptor potential channels and mechanosensation. *Annu. Rev. Neurosci.* 36:519–46
Ellefsen KL, Holt JR, Chang AC, Nourse JL, Arulmoli J, et al. 2019. Myosin-II mediated traction forces evoke localized Piezo1-dependent Ca^{2+} flickers. *Commun. Biol.* 2:298
Emtage L, Gu G, Hartwieg E, Chalfie M. 2004. The mechanosensory channel complex in *C. elegans* touch receptor neurons. *Neuron* 44(5):795–807
Enyedi P, Czirják G. 2010. Molecular background of leak K^+ currents: two-pore domain potassium channels. *Physiol. Rev.* 90(2):559–605

Falleroni F, Torre V, Cojoc D. 2018. Cell mechanotransduction with piconewton forces applied by optical tweezers. *Front. Cell. Neurosci.* 12:130

Faucherre A, Nargeot J, Mangoni ME, Jopling C. 2013. *piezo2b* regulates vertebrate light touch response. *J. Neurosci.* 33(43):17089–94

Falzone ME, Rheinberger J, Lee BC, Peyear T, Sasset L, et al. 2019. Structural basis of Ca^{2+}-dependent activation and lipid transport by a TMEM16 scramblase. *eLife* 8:e43229

Feng S, Dang S, Han TW, Jan YN, Jan LY, et al. 2019. Cryo-EM studies of TMEM16F calcium-activated ion channel suggest features important for lipid article cryo-EM studies of TMEM16F calcium-activated ion channel suggest features important for lipid scrambling. *Cell Rep*. 28(2):567–79.e4

Fettiplace R. 2016. Is TMC1 the hair cell mechanotransducer channel? *Biophys. J.* 111(1):3–9

Fukushige T, Siddiqui ZK, Chou M, Culotti JG, Gogonea CB, et al. 1999. MEC-12, an alpha-tubulin required for touch sensitivity in *C. elegans*. *J. Cell Sci*. 112:395–403

Gao X, Tao Y, Lamas V, Huang M, Yeh WH, et al. 2018. Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. *Nature* 553(7687):217–21

Ge J, Li W, Zhao Q, Li N, Chen M, et al. 2015. Architecture of the mammalian mechanosensitive Piezo1 channel. *Nature* 527(7576):64–69

Geffeney SL, Cueva JG, Glauser DA, Doll JC, Lee THC, et al. 2011. DEG/ENaC but not TRP channels are the major mechanoelectrical transduction channels in a *C. elegans* nociceptor. *Neuron* 71(5):845–57

Gonzales EB, Kawate T, Gouaux E. 2009. Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. *Nature* 460(7255):599–604

Goodman MB, Ernstrom GG, Chelur DS, O'Hagan R, Yao CA, Chalfie M. 2002. MEC-2 regulates *C. elegans* DEG/ENaC channels needed for mechanosensation. *Nature* 415(6875):1039–42

Göpfert MC, Robert D. 2003. Motion generation by *Drosophila* mechanosensory neurons. *PNAS* 100(9):5514–19

Gottlieb P, Folgering J, Maroto R, Raso A, Wood TG, et al. 2008. Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflugers Arch*. 455(6):1097–103

Gottlieb PA, Bae C, Sachs F. 2012. Gating the mechanical channel Piezo1: a comparison between whole-cell and patch recording. *Channels* 6(4):282–89

Guo Y, Wang Y, Zhang W, Meltzer S, Zanini D, et al. 2016. Transmembrane channel-like (*tmc*) gene regulates *Drosophila* larval locomotion. *PNAS* 113(26):7243–48

Guo YR, MacKinnon R. 2017. Structure-based membrane dome mechanism for Piezo mechanosensitivity. *eLife* 6:e33660

György B, Nist-Lund C, Pan B, Asai Y, Karavitaki KD, et al. 2019. Allele-specific gene editing prevents deafness in a model of dominant progressive hearing loss. *Nat. Med.* 25(7):1123–30

