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Physiological and Pathological Functions of Mechanosensitive Ion Channels

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Abstract

Rapid sensation of mechanical stimuli is often mediated by mechanosensitive ion channels. Their opening results from conformational changes induced by mechanical forces. It leads to membrane permeation of selected ions and thereby to electrical signaling. Newly identified mechanosensitive ion channels are emerging at an astonishing rate, including some that are traditionally assigned for completely different functions. In this review, we first provide a brief overview of ion channels that are known to play a role in mechanosensation. Next, we focus on three representative ones, including the transient receptor potential channel V4 (TRPV4), Kv1.1 voltage-gated potassium (Kv) channel, and Piezo channels. Their structures, biophysical properties, expression and targeting patterns, and physiological functions are highlighted. The potential role of their mechanosensation in related diseases is further discussed. In sum, mechanosensation appears to be achieved in a variety of ways by different proteins and plays a fundamental role in the function of various organs under normal and abnormal conditions.

Introduction

Sensing external mechanical forces such as gravity, touch, and sound wave is fundamentally important for our daily lives. Touch and hearing together with sight, taste and smell are the traditionally-recognized five senses in humans. In fact, organisms from single-cellular bacteria to multicellular plants and animals are able to sense and respond to not only external mechanical forces, but internal mechanical forces (such as osmotic pressure and membrane deformation). Mechanical forces have broad effects on cell proliferation, migration and adhesion, morphogenesis, gene expression, fluid homeostasis and vesicular transport ([1, 2] for reviews). They are vital for proper growth, development and health of various organisms. Specialized cells that are sensitive to mechanical forces in animals have been recognized and studied for a long time, from bristle receptors in flies and touch receptors in worms, to cochlear hair cells and skin mechanoreceptors in vertebrates. These cells are capable of converting the internal and/or external mechanical stimuli to electrical signals. However, due to the low abundance of these cells and seemingly lack of general implications for their transduction mechanisms, the progress of determining molecular mechanisms underlying mechanotransduction was slow. Recently, rapid progress was made in studying both invertebrates and vertebrates by identifying the molecular machinery

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responsible for mechanosensation and mechanotransduction, the mechanosensitive ion channels ([3–6] for reviews).

Whereas sight, smell and much of taste are initiated by ligands binding to G-protein-coupled receptors (GPCRs), which activate biochemical signaling cascades, mechanical sensations of touch and hearing are primarily initiated by mechanosensitive ion channels. These channels are the primary transducers that convert mechanical force into an electrical or chemical signal in touch, hearing, and other mechanical senses. In recent several years, a substantial amount of studies across different disciplines, using molecular, biochemistry, genetic, electrophysiology and other state-of-the-art techniques, have examined the structure and function of various mechanosensitive ion channels expressed in different cells in sensing various mechanical stimuli. These channels are surprisingly broadly expressed and can respond to different stimuli such as: touch including gentle touch, texture, light brush of the skin, stretch, vibration, and pressure including noxious pressure ([7, 8] for reviews).

It has been recognized for many years that various mechanical stimuli can induce ionic currents crossing the plasma membrane in different cells. Mechanically activated currents were detected from cochlear hair cell, dorsal root ganglion (DRG) neurons, vascular smooth muscle cells, kidney primary epithelia and mammalian cell lines [9–13]. Many mechanically activated currents are non-selective cationic currents with Na^+ , K^+ and Ca^{2+} permeability [14, 15]. These currents are conducted through different ion channels in the cell membrane, converting mechanical stimuli to electrical signals to enable cells to control their own metabolism and to communicate with the surrounding environment. Since many ion channels are implicated in mechanosensation, an important question is often raised. Are they directly or indirectly involved in mechanosensation and mechanotransduction? Overlapping criteria from different aspects are proposed by different investigators. The criteria for stretch-activated ion channels are more mechanistic, including direct activation by stretch/pressure, rapid channel kinetics, dependence of current kinetics on pressure amplitude, and association of gating with conformational changes ([16, 17] for reviews). Its expression pattern and loss of function phenotype are also important concerns for the physiological significance of a channel being involved in mechanotransduction ([18] for review). So far, only small number of channels has been shown to fulfill all this criteria, which partially results from unavailable experimental results. For instance, although being accepted as important channels sensing mechanical forces, Piezo proteins do not fulfill all the criteria for a stretch-activated ion channel due to the lack of understanding of the association of its gating with conformation changes. In the present review, we focus on mechanosensitive ion channels expressed in eukaryotic cells, which include all the channels with reasonable in vivo and in vitro evidence for a role in mechanosensation (Table 1). We anticipate that this list will continue to grow when more experimental results become available. The most extensively studied mechanosensitive ion channels are the bacterial Msc channels [19], but these channels have no homologues in animals and are not discussed here. As illustrated in the table, unrelated ion channels can be used for sensing different mechanical stimuli, which can occur not only in distant organisms, but also in distinct locations of the same organism.

Compared to extensive structure-function analysis of these mechanosensitive ion channels, their physiological and pathological functions remain poorly understood. In particular, some

ion channels listed in Table 1 were traditionally associated with completely different functions. Furthermore, almost all these channels can be regulated by many other factors besides mechanical forces. The exact role of mechanosensation and mechanotransduction under physiological and especially pathological conditions often remains unclear. In this review, we focus on the physiological functions and related diseases in humans of three different candidates of mechanosensitive ion channels, TRPV4, Kv1.1, and Piezo.

TRPV4 channel in mechanosensation and diseases

The transient receptor potential was first described in fruit fly *Drosophila* in 1969 and the channel was cloned in 1989 [20, 21]. Since then, many TRP channels have been cloned from both invertebrates and vertebrates using genetic screening and they have been extensively characterized. The TRP channel family is normally divided into 7 subfamilies (TRPC, TRPV, TRPM, TRPN, TRPA, TRPP and TRPML) ([22, 23] for reviews). The yeast TRP like gene (TRPY) identified by the Saimi group in 2001 is considered as the eighth subfamily [24]. TRP channels can be activated by light, sound, chemicals, temperature, osmolarity, touch, heat (>27–34°C), the phorbol ester derivative 4 α -phorbol-12,13-didecanoate (4 α -PDD), or lipids downstream of arachidonic acid metabolism [25]. It was postulated that activation by mechanical stimuli may be a common feature to nearly every TRP subfamily ([17, 26] for review). Since TRPV4 is one of the most studied TRP channels and TRPV4 mutations lead to a variety of phenotypic disorders, we focus on TRPV4 in this review.

