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Review

Biophysics and Modeling of Mechanotransduction in Neurons: A Review

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Abstract: Mechanosensing is a key feature through which organisms can receive inputs from the environment and convert them into specific functional and behavioral outputs. Mechanosensation occurs in many cells and tissues, regulating a plethora of molecular processes based on the distribution of forces and stresses both at the cell membrane and at the intracellular organelles levels, through complex interactions between cells' microstructures, cytoskeleton, and extracellular matrix. Although several primary and secondary mechanisms have been shown to contribute to mechanosensation, a fundamental pathway in simple organisms and mammals involves the presence of specialized sensory neurons and the presence of different types of mechanosensitive ion channels on the neuronal cell membrane. In this contribution, we present a review of the main ion channels which have been proven to be significantly involved in mechanotransduction in neurons. Further, we discuss recent studies focused on the biological mechanisms and modeling of mechanosensitive ion channels' gating, and on mechanotransduction modeling at different scales and levels of details.

Keywords: mechanosensing; mechanotransduction; ion channels; neurons; modeling; atomistic modeling; molecular dynamics; multiscale; biomechanics; mechanobiology

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1. Introduction

Mechanosensation is among the most important sensory functions in animals. Indeed, animals' navigation, communication, and survival are critically dependent on their ability to perceive the external and internal forces. To reach this purpose, animals have evolved specialized sensory cells that allow the conversion of mechanical stimuli into electrical signals to be processed by the nervous system. This conversion process is usually referred to as "mechanotransduction", and constitutes the essential basis of touch, hearing, proprioception, and of all the other biological functions that rely on force detection. Mechanotransduction at the single-cell level is allowed by the combination of the morphological and molecular features of the cell and by complex interactions between cell membrane structures, cytoskeleton, and extracellular matrix (ECM). Although several molecular mechanisms have been shown to be affected by the mechanical state of cells, only a small amount contributes to direct mechanical sensing of external stimuli. In particular, the main pathway through which organisms achieve mechanosensing accounts for specialized sensory neurons opportunely enriched with mechano-electrical transduction (MeT) complexes and interfaced with different types of biological structures. A typical example is represented by mammalian hair cells in the cochlea, equipped with hair bundles composed of several rows of variable-length stereocilia. The stereocilium tip is able to convert mechanical forces into electrical signals thanks to MeT complexes formed by stretch-gated ionic channels, allowing fast and effective transduction of the auditory stimulus. This general example

shares common features with a large amount of mechanical–biological sensors based on neuronal transduction. In this review, we focus on a specific aspect of the mechanosensing investigation, that is the multiscale theoretical and computational description of systems and mechanisms. We first present an overview of the main mechanosensitive (MS) channels which have been shown to have an important role in mechanosensation both in simple organisms and in mammals, including the Degenerin/Epithelial Sodium Channels/Acid Sensing Channels (DEG/ENaC/ASIC), Two Pore domain Potassium (K2P), Piezo, Anoctamin, and Transient Receptor Potential (TRP) superfamilies, also discussing the most credited biological and physiological models of ion channel gating. Secondly, concerning the theoretical modeling, we discuss different scales and methods developed to investigate mechanotransduction. All presented methods have multiscale components. Starting from the most recent advances in mechanosensitive ion-channel modeling based on all-atom and/or coarse-grained molecular dynamics, we move to the description of continuum models for pressure-induced channel gating. Finally, we present methods coupling the biomechanics to the electrophysiological modeling of neuron response, with possible applications to biological tissues of increasing size and complexity. In summary, this contribution aims to dissect the molecular basis of mechanotransduction, to present the current opinions on its biological and physiological regulation, and to discuss the physico-mathematical frameworks introduced in the literature to built descriptive and predictive models to explain mechanosensation at the molecular, micro, and macro scales. Literature was searched primarily on PubMed and Google Scholar databases (and on the journals' webpages in some cases), including different specific keywords for each topic on mechanosensation afforded in this review, such as "mechanosensitive ion channels","mechanosensitive" + *channel name*, "mechano-gated ion channels", "mechano-gated" + *channel name*, "neuron mechanosensation/mechanosensing/mechanotransduction", "atomistic model" + *channel name*, "molecular dynamics" + *channel name*, "multiscale/multiphysics model mechanosensation/mechanosensing/mechanotransduction" (where the/symbol denotes all the possible combination of the words). We restricted our research to the papers published in the last twenty years.