Hamilton ES, Schlegel AM, Haswell ES. 2014. United in diversity: mechanosensitive ion channels in plants. *Annu. Rev. Plant Biol.* 66:113–37

Hanukoglu I, Hanukoglu A. 2016. Epithelial sodium channel (ENaC) family: phylogeny, structure-function, tissue distribution, and associated inherited diseases. *Gene* 579(2):95–132

Harkcom WT, Papanikolaou M, Kanda V, Crump SM, Abbott GW. 2019. KCNQ1 rescues TMC1 plasma membrane expression but not mechanosensitive channel activity. *J. Cell. Physiol.* 234(8):13361–69

Haselwandter CA, MacKinnon R. 2018. Piezo's membrane footprint and its contribution to mechanosensitivity. *eLife* 7:e41968

He L, Gulyanon S, Mihovilovic Skanata M, Karagyozov D, Heckscher ES, et al. 2019. Direction selectivity in *Drosophila* proprioceptors requires the mechanosensory channel Tmc. *Curr. Biol.* 29(6):945–956.e3

He L, Si G, Huang J, Samuel ADT, Perrimon N. 2018. Mechanical regulation of stem-cell differentiation by the stretch-activated Piezo channel. *Nature* 555(7694):103–6

Hong GS, Lee B, Wee J, Chun H, Kim H, et al. 2016. Tentonin 3/TMEM150c confers distinct mechanosensitive currents in dorsal-root ganglion neurons with proprioceptive function. *Neuron* 91(1):107–18

Hou C, Tian W, Kleist T, He K, Garcia V, et al. 2014. DUF221 proteins are a family of osmosensitive calcium-permeable cation channels conserved across eukaryotes. *Cell Res*. 24(5):632–35

Howard J, Bechstedt S. 2004. Hypothesis: A helix of ankyrin repeats of the NOMPC-TRP ion channel is the gating spring of mechanoreceptors. *Curr. Biol.* 14(6):R224–26