TRPV4 is also known as vanilloid receptor related osmotically activated channel (VR-OAC), OTRPC4, TRP12, or VRL-2 [27]. It is a nonselective cation channel, first described as an osmosensor detecting hypotonic stimuli, and shares about 40% amino acid identity with TRPV1. Each subunit has 6 transmembrane (TM) segments with a pore forming loop between the 5th and 6th TM segments. Both N and C termini are located in the cytoplasmic side, similar to Kv channels. In addition, the amino terminus of TRPV4 channel contains 6 ankyrin-repeat domains (Fig 1A). Four TRPV4 subunits form a homotetramer in the endoplasmic reticulum (ER), and subsequently the TRPV4 homotetramer is trafficked to the cell membrane [28, 29].

TRPV4 channel is activated not only by 4 α -PDD, but by hypotonic stress, one type of mechanical stimuli (Fig. 1B,C). One study shows that cell swelling caused by hypotonicity activates phospholipase A₂ (PLA₂), thereby triggering Cytochrome P450-dependent downstream reactions, which underlies the mechanism of activation of TRPV4 by hypotonicity [30]. Another study shows the activation of TRPV4 by extracellular hypotonic stress requires its interaction with the water channel aquaporin 5 ([22], [31] for review) It remains unclear whether activation of the TRPV4 channel, a polymodally activated TRPV channel, by shear stress and fluid flow shares the same molecular mechanism. This is because a TRPV4 mutant (Y555A) with a point mutation in its 3rd TM domain is not activated by heat or 4 α -PDD but by cell swelling or arachidonic acid [32]. The channel opening of TRPV4 to hypotonic stress but not isotonic stasis or hypertonic stress occurs within a few seconds to 2 min. Its biophysical properties are characterized with outward rectification, a reversal potential close to 0 mV [33, 34] (Fig. 1B,C).

The trafficking and function of TRPV4 are regulated by various binding partners. For instance, TRPV4 binds to protein kinase C and casein kinase substrate in neurons protein 3 (PACSIN3) via its N-terminal proline-rich domain. PACSIN3 is a protein involved in synaptic vesicular membrane trafficking and endocytosis, and it binds to TRPV4 via its C-terminal Src homology 3 domain. The binding not only increases the TRPV4 expression level in the plasma membrane but also inhibits its basal activity in a stimulus-specific manner [35, 36]. The interaction between microtubule-associated protein 7 (MAP7) and TRPV4 via the TRPV4 C-terminus enhances its cell surface expression level and increases TRPV4 current density in CHO cells [37]. In addition, inositol triphosphate (IP3) directly interacts with the C-terminal domain of TRPV4 channels in ciliated epithelia. The function of this interaction is to sensitize TRPV4 channels to mechanical and osmotic stimuli [38, 39]. Moreover, the physical interaction between TRPV4 and $\alpha 2$ integrin and Src tyrosine kinase was implicated in mechanical hyperalgesia [40]. These direct and/or indirect interactions between TRPV4 and its associating proteins suggest that TRPV4 location and function are fine tuned by its binding proteins under physiological and pathological conditions.

TRPV4 channels are highly expressed in DRG neurons (Fig. 1D), kidney, lung, spleen, testis, heart, keratinocytes, heart, liver, endothelia, cochlea, sweat glands, and osmosensory cells in the brain ([22] for review). TRPV4 expression in some regions of the central nervous system (CNS) remains controversial. One study by Shibasaki et al. showed TRPV4 was strongly expressed in adult mouse hippocampal neurons using *in situ* hybridization and functional analysis including the intracellular Ca^{2+} response to hypotonic stress and patch clamp recording of the current activated by 4- α -PDD, which is a specific TRPV4 agonist [41]. On the other hand, in a different study, TRPV4 staining was observed in rat astrocytes and the choroid plexus near the hippocampus using immunostaining [42]. These conflicting findings may result from the antibody quality and/or potential unknown mechanisms regulating TRPV4 expression. This interesting discrepancy needs to be clarified in future studies.

Mutations of TRPV4 have been implicated in several diseases in humans including both the skeleton and nervous systems, which is a testament of important functions of TRPV4 channels. Familial digital arthropathy-brachydactyly (FDAB) is a dominantly inherited condition associated with TRPV4 mutations within its N-terminus. The patients suffering from FDAB have aggressive osteoarthropathy of the fingers and toes and consequent shortening of the middle and distal phalanges [28]. Lamande and colleagues identified three TRPV4 mutations (G270V, R271P and F273L) that are associated with FDAB using genome-wide microsatellite linkage scan [28]. All three TRPV4 mutants had a poor cell surface expression due to impaired transport from the ER to the Golgi. Function analysis using calcium imaging indicated that all three mutants had a defect either in response to a TRPV4 agonist or hypotonic stress. Together, the reduced cell surface expression and impaired function caused by the three mutations in the N-terminal ankyrin-repeat-containing domain of TRPV4 are most likely the pathogenic mechanism underlying FDAB. In addition, TRPV4 mutations are involved in Charcot-Marie-Tooth disease 2C (CMT2C), which is an autosomal dominant neuropathy characterized by limb, diaphragm, and laryngeal muscle

weakness. The CMT2C associated with TRPV4 mutants (R269C and R269H) resulted in significant cellular toxicity and increased cell death. Channel function analysis revealed a gain-of-function for these mutants. Both basal and activated channel activities were significantly increased. However, there was no change in membrane location for these mutants when expressed in *Xenopus oocytes* [43, 44]. Similar to CMT2C, the TRPV4 mutant (R316C) causing scapuloperoneal spinal muscular atrophy (SPSMA) did not change the channel expression level in the plasma membrane. Notably, this mutation also increased channel activities, thereby altering intracellular calcium homeostasis and leading to peripheral neuropathies [43, 45, 46]. Besides the diseases associated with TRPV4 mutations described above, more than 50 mutations in TRPV4 gene have been identified, which are involved not only in skeletal dysplasias including FDAB but also in motor and sensory neuropathies including CMT2C and SPSMA.