The paper is organized as explained below. Section 2 deals with the mechanosensitive (MS) ion channels expressed in neurons, also discussing their functional role and gating mechanisms. In Section 3, we review different modeling approaches that have been applied to describe animals' mechanosensing, from the atomistic to the tissue scale. In Sections 3.1 and 3.2, we describe the recent advances in particle-based modeling of MS ion channels coupled to the lipid membrane and the continuum models of MS channels coupled to the membrane, respectively. Section 3.3 describes multiscale modeling studies merging biomechanics to neurons' electrical dynamics at different levels of detail. Finally, Section 4 is devoted to conclusions and outlooks.

2. Ion Channels in Neurons Mechanosensing

Specialized mechanosensory cells and neurons can sense a wide variety of stimuli, including shear stress, vibrations, and sound waves, that induce perturbations of the cellular environment. Mechanical stimuli are translated into electrical signals by a specific category of ion channels, the mechanosensitive ion channels, endowed with the ability to open or close in response to the applied force. The heterogeneity of the mechanical stimuli and of the channels involved in mechanotransduction have manifested the need for specific criteria to establish whether an ion channel is mechanosensitive or not. Four different criteria have been proposed and applied to classify MS channels [1].

1. *Temporal and spatial expression in mechanosensory cells.* The channel must be expressed in specialized mechanosensory cells, and should not be necessary for cell maturation or integrity [1].
2. *Direct involvement in the mechanical response.* The channel must be critical for mechanosensitivity, in the sense that its loss abolishes the ability of the cell to respond to mechanical stimulations. The fulfillment of this criterion is a necessary

but not sufficient condition to establish the mechanosensitive nature of a candidate channel. Indeed, its participation in mechanosensation could be indirect, as in the case of channels involved in cell development or in the signaling downstream of the stimulus [1]. Furthermore, a cell can compensate for the loss of a subunit by forming heteromers with other members of the same family of channels [2].

3. *Channel alterations alter the mechanical response.* Alterations of the channel biophysical properties, such as ion selectivity, activation, or inactivation, result in alterations of the physiological response to mechanical stimuli. In addition, in this case, the satisfaction of the criterion does not guarantee that the considered channel is mechanosensitive; indeed, multiple auxiliary subunits could modify the biophysical properties of the channel [2].
4. *Heterologous expression induces mechanical responses in the host cell.* This condition is one of the most difficult to meet. Various pore-forming subunits do not show mechanosensitive properties when heterologously expressed, as their ability to sense mechanical forces could be determined by the specific lipid composition of the bilayer [2] or by auxiliary subunits that anchor the channel to the intracellular matrix [2].

The majority of the identified MS channels belongs to the following families: TRP, DEG/ENaC/ASIC, K2P, Piezo, and Anoctamin.

Two models have been proposed to explain how the channel responds to mechanical stimuli: the "force-from-tether" and the "force-from-lipid" model. The main difference between the two models is that in the first, the channel has to be bound to intra/extracellular structures such as the cytoskeleton and/or the extracellular matrix [3] (Figures 1B and 2B), while in the second, the force is transmitted to the channels directly through the lipid bilayer (Figure 3B,D).