Huang M, Chalfie M. 1994. Gene interactions affecting mechanosensory transduction in *Caenorhabditis elegans*. *Nature* 367(6462):467–70
Ibsen S, Tong A, Schutt C, Esener S, Chalasani SH. 2015. Sonogenetics is a non-invasive approach to activating neurons in *Caenorhabditis elegans*. *Nat. Commun.* 6:8264
Imtiaz A, Maqsood A, Rehman AU, Morell RJ, Holt JR, et al. 2016. Recessive mutations of *TMC1* associated with moderate to severe hearing loss. *Neurogenetics* 17(2):115–23
Jaggers OB, Ridone P, Martinac B, Baker MAB. 2019. Fluorescence microscopy of Piezo1 in droplet hydrogel bilayers. *Channels* 13(1):102–9
Jaramillo F, Hudspeth AJ. 1991. Localization of the hair cell's transduction channels at the hair bundle's top by iontophoretic application of a channel blocker. *Neuron* 7(3):409–20
Jasti J, Furukawa H, Gonzales EB, Gouaux E. 2007. Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. *Nature* 449(7160):316–23
Jia Y, Zhao Y, Kusakizako T, Wang Y, Pan C, et al. 2020. TMC1 and TMC2 proteins are pore-forming subunits of mechanosensitive ion channels. *Neuron*. 105:310–21.e3
Jin P, Bulkley D, Guo Y, Zhang W, Guo Z, et al. 2017. Electron cryo-microscopy structure of the mechanotransduction channel NOMPC. *Nature* 547(7661):118–22
Jojoa-Cruz S, Saotome K, Murthy SE, Tsui CCA, Sansom MS, et al. 2018. Cryo-EM structure of the mechanically activated ion channel OSCA1.2. *eLife* 7:e41845
Kang L, Gao J, Schafer WR, Xie Z, Xu XZS. 2010. *C. elegans* TRP family protein TRP-4 is a pore-forming subunit of a native mechanotransduction channel. *Neuron* 67(3):381–91
Kawashima Y, Géléoc GSG, Kurima K, Labay V, Lelli A, et al. 2011. Mechanotransduction in mouse inner ear hair cells requires transmembrane channel–like genes. *J. Clin. Investig.* 121(12):4796–809
Kernan M, Cowan D, Zuker C. 1994. Genetic dissection of mechanosensory transduction: mechanoreception-defective mutations of *Drosophila*. *Neuron* 12(6):1195–206
Kim SE, Coste B, Chadha A, Cook B, Patapoutian A. 2012. The role of *Drosophila* Piezo in mechanical nociception. *Nature* 483(7388):209–12
Kleyman TR, Kashlan OB, Hughey RP. 2018. Epithelial Na^+ channel regulation by extracellular and intracellular factors. *Annu. Rev. Physiol.* 80:263–81
Koser DE, Thompson AJ, Foster SK, Dwivedy A, Pillai EK, et al. 2016. Mechanosensing is critical for axon growth in the developing brain. *Nat. Neurosci.* 19(12):1592–98
Kung C. 2005. A possible unifying principle for mechanosensation. *Nature* 436(7051):647–54
Kurima K, Ebrahim S, Pan B, Sedlacek M, Sengupta P, et al. 2015. TMC1 and TMC2 localize at the site of mechanotransduction in mammalian inner ear hair cell stereocilia. *Cell Rep.* 12(10):1606–17
Labay V, Weichert RM, Makishima T, Griffith AJ. 2010. Topology of transmembrane channel–like gene 1 protein. *Biochemistry* 49(39):8592–98
Lee G, Abdi K, Jiang Y, Michaely P, Bennett V, Marszalek PE. 2006. Nanospring behaviour of ankyrin repeats. *Nature* 440(7081):246–49
Lehnert BP, Baker AE, Gaudry Q, Chiang AS, Wilson RI. 2013. Distinct roles of TRP channels in auditory transduction and amplification in *Drosophila*. *Neuron* 77(1):115–28
Lewis AH, Grandl J. 2015. Mechanical sensitivity of Piezo1 ion channels can be tuned by cellular membrane tension. *eLife* 4:e12088
Li C, Rezania S, Kammerer S, Sokolowski A, Devaney T, et al. 2015. Piezo1 forms mechanosensitive ion channels in the human MCF-7 breast cancer cell line. *Sci. Rep.* 5:8364
Li J, Hou B, Tumova S, Muraki K, Bruns A, et al. 2014. Piezo1 integration of vascular architecture with physiological force. *Nature* 515(7526):279–82
Li W, Feng Z, Sternberg PW, Xu XZS. 2006. A *C. elegans* stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. *Nature* 440(7084):684–87
Liang X, Madrid J, Gärtner R, Verbavatz JM, Schiklenk C, et al. 2013. A NOMPC-dependent membrane-microtubule connector is a candidate for the gating spring in fly mechanoreceptors. *Curr. Biol.* 23(9):755–63
Liang X, Madrid J, Saleh HS, Howard J. 2011. NOMPC, a member of the TRP channel family, localizes to the tubular body and distal cilium of *Drosophila* campaniform and chordotonal receptor cells. *Cytoskeleton* 68(1):1–7

Liao M, Cao E, Julius D, Cheng Y. 2013. Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature* 504(7478):107–12

Lin Y-C, Guo YR, Miyagi A, Levring J, MacKinnon R, Scheuring S. 2019. Force-induced conformational changes in PIEZO1. *Nature* 573:230–34

Liu C, Montell C. 2015. Forcing open TRP channels: mechanical gating as a unifying activation mechanism. *Biochem. Biophys. Res. Commun.* 460(1):22–25

Liu X, Wang J, Sun L. 2018. Structure of the hyperosmolality-gated calcium-permeable channel OSCA1.2. *Nat. Commun.* 9:5060