Important functions of TRPV4 channels have also been demonstrated with clear phenotypes of TRPV4 knockout (KO) mice. TRPV4 KO mice display a variety of abnormal behaviors and characteristics. For instance, less fluid intake, lower plasma levels of antidiuretic hormone, impairment of osmotic sensation [27], and reduced sensation of tail to pressure and acidic nociception [37] were observed in TRPV4 KO mice. In addition, other phenotypic changes such as defects in the alveolar barrier, abnormal secretion of renal tubular K^+ , and defect in response to arterial shear were also found in TRPV4 KO mice [47]. Interestingly, a recent study by O'Connor et al. showed that mice with a deleted TRPV4 gene have more severe osteoarthritis when fed with a high-fat diet [48]. In conclusion, human diseases associated with TRPV4 mutations and phenotypes observed in TRPV4 KO mice indicate that TRPV4 plays very important roles under both physiological and pathological conditions. These findings will contribute to the development of new therapeutic strategies using TRPV4 as a target.

Kv1.1 channel can sense mechanical stimuli

The potassium channel superfamily contains three major groups based on the secondary structure of channel subunits. Kv channels belong to the first group of the superfamily. Each pore-forming subunit in this group contains 6 or 7 TM segments and a pore-forming loop between the 5th and 6th TM segments (Fig. 2A). A functional Kv channel complex contains 4 subunits, which could be homotetramer or heterotetramer. The four best-known subfamilies of Kv channels based on their homology to those in fruit fly *Drosophila*, are Kv1 (Shaker; KCNA), Kv2 (Shab; KCNB), Kv3 (Shaw; KCNC), and Kv4 (Shal; KCND) ([49] for review). In vertebrates, there are about 40 Kv channels that are divided into 12 subfamilies, from Kv1 to Kv12. Kv channels are broadly expressed in the nervous system and other tissues [50–53] (Fig. 2C).

Kv1 channels are conventionally considered as channels mainly activated by depolarizing changes in membrane potential. However, the emerging evidence has shown that some Kv channels are also sensitive to mechanical stimuli. It was previously shown that membrane stretch accelerates activation and slows inactivation in Shaker channels with S3–S4 linker deletions [54]. Shaker channel's sensitivity to tension was proposed to be caused by the following possibilities. The first is that Shaker channels are sufficiently distensible that

stretch produced a novel channel state. The second is that Shaker channels expand in the plane of the membrane during voltage gating [54]. Later, it was found that the membrane tension accelerates rate-limiting voltage-dependent activation and slows the inactivation step [55]. These studies provided some clues to support that the conventional Kv1 channel could be a mechanically-activated channel. Recently, Hao et al. identified a mechanosensitive K⁺ current ($I_{K_{mech}}$) in mouse DRG neurons, which is carried by Kv1.1–Kv1.2 heteromers (Fig. 2B) [8]. The $I_{K_{mech}}$ with reversal potential (E_{rev}) around at 0 mV and using CsCl-based internal solution is a non-selective cationic current. The $I_{K_{mech}}$ is activated gradually by increasing mechanical stimuli. To identify $I_{K_{mech}}$, they used pharmacological inhibitors, reconstitution of Kv1 channels in HEK293 cells, and Kv1.1 knockout mice. Their studies showed that the $I_{K_{mech}}$ was not mediated by K_{2P}-, KCNQ-, EAG-, or Ca²⁺ dependent K⁺ channels which are the known candidates of mechanosensitive channels. Taken together, this recently published paper has revealed an unexpected function of the Kv1.1 subunits acting as a mechanosensitive brake and offered physiological relevance to the mechanosusceptibility of Kv1.1 channels.

Since Kv1 channels regulate neuronal excitability by controlling the initiation and shaping of action potentials, disruption of the expression and/or function of Kv1 channels can lead to severe diseases. For instance, the mutations in KCNA1 and KCNA2 (the genes encoding Kv1.1 and Kv1.2) are associated with neurological diseases including epilepsy and ataxia with distinct phenotypes both in humans and rodents. Kv1.1 KO mice exhibit a limbic seizure phenotype which is characterized by facial twitching, immobility, head nodding, and unilateral and bilateral forelimb clonus with symptoms appearing 2–3 weeks after birth. In contrast, Kv1.2 KO mice develop a more severe brainstem seizure phenotype around 15 days after birth. The difference in function, developmental expression, subcellular location and compensation from the remaining channels may be the underlying mechanisms responsible for distinct seizure phenotypes ([56] for review). The study by Simeone et al. showed loss of Kv1.1 leads to enhanced synaptic release in the hippocampal CA3 region, thereby reducing spike timing precision of neurons, which provides further insight into the mechanism underlying temporal lobe epilepsy [57]. Interestingly, the work by Zenker et al. implicated that lower expression level of juxtaparanodal Kv1.2 and reduced nerve conduction velocity mediate peripheral nerve hyperexcitability in type 2 diabetes mellitus (T2DM) [58]. In addition, the study by Wijst and colleagues described another phenotype associated with a mutation in Kv1.1 (N255D) which results in autosomal dominant hypomagnesemia [59, 60]. Kv1.1 colocalizes with epithelial Mg²⁺ channel TRPM6, a member of TRP family, in distal convoluted tubule (DCT) and regulates Mg²⁺ influx by setting the membrane potential. Therefore, the Kv1.1 N255D mutation, which results in non-functional channels, loses the ability to regulate Mg²⁺, thereby causing autosomal dominant hypomagnesemia. The patients with this disease have phenotypic tetany, cardiac arrhythmias, and seizures due to the lower level of intracellular Mg²⁺. Together, these findings have shown that Kv1 channels underlie multiple human neurological diseases.

Depolarization induced Kv1 channel activation is characterized by typical delayed rectifying currents, which are known to play important roles in setting membrane potentials and shaping action potentials. As discussed above, these channels are also activated by mechanical force. The currents induced by mechanical forces are inhibited by Gd³⁺, which

is a nonspecific mechanosensitive ion channel blocker. These findings raise some intriguing questions. Is there any relationship between the voltage- and mechanical-sensing of Kv1 channels? Is disrupted mechanosensitivity of Kv1 channels is also involved in the diseases we discussed above? The recent work suggests that the mechanical forces facilitate voltage-dependent activation of Kv1.1 channels [8], which provide a clue for answering these questions. However, more studies need to be done to elucidate the communication between voltage- and mechanical-sensitivity, and their distinct roles in diseases in humans.