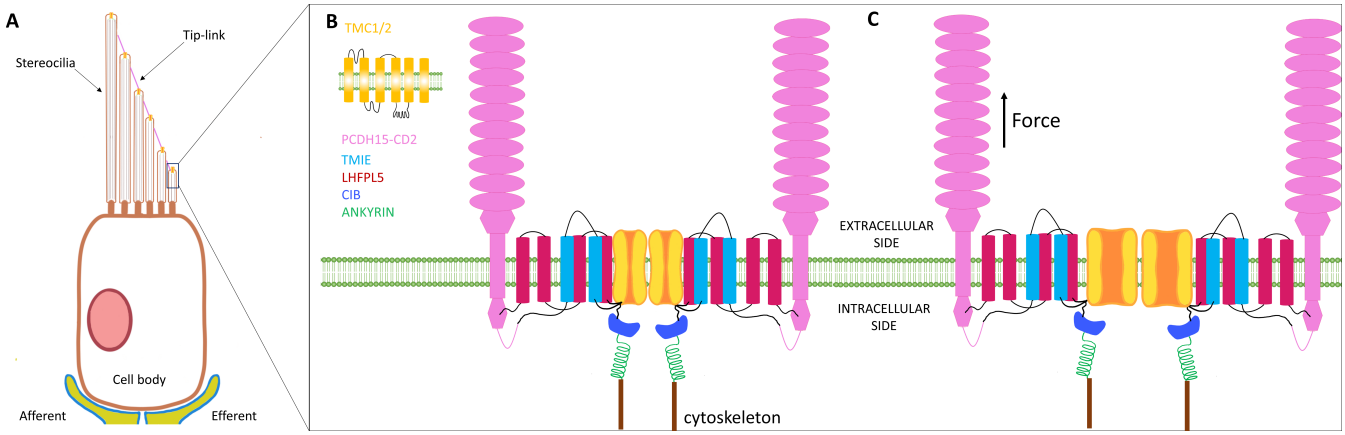

Figure 1. Mechanotransduction in mammalian hair cells. (**A**) Schematic representation of mammalian hair cells. Each hair cell is equipped with a set of interconnected stereocilia with variable length. The bending of the highest stereocilium induces the defection of the other cilia in the bundle and initiates the auditory transduction processes. (**B**,**C**) Model for mechano-electrical transduction (MeT) (adapted from [4,5]). The conversion of mechanical stimuli into electrical signal happens at the tip of the stereocilia where MeT complexes are localized. MeT complexes have specular stoichiometry and are composed of two Transmembrane Channels (TMCs) that interact with the tip link (PCDH15-CD2, in pink) through the TMIE and the LHFPL5 proteins [5–11]. In addition, the channel directly interacts with the Calcium Binding Protein-2 (CIB-2) [9,12]. A recent study on the *Caenorhabditis elegans* (*C. elegans*) model organism showed that the connection of the complex with the cytoskeleton is realized through the binding of the CIB protein with ankyrin protein UNC-44 [9]. However, in mammals, this aspect is still poorly explored, and the target of CIB-2 proteins is unknown.

In the following, we briefly describe the MS ion channels that have been identified in the literature, with a particular focus on their functions in mechanosensory neurons. The complete list of the mechanosensitive channels here reviewed is reported in Tables 1 and 2.

2.1. DEG/ENaC/ASIC

The DEG/ENaC/ASIC is a superfamily of voltage-insensitive, Na^+ permeable, and amiloride-sensitive channels. These channels are found in both vertebrates and invertebrates and include the nematode degenerin (DEG), the *Drosophila* Pickpocket (ppk), Ripped Pocket (rpk) and Balboa, and the vertebrate Epithelial Sodium Channels (ENaC) and Acid-Sensitive Channels (ASIC). Channels of the DEG/ENaC family share a similar structure with two transmembrane domains and a large extracellular domain [13–17] (Figure 2A) and assemble in homo- or hetero-trimers with a chalice-like shape [13,15,18,19]. DEG/ENaC channels are involved in vertebrate and invertebrate mechanosensation.

Among the most studied mechanosensitive DEG/ENaC/ASIC channels there are the *C. elegans* MEC-4 and MEC-10. MEC-4 and MEC-10 assemble in homo- or hetero-trimers and are expressed in mechanosensory neurons responsible for touch sensation [18–20] (Figure 2A). DEG/ENaC mutants display various defects in locomotion and nociception, confirming the critical role of DEG/ENaC channels in mechanonociception, proprioception, and also ultrasound response [19,21–23]. In addition, when heterologously expressed in *Xenopus* oocytes, MEC-4 and MEC-10 channels could respond to laminar shear stress with a predominant role of MEC-10 on MEC-4 [24,25].

Drosophila expresses three DEG/ENaC channels encoded by the *pickpocket*, *ripped pocket*, and *balboa* genes [13,26]. These channels are expressed in nociceptive class IV multidendritic (md) neurons of the fly peripheral nervous system. PPK and Balboa can form heterotrimeric channels that play a critical role in mechanonociception [13]. In addition, PPK mutant flies display impaired locomotion [27].