Lolicato M, Arrigoni C, Mori T, Sekioka Y, Bryant C, et al. 2017. K2P2.1 (TREK-1)-activator complexes reveal a cryptic selectivity filter binding site. *Nature* 547(7663):364–68

Lolicato M, Riegelhaupt PM, Arrigoni C, Clark KA, Minor DL. 2014. Transmembrane helix straightening and buckling underlies activation of mechanosensitive and thermosensitive K2P channels. *Neuron* 84(6):1198–212

Maingret F, Fosset M, Lesage F, Lazdunski M, Honoré E. 1999. TRAAK is a mammalian neuronal mechano-gated K^+ channel. *J. Biol. Chem.* 274(3):1381–87

Maity K, Heumann JM, McGrath AP, Kopcho NJ, Hsu P-K, et al. 2019. Cryo-EM structure of OSCA1.2 from *Oryza sativa* elucidates the mechanical basis of potential membrane hyperosmolality gating. *PNAS* 116(28):14309–18

Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP. 2005. TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat. Cell Biol.* 7(2):179–85

Martino F, Perestrelo AR, Vinarský V, Pagliari S, Forte G. 2018. Cellular mechanotransduction: from tension to function. *Front. Physiol.* 9:824

Medrano-Soto A, Moreno-Hagelsieb G, McLaughlin D, Ye ZS, Hendargo KJ, Saier MH. 2018. Bioinformatic characterization of the Anoctamin Superfamily of Ca^{2+}-activated ion channels and lipid scramblases. *PLOS ONE* 13(3):e0192851

Montell C. 2001. Physiology, phylogeny, and functions of the TRP superfamily of cation channels. *Sci. STKE* 2001(90):re1

Murthy SE, Dubin AE, Patapoutian A. 2017. Piezos thrive under pressure: mechanically activated ion channels in health and disease. *Nat. Rev. Mol. Cell Biol.* 18(12):771–83

Murthy SE, Dubin AE, Whitwam T, Jojoa-Cruz S, Cahalan SM, et al. 2018. OSCA/TMEM63 are an evolutionarily conserved family of mechanically activated ion channels. *eLife* 7:e41844

Noël J, Zimmermann K, Busserolles J, Deval E, Alloui A, et al. 2009. The mechano-activated K^+ channels TRAAK and TREK-1 control both warm and cold perception. *EMBO J.* 28(9):1308–18

Noreng S, Bharadwaj A, Posert R, Yoshioka C, Baconguis I. 2018. Structure of the human epithelial sodium channel by cryo-electron microscopy. *eLife* 7:e39340

O'Hagan R, Chalfie M, Goodman MB. 2005. The MEC-4 DEG/ENaC channel of *Caenorhabditis elegans* touch receptor neurons transduces mechanical signals. *Nat. Neurosci.* 8(1):43–50

Pacentine IV, Nicolson T. 2019. Subunits of the mechano-electrical transduction channel, Tmc1/2b, require Tmie to localize in zebrafish sensory hair cells. *PLOS Genet.* 15(2):e1007635

Pan B, Akyuz N, Liu XP, Asai Y, Nist-Lund C, et al. 2018. TMC1 forms the pore of mechanosensory transduction channels in vertebrate inner ear hair cells. *Neuron* 99(4):736–53.e6

Pan B, Géléoc GS, Asai Y, Horwitz GC, Kurima K, et al. 2013. TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron* 79(3):504–15

Patel AJ, Honoré E, Maingret F, Lesage F, Fink M, et al. 1998. A mammalian two pore domain mechano-gated S-like K^+ channel. *EMBO J.* 17(15):4283–90

Pathak MM, Nourse JL, Tran T, Hwe J, Arulmoli J, et al. 2014. Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. *PNAS* 111(45):16148–53