Piezo proteins are pore-forming subunits of mechanosensitive ion channels

The Piezo proteins were first identified as potential mechanosensitive ion channels in the cell line Neuro2A (N2A, a glial tumor cell line) by Patapoutian and colleagues using a combination of patch clamp, whole cell stimulation/recording and molecular biology [61, 62]. The currents induced by mechanical indentation in the N2A cell line are characterized by relatively high amplitude currents (200 pA at -80 mV) with E_{rev} around 0 mV, which means the current induced by mechanical stimuli is a non-selective cationic current ([16] for review). To identify the genes encoding for these potential mechanosensitive ion channels, the investigators systematically knocked down the transcript of individual transmembrane proteins with short interfering RNAs in N2A cell lines. Finally, they identified a protein named Piezo1, which initially was termed as Fam38A. They also found another homologous gene encoding for Piezo2 protein from DRG neurons subsequently. The currents mediated by Piezo2 were similar to Piezo1 with different kinetics and conductance.

Piezo proteins have 2100 to 4700 amino acids, which contain approximately 24–39 TM segments showing no homology to other already known voltage sensitive channels (Fig. 2D,E) ([16, 26, 62] for reviews). Piezo1 is a membrane protein and it is highly expressed in the lung, bladder and skin. Piezo2 displays a similar tissue expression pattern as Piezo1, but has a higher expression level in DRG neurons (Fig. 2F) [61]. As we described above, currents mediated by Piezo proteins are non-selective cationic currents, and their structures do not resemble any of the known voltage-sensing ion channels. These findings raised an interesting question. Are Piezos pore-forming subunits of mechanically activated channels? To address this question, Coste et al. reconstituted purified mouse Piezo1 (MmPiezo1) into lipid bilayers in two different configurations: droplet interface lipid bilayer and proteoliposomes. In both settings, a brief and discrete channel opening that was sensitive to ruthenium red (RR), a general blocker of TRP channels, was recorded. In addition, the reconstituted MmPiezo 1 constitutively remained active in lipid bilayer configuration and had the ability to conduct sodium ions [10]. When overexpressed in HEK293 cells, *Drosophila melanogaster* DmPiezo or MmPiezo formed mechanically activated channels with distinct biophysical and pore-related properties. For instance, MmPiezo is sensitive to RR but not DmPiezo (Fig. 2E). This study validated MmPiezo1 protein as a real ion channel that conducts both K^+ and Na^+ . As of pore-forming channel subunits, the currents mediated by both Piezo1 and Piezo2 can be pharmacologically inhibited by mechanosensitive channel blockers RR and Gd^{3+} . The spider toxin GsMTx4 selectively inhibits Piezo1 current [16]. Both Piezo currents inactivate in a voltage-dependent manner.

Hereditary xerocytosis (HX, MIM 194380), also known as dehydrated hereditary stomatocytosis (DHSt), is an autosomal dominant hemolytic anemia associated with Piezo1 mutations. The patients affected by HX have primary erythrocyte dehydration with mild to severe compensated hemolytic anemia due to leak of monovalent cation contents (decreased intraerythrocytic K^+ and increased intraerythrocytic Na^+). Usually, the patients also have moderate splenomegaly and increased mean corpuscular hemoglobin concentration. The location on chromosome 16 (16q23–q24) associated with this inherited disease was first found in an Irish family, and was further confirmed in other patients from different countries using high resolution single nucleotide polymorphism typing and whole-exome sequencing [63]. The mutation sites within exons encoding the Piezo1 protein expressed in human erythrocytes were first reported by Zarychanski et al. using copy number analyses, linkage study, and exome sequencing [63]. The mutations at amino acids from 2225 to 2456 are the molecular mechanism underlying HX. These mutations may impair Piezo1 proteins interacting with other proteins and/or trafficking to the erythrocyte membrane, so that the mutant Piezo1 fails to maintain erythrocyte volume homeostasis. This novel finding provides some clues to search for the genetic cause of other phenotypes associated with Piezo proteins [63]. Another study by Lolascón and colleagues suggests that R2456H and R2488Q mutations in Piezo1 is the genetic cause of autosomal dominant dehydrated hereditary stomatocytosis (DHSt), also known as HX [64]. In this recent study, the channel function of these two mutations of Piezo1 (R2456H and R2488Q) were tested both in *Xenopus oocytes* and human erythrocytes. The R2456H mutation expressed in the oocytes resulted in elevated single channel conductance, while the R2488Q mutation caused increased currents elicited by hydrostatic pressure. This functional analysis revealed the causative relationship between the altered mechanotransduction of Piezo1 mutations and the imbalance of intracellular ion contents in red blood cells of DHSt (or HX). Subsequently, the Patapoutian group demonstrated that two distinct Piezo2 mutations, E2727del and I802F, cause Distal Arthrogryposis Type 5 (DA5) [65]. Patients with DA5 have multiple distal contractures, ophthalmoplegia with ptosis, characteristic facies, and a lung disorder with pulmonary hypertension. Patch clamp recording reveals that both these two mutations have faster recovery from inactivation. In addition, E2727del shows a slowed inactivation. In conclusion, the enhanced function of mutated Piezo2 with faster recovery from inactivation underlies the mechanism of DA5 [65].

The roles of Piezo proteins were also tested in *D. melanogaster* (DmPiezo) KO larvae. DmPiezo knockout flies were created by deletion of all 31 coding exons using genomic recombination. The KO flies did not display disorders in coordination and bristle mechanoreceptor potential, and the flies were viable and fertile. Behavioral testing indicated the responses to noxious mechanical stimuli were significantly impaired in KO larvae. However, other sensations such as light touch remained unchanged [66]. Unfortunately, Piezo2 KO mice are not available so far, because Piezo2 constitutive KO mice died at birth. Development of conditional KO mice may be suitable for studying the role of Piezo2 *in vivo* [67]. Taken together, these recent findings shed light on the relationship between Piezo channel function and human disease, but more studies are needed to better understand the roles of this channel in future investigation.