Concerning the mammalian members of the superfamily, ASIC channels are encoded by the genes *Accn1-4* and are widely expressed throughout the sensory neurons and afferent mechanoreceptors, such as Panician, Merkel, and Meissner corpuscles [28–31], Ruffini endings [32], aortic baroreceptor neurons [33], visceral mechanoreceptors [34], and dorsal root ganglia neurons [35–37]. Mammalian ASICs participate in cutaneous touch [30], mechanonociception [29,38] and proprioception [29,38], autonomic regulation of gastrointestinal function and blood circulation [29,33,34,38–40]. Moreover, Barth and Fronius have demonstrated that human ASIC channels generate shear force-induced currents in specific environmental conditions, such as acidic PH or presence of non-proton ligands, suggesting that human ASICs, similarly to the nematode DEGs, are intrinsically able to detect mechanical stimuli [41].

There are several pieces of evidence that DEG/ENaC/ASIC channels give rise to mechanically activated currents. However, the gating mechanisms are still not completely understood. The nematode DEG channels are proposed to rely on a "force-from-tether" model to transduce mechanical stimuli [42]. In this scheme, the pore-forming subunits (MEC-4 and MEC-10) may interact with the extracellular proteins MEC-1, MEC-5, and MEC-9 [43] and with the special tubulins MEC-7 and MEC-12 [42,44]. However, evidence of in vivo co-localization of MEC-4 with MEC-5 proteins and of MEC-7 and MEC-12 with the complex is still missing [42,45]. Moreover, MEC-4 and MEC-10 subunits associate with the stomatin-related protein MEC-2 and the paraoxonase-like protein MEC-6, which are critical regulators of the channel activity [20,46–48]. Concerning the mammalian ASIC channel, both the "force-from-lipid" and the "force-from-tether" models have been proposed to explain the mechanical gating [38]. Similarly to their nematode homologs, ASIC channels associate with stomatin-domain proteins, STOLM1 and STOLM3 [49–54], suggesting that they could rely on a force-from-tether mechanism. However, this aspect still deserves further investigation, in particular, to highlight eventual interactions with extracellular and intracellular matrix proteins.