Paulino C, Kalienkova V, Lam AKM, Neldner Y, Dutzler R. 2017a. Activation mechanism of the calcium-activated chloride channel TMEM16A revealed by cryo-EM. *Nature* 552(7685):421–25

Paulino C, Neldner Y, Lam AKM, Kalienkova V, Brunner JD, et al. 2017b. Structural basis for anion conduction in the calcium-activated chloride channel TMEM16A. *eLife* 6:e26232

Pereira V, Busserolles J, Christin M, Devilliers M, Poupon L, et al. 2014. Role of the TREK2 potassium channel in cold and warm thermosensation and in pain perception. *Pain* 155(12):2534–44

Poole K, Herget R, Lapatsina L, Ngo HD, Lewin GR. 2014. Tuning Piezo ion channels to detect molecular-scale movements relevant for fine touch. *Nat. Commun.* 5:3520

Qi Y, Andolfi L, Frattini F, Mayer F, Lazzarino M, Hu J. 2015. Membrane stiffening by STOML3 facilitates mechanosensation in sensory neurons. *Nat. Commun.* 6:8512

Qiu X, Müller U. 2018. Mechanically gated ion channels in mammalian hair cells. *Front. Cell. Neurosci.* 12:100

Ramdya P, Lichocki P, Cruchet S, Frisch L, Tse W, et al. 2015. Mechanosensory interactions drive collective behaviour in *Drosophila*. *Nature* 519(7542):233–36

Retailleau K, Duprat F, Arhatte M, Ranade SS, Peyronnet R, et al. 2015. Piezo1 in smooth muscle cells is involved in hypertension-dependent arterial remodeling. *Cell Rep*. 13(6):1161–71

Saotome K, Murthy SE, Kefauver JM, Whitwam T, Patapoutian A, Ward AB. 2018. Structure of the mechanically activated ion channel Piezo1. *Nature* 554(7693):481–86

Saotome K, Singh AK, Yelshanskaya MV, Sobolevsky AI. 2016. Crystal structure of the epithelial calcium channel TRPV6. *Nature* 534(7608):506–11

Savage C, Hamelin M, Culotti JG, Coulson A, Albertson DG, Chalfie M. 1989. *mec-7* is a β-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans*. *Genes Dev*. 3(6):870–81

Scholz N, Monk KR, Kittel RJ, Langenhan T. 2016. Adhesion GPCRs as a putative class of metabotropic mechanosensors. In *Handbook of Experimental Pharmacology*, Vol. 234: *Adhesion G Protein–Coupled Receptors*, ed. T Langenhan, T Schöneburg, pp. 221–47. Cham, Switz.: Springer

Schroeder BC, Cheng T, Jan YN, Jan LY. 2008. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell* 134(6):1019–29

Schüler A, Schmitz G, Reft A, Özbek S, Thurm U, Bornberg-Bauer E. 2015. The rise and fall of TRP-N, an ancient family of mechanogated ion channels, in metazoa. *Genome Biol. Evol.* 7(6):1713–27

Segel M, Neumann B, Hill MFE, Weber IP, Viscomi C, et al. 2019. Niche stiffness underlies the ageing of central nervous system progenitor cells. *Nature* 573:130–34

Shi S, Luke CJ, Miedel MT, Silverman GA, Kleyman TR. 2016. Activation of the *Caenorhabditis elegans* degenerin channel by shear stress requires the MEC-10 subunit. *J. Biol. Chem.* 291(27):14012–22

Shin J-B, Adams D, Paukert M, Siba M, Sidi S, et al. 2005. *Xenopus* TRPN1 (NOMPC) localizes to microtubule-based cilia in epithelial cells, including inner-ear hair cells. *PNAS* 102(35):12572–77

Sidi S, Friedrich RW, Nicolson T. 2003. NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. *Science* 301(5629):96–99

Sloan-Heggen CM, Bierer AO, Shearer AE, Kolbe DL, Nishimura CJ, et al. 2016. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum. Genet.* 135(4):441–50