Future Perspectives

Recent studies reveal a variety of ion channels that are able to convert mechanical forces to electrical signaling. They include some TRP channels, some voltage-gated Na⁺, K⁺ and Ca²⁺ channels, and Piezo channels (Table 1). The diversity of these mechanosensitive ion channels raises some interesting questions. For instance, what are the channel parts that detect mechanical stimuli and are these channel parts conserved? Since many ion channels are linked to intracellular cytoskeletons via adaptor proteins and/or linked to the extracellular matrix via extracellular domains and sugar chains [52, 68–70], should their channel activities generally be influenced by mechanical forces? Existing evidence does suggest that cell membrane compression, expansion, bending and tension caused by mechanical forces applied to cell membrane intracellularly and/or extracellularly can lead to changes in the structural configuration of these ion channels, thereby modulating their opening and membrane conductance ([2] for review). This could be a common mechanism underlying mechanosensation. In terms of channel structure, TRPV4 has 6 TM segments with a pore forming loop between the 5th and 6th TM segments. Similarly, Kv1.1 channels also contain 6 TM segments and a pore-forming loop between the last two TM segments. However, the two may use completely different mechanisms in response to mechanical forces. Moreover, Piezo proteins contain approximately 24–39 TM segments and show no homology to TRPV4 and Kv1.1 channels. Thus, because of little homology in primary sequences, mechanosensitive ion channels likely differ in their mechanosensation and intracellular signal transduction. The structure and function analysis will remain an exciting topic for better understanding of mechanosensitive ion channels.

Mechanosensitive ion channels play important roles in cellular functions, such as gene expression, cell division, migration, cell adhesion, and fluid homeostasis ([2] for review). Extensive studies have been focused on their functions in kidney, cochlear hair cells, and classical sensory neurons such as DRG. Despite broad expression in the brain, a complex mechanosensitive organ, functions of mechanosensitive ion channels in the CNS remain poorly understood. Various neurological diseases in humans are associated with mutations in mechanosensitive ion channels. For instance, TRPV4 mutations are associated with FDAB [28], CMT2C [43, 44], SPSMA [45, 46]. These mutations impair the TRPV4 channel function and/or cell membrane location. Kv1.1 mutations cause various phenotypic seizures [56], T2DM [58] and autosomal dominant hypomagnesemia [59, 60]. Function analysis reveals these Kv1.1 mutants have defects in channel properties and/or subcellular mislocation. Piezo mutations are involved in HX [63] and DA5 [65]. Although it is clear that mutations in mechanosensitive channels can cause diseases in humans, whether mechanosensation plays a major role is often unclear. This is because mechanosensitive channels can also be regulated by many other factors. Therefore, besides the regulation of mechanosensitive channel activity and localization, the exact role of mechanosensation in a variety of physiological and pathological processes is an exciting research direction in the future. The research not only helps us to better understand pathogenic mechanisms in a number of diseases with symptoms beyond touch and hearing, but also reveals the role of gravity and tissue pressure in the development and functioning of our nervous system.

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References

1. Eyckmans J, et al. A hitchhiker's guide to mechanobiology. *Dev Cell*. 2011; 21(1):35–47. [PubMed: 21763607]
2. Tyler WJ. The mechanobiology of brain function. *Nat Rev Neurosci*. 2012; 13(12):867–78. [PubMed: 23165263]
3. Gillespie PG, Walker RG. Molecular basis of mechanosensory transduction. *Nature*. 2001; 413(6852):194–202. [PubMed: 11557988]
4. Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol*. 2006; 7(4):265–75. [PubMed: 16607289]
5. Sachs F. Stretch-activated ion channels: what are they? *Physiology (Bethesda)*. 2010; 25(1):50–6. [PubMed: 20134028]
6. Haswell ES, Phillips R, Rees DC. Mechanosensitive channels: what can they do and how do they do it? *Structure*. 2011; 19(10):1356–69. [PubMed: 22000509]
7. Roudaut Y, et al. Touch sense: functional organization and molecular determinants of mechanosensitive receptors. *Channels (Austin)*. 2012; 6(4):234–45. [PubMed: 23146937]
8. Hao J, et al. Kv1.1 channels act as mechanical brake in the senses of touch and pain. *Neuron*. 2013; 77(5):899–914. [PubMed: 23473320]
9. Corey DP, Hudspeth AJ. Response latency of vertebrate hair cells. *Biophysical Journal*. 1979; 26(3):499–506. [PubMed: 318064]
10. Coste B, et al. Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature*. 2012; 483(7388):176–81. [PubMed: 22343900]
11. McCarter GC, Reichling DB, Levine JD. Mechanical transduction by rat dorsal root ganglion neurons in vitro. *Neuroscience Letters*. 1999; 273(3):179–82. [PubMed: 10515188]
12. Davis MJ, Donovitz JA, Hood JD. Stretch-activated single-channel and whole cell currents in vascular smooth muscle cells. *American Journal of Physiology*. 1992; 262(4 Pt 1):C1083–8. [PubMed: 1373561]
13. Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *Journal of Membrane Biology*. 2001; 184(1):71–9. [PubMed: 11687880]
14. Maroto R, et al. TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol*. 2005; 7(2):179–85. [PubMed: 15665854]
15. Hao J, Delmas P. Multiple desensitization mechanisms of mechanotransducer channels shape firing of mechanosensory neurons. *J Neurosci*. 2010; 30(40):13384–95. [PubMed: 20926665]
16. Nilius B, Honore E. Sensing pressure with ion channels. *Trends Neurosci*. 2012; 35(8):477–86. [PubMed: 22622029]
17. Christensen AP, Corey DP. TRP channels in mechanosensation: direct or indirect activation? *Nat Rev Neurosci*. 2007; 8(7):510–21. [PubMed: 17585304]
18. Arnadottir J, Chalfie M. Eukaryotic mechanosensitive channels. *Annu Rev Biophys*. 2010; 39:111–37. [PubMed: 20192782]
19. Sukharev SI, et al. A large-conductance mechanosensitive channel in *E. coli* encoded by *mscL* alone. *Nature*. 1994; 368(6468):265–8. [PubMed: 7511799]
20. Cosens DJ, Manning A. Abnormal electroretinogram from a *Drosophila* mutant. *Nature*. 1969; 224(5216):285–7. [PubMed: 5344615]
21. Montell C, Rubin GM. Molecular characterization of the *Drosophila* *trp* locus: a putative integral membrane protein required for phototransduction. *Neuron*. 1989; 2(4):1313–23. [PubMed: 2516726]

22. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem.* 2007; 76:387–417. [PubMed: 17579562]
23. Liedtke W, Kim C. Functionality of the TRPV subfamily of TRP ion channels: add mechano-TRP and osmo-TRP to the lexicon! *Cell Mol Life Sci.* 2005; 62(24):2985–3001. [PubMed: 16314934]
24. Palmer CP, et al. A TRP homolog in *Saccharomyces cerevisiae* forms an intracellular Ca²⁺-permeable channel in the yeast vacuolar membrane. *Proc Natl Acad Sci U S A.* 2001; 98(14):7801–5. [PubMed: 11427713]
25. Guler AD, et al. Heat-evoked activation of the ion channel, TRPV4. *J Neurosci.* 2002; 22(15):6408–14. [PubMed: 12151520]
26. Xiao R, Xu XZ. Mechanosensitive channels: in touch with Piezo. *Curr Biol.* 2010; 20(21):R936–8. [PubMed: 21056836]
27. Liedtke W, Friedman JM. Abnormal osmotic regulation in *trpv4*^{−/−} mice. *Proc Natl Acad Sci U S A.* 2003; 100(23):13698–703. [PubMed: 14581612]
28. Lamande SR, et al. Mutations in TRPV4 cause an inherited arthropathy of hands and feet. *Nat Genet.* 2011; 43(11):1142–6. [PubMed: 21964574]
29. Lei L, et al. A TRPV4 channel C-terminal folding recognition domain critical for trafficking and function. *J Biol Chem.* 2013; 288(15):10427–39. [PubMed: 23457335]
30. Watanabe H, et al. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature.* 2003; 424(6947):434–8. [PubMed: 12879072]
31. Sidhaye VK, et al. Transient receptor potential vanilloid 4 regulates aquaporin-5 abundance under hypotonic conditions. *Proc Natl Acad Sci U S A.* 2006; 103(12):4747–52. [PubMed: 16537379]
32. Vriens J, et al. Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. *Proc Natl Acad Sci U S A.* 2004; 101(1):396–401. [PubMed: 14691263]
33. Liedtke W, et al. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell.* 2000; 103(3):525–35. [PubMed: 11081638]
34. Strotmann R, et al. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Cell Biol.* 2000; 2(10):695–702.
35. D'Hoedt D, et al. Stimulus-specific modulation of the cation channel TRPV4 by PACSIN 3. *J Biol Chem.* 2008; 283(10):6272–80. [PubMed: 18174177]
36. Cuajungco MP, et al. PACSINs bind to the TRPV4 cation channel. PACSIN 3 modulates the subcellular localization of TRPV4. *J Biol Chem.* 2006; 281(27):18753–62. [PubMed: 16627472]
37. Suzuki M, et al. Impaired pressure sensation in mice lacking TRPV4. *J Biol Chem.* 2003; 278(25):22664–8. [PubMed: 12692122]
38. Fernandes J, et al. IP₃ sensitizes TRPV4 channel to the mechano- and osmotransducing messenger 5'-6'-epoxyeicosatrienoic acid. *J Cell Biol.* 2008; 181(1):143–55. [PubMed: 18378772]
39. Garcia-Elias A, et al. IP₃ receptor binds to and sensitizes TRPV4 channel to osmotic stimuli via a calmodulin-binding site. *J Biol Chem.* 2008; 283(46):31284–8. [PubMed: 18826956]
40. Alessandri-Haber N, et al. Interaction of transient receptor potential vanilloid 4, integrin, and SRC tyrosine kinase in mechanical hyperalgesia. *J Neurosci.* 2008; 28(5):1046–57. [PubMed: 18234883]
41. Shibasaki K, et al. Effects of body temperature on neural activity in the hippocampus: regulation of resting membrane potentials by transient receptor potential vanilloid 4. *J Neurosci.* 2007; 27(7):1566–75. [PubMed: 17301165]
42. Bai JZ, Lipski J. Differential expression of TRPM2 and TRPV4 channels and their potential role in oxidative stress-induced cell death in organotypic hippocampal culture. *Neurotoxicology.* 2010; 31(2):204–14. [PubMed: 20064552]
43. Deng HX, et al. Scapuloperoneal spinal muscular atrophy and CMT2C are allelic disorders caused by alterations in TRPV4. *Nat Genet.* 2010; 42(2):165–9. [PubMed: 20037587]
44. Landourey G, et al. Mutations in TRPV4 cause Charcot-Marie-Tooth disease type 2C. *Nat Genet.* 2010; 42(2):170–4. [PubMed: 20037586]