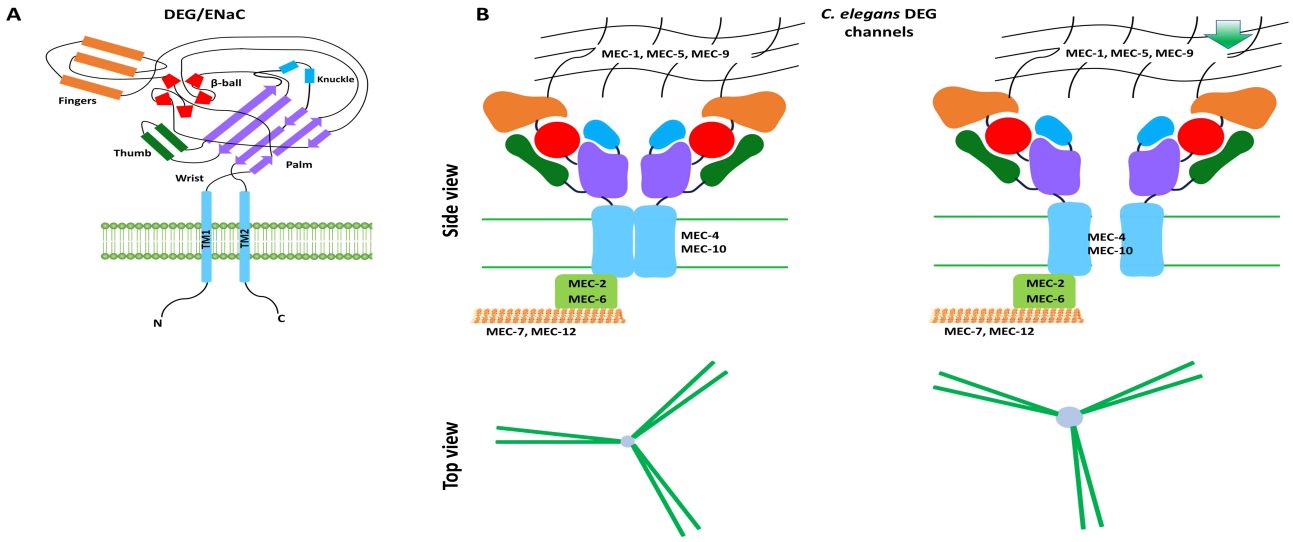

Figure 2. Degenerin (DEG)/Epithelial Sodium Channel (ENaC)/Acid-Sensitive Channel (ASIC) structure and gating. (**A**) Representative cartoon of DEG/ENaC/ASIC channel monomers. DEG/ENaC/ASIC channel monomers share a similar structure composed of two transmembrane domains, N and C terminals, and a large extracellular loop resembling a hand. The wrist connects the transmembrane domain to the seven stranded β-sheet palm and the α-helical thumb, while the β-ball is surrounded by the fingers and knuckle [14–16] (adapted from [15,55]). (**B**) Hypothetical gating mechanism of *C. elegans* DEG channels. The molecular bases of mammalian ASIC channels mechanical gating are still poorly explored. In the case of *C. elegans*, it has been established that the pore-forming subunits MEC-4 and MEC-10 interact with the paraoxonase-like and the stomatin-like proteins MEC-2 and MEC-6 [46–48] and with the special tubulins MEC-7 and MEC-12 [44]. In addition, the extracellular matrix components, MEC-1, MEC-5, and MEC-9, might be critical for force transduction [42,43] by directly associating with the pore-forming subunits (adapted from [38,55]).

2.2. K2P

The two-pore domain K2P channels are a family of tetrameric channels with two-pore forming loops [56] (Figure 3A,B). Some members of the family, such as TREK-1, TREK-2, and TRAAK, form thermosensitive and mechanosensitive channels expressed in different organs and tissues, including the central and peripheral nervous system [57–60]. In particular, TREK and TRAAK channels are expressed in dorsal root ganglia neurons where they regulate the threshold for mechanical responses [56,61]. Mice lacking K2P channels do not show significant sensory function impairment; rather they show hypersensitivity to mechanical and thermal stimuli (mechanical and thermal allodynia) [58,59]. When heterologously expressed, TREK and TRAAK show stretch-activated currents [57,60,62,63]. These currents are equally induced by the application of positive and negative pressure and are not altered by the disruption of intracellular components (e.g., the cytoskeleton) [60,62,63]. Moreover, these currents do not require the integrity of intracellular components, suggesting that K2P channels mechanical gating relies on force-from-lipid mechanism [60,62,63] (Figure 3B). *Drosophila* orthologue of TREK channels, ORK1, is critical for the regulation of sleep and cardiac rhythm, similarly to its mammalian counterpart [64–66]. However, in contrast to mammalian K2P, there is still no report of a direct activation by mechanical stimuli.