Solis AG, Bielecki P, Steach HR, Sharma L, Harman CCD, et al. 2019. Mechanosensation of cyclical force by PIEZO1 is essential for innate immunity. *Nature* 573:69–74

Song Y, Li D, Farrelly O, Miles L, Li F, et al. 2019. The mechanosensitive ion channel Piezo inhibits axon regeneration. *Neuron* 102(2):373–89.e6

Spassova MA, Hewavitharana T, Xu W, Soboloff J, Gill DL. 2006. A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *PNAS* 103(44):16586–91

Sugimoto A, Miyazaki A, Kawarabayashi K, Shono M, Akazawa Y, et al. 2017. Piezo type mechanosensitive ion channel component 1 functions as a regulator of the cell fate determination of mesenchymal stem cells. *Sci. Rep.* 7:17696

Sun L, Gao Y, He J, Cui L, Meissner J, et al. 2019. Ultrastructural organization of NompC in the mechanoreceptive organelle of *Drosophila* campaniform mechanoreceptors. *PNAS* 116(15):7343–52

Syeda R, Florendo MN, Cox CD, Kefauver JM, Santos JS, et al. 2016. Piezo1 channels are inherently mechanosensitive. *Cell Rep*. 17(7):1739–46

Taberner FJ, Prato V, Schaefer I, Schrenk-Siemens K, Heppenstall PA, Lechner SG. 2019. Structure-guided examination of the mechanogating mechanism of PIEZO2. *PNAS* 116(28):14260–69

Teng J, Loukin S, Anishkin A, Kung C. 2014. The force-from-lipid (FFL) principle of mechanosensitivity, at large and in elements. *Pflugers Arch*. 467(1):27–37

Turner HN, Armengol K, Patel AA, Himmel NJ, Sullivan L, et al. 2016. The TRP channels Pkd2, NompC, and Trpm act in cold-sensing neurons to mediate unique aversive behaviors to noxious cold in *Drosophila*. *Curr. Biol.* 26(23):3116–28

Walker RG, Willingham AT, Zuker CS. 2000. A *Drosophila* mechanosensory transduction channel. *Science* 287(5461):2229–34

Wang L, Zhou H, Zhang M, Liu W, Deng T, et al. 2019. Structure and mechanogating of the mammalian tactile channel PIEZO2. *Nature* 573:225–29

Wang X, Li G, Liu J, Liu J, Xu XZS. 2016. TMC-1 mediates alkaline sensation in *C. elegans* through nociceptive neurons. *Neuron* 91(1):146–54

Wang Y, Guo Y, Liu C, Wang L, Zhang A, et al. 2019. Push-to-open: the gating mechanism of the tethered mechanosensitive ion channel NompC. bioRxiv 853721. **https://doi.org/10.1101/853721**

Whitlock JM, Hartzell HC. 2016. Anoctamins/TMEM16 proteins: chloride channels flirting with lipids and extracellular vesicles. *Annu. Rev. Physiol.* 79:119–43

Wilson ME, Maksaev G, Haswell ES. 2013. MscS-like mechanosensitive channels in plants and microbes. *Biochemistry* 52(34):5708–22

Woo SH, Ranade S, Weyer AD, Dubin AE, Baba Y, et al. 2014. Piezo2 is required for Merkel-cell mechanotransduction. *Nature* 509(7502):622–26

Wu J, Goyal R, Grandl J. 2016. Localized force application reveals mechanically sensitive domains of Piezo1. *Nat. Commun.* 7:12939

Wu Z, Grillet N, Zhao B, Cunningham C, Harkins-Perry S, et al. 2017. Mechanosensory hair cells express two molecularly distinct mechanotransduction channels. *Nat. Neurosci.* 20(1):24–33

Wu Z, Müller U. 2016. Molecular identity of the mechanotransduction channel in hair cells: not quiet there yet. *J. Neurosci.* 36(43):10927–34