45. Zimon M, et al. Dominant mutations in the cation channel gene transient receptor potential vanilloid 4 cause an unusual spectrum of neuropathies. *Brain*. 2010; 133(Pt 6):1798–809. [PubMed: 20460441]
46. Auer-Grumbach M, et al. Alterations in the ankyrin domain of TRPV4 cause congenital distal SMA, scapuloperoneal SMA and HMSN2C. *Nat Genet*. 2010; 42(2):160–4. [PubMed: 20037588]
47. Zhang DX, et al. Transient receptor potential vanilloid type 4-deficient mice exhibit impaired endothelium-dependent relaxation induced by acetylcholine in vitro and in vivo. *Hypertension*. 2009; 53(3):532–8. [PubMed: 19188524]
48. O'Connor CJ, et al. Increased susceptibility of Trpv4-deficient mice to obesity and obesity-induced osteoarthritis with very high-fat diet. *Ann Rheum Dis*. 2013; 72(2):300–4. [PubMed: 23178209]
49. Gu C, Barry J. Function and mechanism of axonal targeting of voltage-sensitive potassium channels. *Prog Neurobiol*. 2011; 94(2):115–32. [PubMed: 21530607]
50. Gu C, et al. The microtubule plus-end tracking protein EB1 is required for Kv1 voltage-gated K⁺ channel axonal targeting. *Neuron*. 2006; 52(5):803–16. [PubMed: 17145502]
51. Xu M, et al. The axon-dendrite targeting of Kv3 (Shaw) channels is determined by a targeting motif that associates with the T1 domain and ankyrin G. *Journal of Neuroscience*. 2007; 27(51):14158–70. [PubMed: 18094255]
52. Barry J, et al. Activation of conventional kinesin motors in clusters by Shaw voltage-gated K⁺ channels. *Journal of Cell Science*. 2013; 126(Pt 9):2027–41. [PubMed: 23487040]
53. Gu C, Gu Y. Clustering and activity tuning of Kv1 channels in myelinated hippocampal axons. *Journal of Biological Chemistry*. 286(29):25835–47. [PubMed: 21602278]
54. Tabarean IV, Morris CE. Membrane stretch accelerates activation and slow inactivation in Shaker channels with S3–S4 linker deletions. *Biophys J*. 2002; 82(6):2982–94. [PubMed: 12023221]
55. Laitko U, Morris CE. Membrane tension accelerates rate-limiting voltage-dependent activation and slow inactivation steps in a Shaker channel. *J Gen Physiol*. 2004; 123(2):135–54. [PubMed: 14744987]
56. Robbins CA, Tempel BL. Kv1.1 and Kv1.2: similar channels, different seizure models. *Epilepsia*. 2012; 53(Suppl 1):134–41. [PubMed: 22612818]
57. Simeone TA, et al. Loss of the Kv1.1 potassium channel promotes pathologic sharp waves and high frequency oscillations in in vitro hippocampal slices. *Neurobiol Dis*. 2013; 54:68–81. [PubMed: 23466697]
58. Zenker J, et al. Altered distribution of juxtaparanodal kv1.2 subunits mediates peripheral nerve hyperexcitability in type 2 diabetes mellitus. *J Neurosci*. 2012; 32(22):7493–8. [PubMed: 22649228]
59. van der Wijst J, et al. Functional analysis of the Kv1.1 N255D mutation associated with autosomal dominant hypomagnesemia. *J Biol Chem*. 2010; 285(1):171–8. [PubMed: 19903818]
60. Glaudemans B, et al. A missense mutation in the Kv1.1 voltage-gated potassium channel-encoding gene KCNA1 is linked to human autosomal dominant hypomagnesemia. *J Clin Invest*. 2009; 119(4):936–42. [PubMed: 19307729]
61. Coste B, et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science*. 2010; 330(6000):55–60. [PubMed: 20813920]
62. Gottlieb PA, Sachs F. Piezo1: properties of a cation selective mechanical channel. *Channels (Austin)*. 2012; 6(4):214–9. [PubMed: 22790400]
63. Zarychanski R, et al. Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis. *Blood*. 2012; 120(9):1908–15. [PubMed: 22529292]
64. Andolfo I, et al. Multiple clinical forms of dehydrated hereditary stomatocytosis arise from mutations in PIEZO1. *Blood*. 2013; 121(19):3925–35. S1–12. [PubMed: 23479567]
65. Coste B, et al. Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause a subtype of Distal Arthrogryposis. *Proc Natl Acad Sci U S A*. 2013; 110(12):4667–72. [PubMed: 23487782]
66. Kim SE, et al. The role of Drosophila Piezo in mechanical nociception. *Nature*. 2012; 483(7388):209–12. [PubMed: 22343891]

67. Dubin AE, et al. Inflammatory signals enhance piezo2-mediated mechanosensitive currents. *Cell Rep.* 2012; 2(3):511–7. [PubMed: 22921401]
68. Barry J, Gu C. Coupling mechanical forces to electrical signaling: molecular motors and the intracellular transport of ion channels. *Neuroscientist.* 2013; 19(2):145–59. [PubMed: 22910031]
69. Barry J, G Y, Jukkola P, O'Neill B, Gu H, Mohler PJ, Rajamani KT, Gu C. Ankyrin-G directly binds to Kinesin-1 to transport voltage-gated Na⁺ channels into axons. *Developmental Cell.* 2014; 28 In press.
70. Xu M, et al. Kinesin I transports tetramerized Kv3 channels through the axon initial segment via direct binding. *Journal of Neuroscience.* 30(47):15987–6001. [PubMed: 21106837]
71. Kindt KS, et al. *Caenorhabditis elegans* TRPA-1 functions in mechanosensation. *Nat Neurosci.* 2007; 10(5):568–77. [PubMed: 17450139]
72. Brierley SM, et al. TRPA1 contributes to specific mechanically activated currents and sensory neuron mechanical hypersensitivity. *J Physiol.* 2011; 589(Pt 14):3575–93. [PubMed: 21558163]
73. Spassova MA, et al. A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A.* 2006; 103(44):16586–91. [PubMed: 17056714]
74. Feng NH, et al. Transient receptor potential vanilloid type 1 channels act as mechanoreceptors and cause substance P release and sensory activation in rat kidneys. *Am J Physiol Renal Physiol.* 2008; 294(2):F316–25. [PubMed: 18032552]
75. Morita H, et al. Membrane stretch-induced activation of a TRPM4-like nonselective cation channel in cerebral artery myocytes. *J Pharmacol Sci.* 2007; 103(4):417–26. [PubMed: 17420615]
76. Numata T, Shimizu T, Okada Y. TRPM7 is a stretch- and swelling-activated cation channel involved in volume regulation in human epithelial cells. *Am J Physiol Cell Physiol.* 2007; 292(1):C460–7. [PubMed: 16943238]
77. Kang L, et al. *C. elegans* TRP family protein TRP-4 is a pore-forming subunit of a native mechanotransduction channel. *Neuron.* 67(3):381–91. [PubMed: 20696377]
78. Berrouit J, Jin M, O'Neil RG. Critical role of TRPP2 and TRPC1 channels in stretch-induced injury of blood-brain barrier endothelial cells. *Brain Res.* 2012; 1436:1–12. [PubMed: 22192412]
79. Zhao H, Sokabe M. Tuning the mechanosensitivity of a BK channel by changing the linker length. *Cell Res.* 2008; 18(8):871–8. [PubMed: 18663377]
80. Maingret F, et al. Mechano- or acid stimulation, two interactive modes of activation of the TREK-1 potassium channel. *J Biol Chem.* 1999; 274(38):26691–6. [PubMed: 10480871]
81. Brohawn SG, del Marmol J, MacKinnon R. Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K⁺ ion channel. *Science.* 2012; 335(6067):436–41. [PubMed: 22282805]
82. Lin W, et al. Dual stretch responses of mHCN2 pacemaker channels: accelerated activation, accelerated deactivation. *Biophys J.* 2007; 92(5):1559–72. [PubMed: 17142286]
83. Beyder A, et al. Mechanosensitivity of Nav1.5, a voltage-sensitive sodium channel. *J Physiol.* 2010; 588(Pt 24):4969–85. [PubMed: 21041530]
84. Kraichely RE, et al. Lysophosphatidyl choline modulates mechanosensitive L-type Ca²⁺ current in circular smooth muscle cells from human jejunum. *Am J Physiol Gastrointest Liver Physiol.* 2009; 296(4):G833–9. [PubMed: 19179622]
85. Calabrese B, et al. Mechanosensitivity of N-type calcium channel currents. *Biophys J.* 2002; 83(5):2560–74. [PubMed: 12414690]
86. Hilaire C, et al. Neurotrophin-4 modulates the mechanotransducer Cav3.2 T-type calcium current in mice down-hair neurons. *Biochem J.* 2012; 441(1):463–71. [PubMed: 21892923]
87. Zhang WK, et al. Mechanosensitive gating of CFTR. *Nat Cell Biol.* 2010; 12(5):507–12. [PubMed: 20400957]
88. Hong K, Driscoll M. A transmembrane domain of the putative channel subunit MEC-4 influences mechanotransduction and neurodegeneration in *C. elegans*. *Nature.* 1994; 367(6462):470–3. [PubMed: 8107806]
89. Arnadottir J, et al. The DEG/ENaC protein MEC-10 regulates the transduction channel complex in *Caenorhabditis elegans* touch receptor neurons. *Journal of Neuroscience.* 31(35):12695–704. [PubMed: 21880930]