Xiong W, Grillet N, Elledge HM, Wagner TFJ, Zhao B, et al. 2012. TMHS is an integral component of the mechanotransduction machinery of cochlear hair cells. *Cell* 151(6):1283–95

Xu J, Mathur J, Vessières E, Hammack S, Nonomura K, et al. 2018. GPR68 senses flow and is essential for vascular physiology. *Cell* 173(3):762–75.e16

Yan C, Wang F, Peng Y, Williams CR, Jenkins B, et al. 2018. Microtubule acetylation is required for mechanosensation in *Drosophila*. *Cell Rep.* 25(4):1051–65.e6

Yan Z, Zhang W, He Y, Gorczyca D, Xiang Y, et al. 2013. *Drosophila* NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature* 493(7431):221–25

Yang H, Kim A, David T, Palmer D, Jin T, et al. 2012. TMEM16F forms a Ca^{2+}-activated cation channel required for lipid scrambling in platelets during blood coagulation. *Cell* 151(1):111–22

Yang YD, Cho H, Koo JY, Tak MH, Cho Y, et al. 2008. TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature* 455(7217):1210–15

Yoder N, Gouaux E. 2018. Divalent cation and chloride ion sites of chicken acid sensing ion channel 1a elucidated by x-ray crystallography. *PLOS ONE* 13(8):e0202134

Yoder N, Yoshioka C, Gouaux E. 2018. Gating mechanisms of acid-sensing ion channels. *Nature* 555(7696):397–401

Yuan F, Yang H, Xue Y, Kong D, Ye R, et al. 2014. OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in *Arabidopsis*. *Nature* 514(7522):367–71

Zhang M, Li X, Zheng H, Wen X, Chen S, et al. 2018a. Brv1 is required for *Drosophila* larvae to sense gentle touch. *Cell Rep.* 23(1):23–31

Zhang M, Wang D, Kang Y, Wu JX, Yao F, et al. 2018b. Structure of the mechanosensitive OSCA channels. *Nat. Struct. Mol. Biol.* 25(9):850–58

Zhang W, Cheng LE, Kittelmann M, Li J, Petkovic M, et al. 2015. Ankyrin repeats convey force to gate the NOMPC mechanotransduction channel. *Cell* 162(6):1391–403

Zhang W, Yan Z, Li B, Jan LY, Jan YN. 2014. Identification of motor neurons and a mechanosensitive sensory neuron in the defecation circuitry of *Drosophila* larvae. *eLife* 3:e03293

Zhang WK, Wang D, Duan Y, Loy MMT, Chan HC, Huang P. 2010. Mechanosensitive gating of CFTR. *Nat. Cell Biol.* 12(5):507–12

Zhang YV, Aikin TJ, Li Z, Montell C. 2016. The basis of food texture sensation in *Drosophila*. *Neuron* 91(4):863–77

Zhang Z, Chen J. 2016. Atomic structure of the cystic fibrosis transmembrane conductance regulator. *Cell* 167(6):1586–97.e9

Zhao B, Wu Z, Grillet N, Yan L, Xiong W, et al. 2014. TMIE is an essential component of the mechanotransduction machinery of cochlear hair cells. *Neuron* 84(5):954–67

Zhao Q, Wu K, Chi S, Geng J, Xiao B. 2017. Heterologous expression of the Piezo1-ASIC1 chimera induces mechanosensitive currents with properties distinct from Piezo1. *Neuron* 94(2):274–77

Zhao Q, Zhou H, Chi S, Wang Y, Wang J, et al. 2018. Structure and mechanogating mechanism of the Piezo1 channel. *Nature* 554(7693):487–92

Zhou Y, Cao L-H, Sui X-W, Guo X-Q, Luo D-G. 2019. Mechanosensory circuits coordinate two opposing motor actions in *Drosophila* feeding. *Sci. Adv.* 5:eaaw5141