90. McIlwrath SL, et al. The sensory mechanotransduction ion channel ASIC2 (acid sensitive ion channel 2) is regulated by neurotrophin availability. *Neuroscience*. 2005; 131(2):499–511. [PubMed: 15708491]
91. Singh P, et al. N-methyl-D-aspartate receptor mechanosensitivity is governed by C terminus of NR2B subunit. *J Biol Chem*. 2012; 287(6):4348–59. [PubMed: 22179603]
92. Bao L, Sachs F, Dahl G. Connexins are mechanosensitive. *Am J Physiol Cell Physiol*. 2004; 287(5):C1389–95. [PubMed: 15475518]
93. Pan B, et al. TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron*. 2013; 79(3):504–15. [PubMed: 23871232]
94. Jang Y, et al. Axonal neuropathy-associated TRPV4 regulates neurotrophic factor-derived axonal growth. *J Biol Chem*. 2012; 287(8):6014–24. [PubMed: 22187434]
95. Rasband MN, Trimmer JS. Subunit composition and novel localization of K⁺ channels in spinal cord. *Journal of Comparative Neurology*. 2001; 429(1):166–76. [PubMed: 11086297]
96. Nilius B. Pressing and squeezing with Piezos. *EMBO Rep*. 2010; 11(12):902–3. [PubMed: 21052093]

Figure 1. TRPV4 structural diagram, channel activity and expression in DRG

(A) TRPV4 structural diagram. TM segments and ankyrin repeats are shown as teal barrels and blue barrels, respectively. The mutation sites for FDAB are shown as orange circles. The mutation sites for the spectrum of skeletal dysplasias are shown as red circles. The mutation sites for peripheral neuropathies are shown as purple circles [28]. (B) Current traces activated by 4 α -PDD recorded from WT (the top panel), TRPV4 KO (the middle panel) hippocampal neurons. The current-voltage relationship in both WT and KO neurons is provided in the bottom panel [41]. (C) Representative current trace activated by hypotonicity with Ca²⁺ [33]. (D) TRPV4 is expressed in adult mouse DRG neurons. TRPV4 proteins enrich in soma and nerve fibers. Scale bar, 25 μ m [94].

Figure 2. Kv1 and piezo channels: Schematic structures, mechanically activated currents and immunohistochemistry

(A) Kv1 α subunit structural diagram [49]. (B) Currents induced by mechanical stimuli using CsCl pipette solution at the indicated holding potentials (20 mV increments) [8]. (C) Double staining of Kv1.2 (green) and Nav channel (red) in myelinated axons in rat spinal cord [95]. (D) Schematic structure of Piezo1 with 30 transmembrane domains [96]. (E) Mechanosensitive currents recorded from N2A cells (upper) and HEK293T cell expressing Piezo1 (bottom), respectively [61]. (F) In situ hybridization images for Piezo2 in DRG neurons using antisense (left) and sense probes (right) [61].

Table 1

Eukaryotic Mechanosensitive Ion Channels and Receptors.

Channel family	Channel isoforms	References
TRP channels	TRPA1	[71, 72]
	TRPC1	[14]
	TRPC6	[73]
	TRPV1	[74]
	TRPV4	[27, 33, 37]
	TRPM4	[75]
	TRPM7	[76]
	TRPN	[77]
	TRPP2	[78]
K⁺ channels	Shaker (Kv1.1)	[8]
	Ca ²⁺ -activated K ⁺ (BK)	[79]
	TREK1 (K _{2p} 2.1)	[80]
	TRAAK (K _{2p} 4.1)	[81]
	HCN2	[82]
Na⁺ channels	Nav1.5	[83]
Ca²⁺ channels	L-type	[84]
	N-type	[85]
	T-type	[86]
Cl⁻ channels	CFTR	[87]
DEG/ENaC	C. elegans MEC (MEC-4, MEC-10)	[88, 89]
	ASIC	[90]
Other channels and receptors	Piezo	[10]
	NMDA receptor	[91]
	Connexin (Gap junction)	[92]
	TMC1 and TMC2	[93]