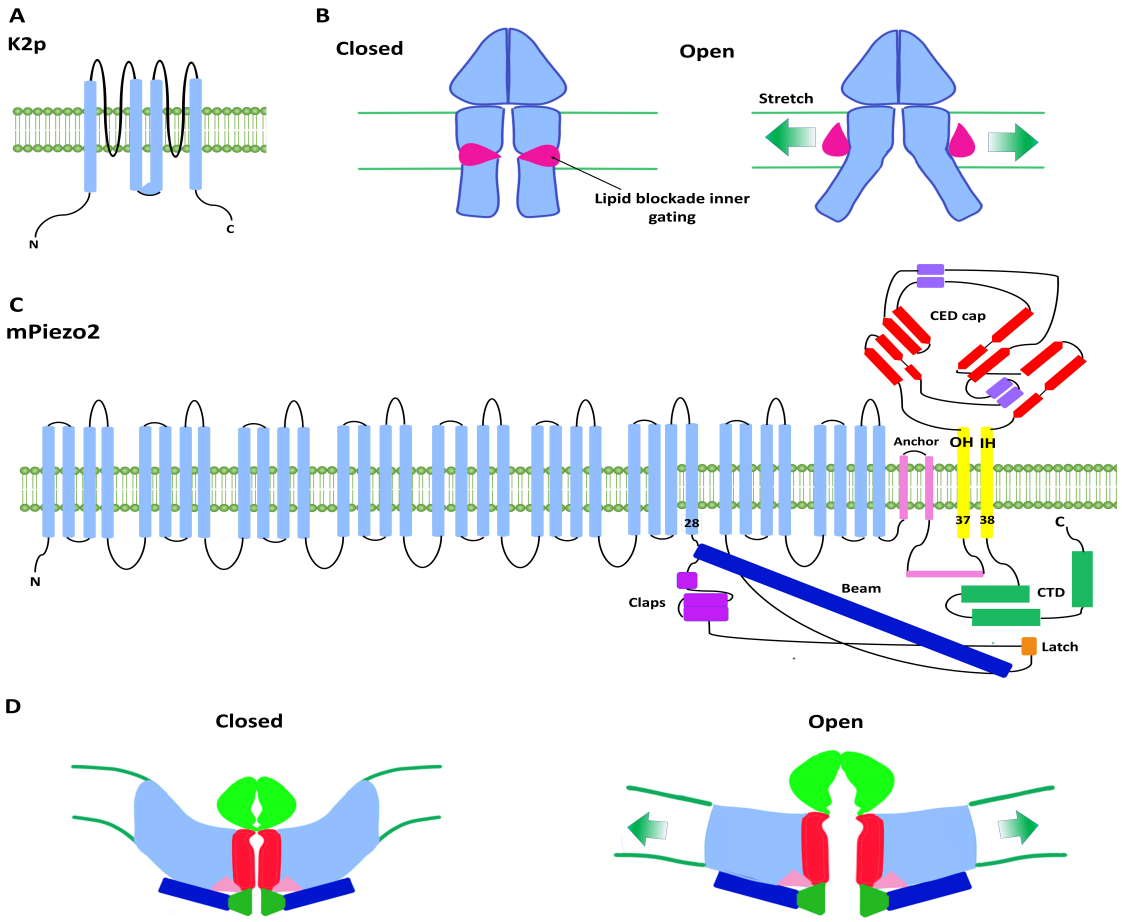

Figure 3. Two Pore domain Potassium channels (K2P) and Piezo channels structure and gating. (**A**) Schematic diagram of K2P monomers. K2P channel monomers have four transmembrane domains (light blue) and two pore-forming regions (in black). They assemble in tetramers to form potassium channels such as the mechanosensitive TREEK-1/2 and TRAAK (adapted from [3]). (**B**) Model of mechanosensitive K2P gating. Mechanosensitive K2P channels rely on the force-from-lipid model to sense mechanical stimuli. The channel opens in response to membrane stretch thanks to its direct interaction with membrane lipids (fuchsia) (adapted from [67]). (**C**) Cartoon representation of mouse Piezo2 channel monomers. Piezo2 channels are predicted to have up to 38 transmembrane domains [3]. In addition to the transmembrane domains, Piezo channels possess also a C-Terminal-Extracellular domain (CED) that constitutes the central dome, as well as a beam and an anchor that connect the central pore of the channel with the mechanotransduction module (adapted from [68]). (**D**) Model of Piezo channel gating. Piezo channels assemble in homotrimeric structures that sense the stretch of the lipid bilayer (adapted from [69]).

2.3. PIEZO

Piezo channels are large homotrimeric channels with a peculiar structure that resembles a three-bladed propeller [70] (Figure 3C). Vertebrates have two Piezo channels, Piezo-1 and Piezo-2, that, despite the similar structure, share only 42% of sequence homology [68]. Piezo-1 is mainly found in non-neuronal cells [55]. In contrast, Piezo-2 channels are primarily expressed in mechanosensory cells (Merkel cells, hair follicles, and hair cells of the auditory system) and neurons (dorsal root ganglion neurons) [71–73]. The two channels have only one homologue in *Drosophila* and *C. elegans* [74]. The *Drosophila Dmpiezo* gene shows an expression pattern similar to that of mice (it is found in non-neuronal and neuronal tissues), and the corresponding channel plays a critical role in noxious mechanosensation [75]. In *C. elegans*, Piezo channels are encoded by the gene *pezo-1*, which is widely expressed throughout the reproductive tissues [76].

Piezo channels generate mechanically-activated currents in response to different mechanical stimuli, including pipet pressure [74,77], Myosin-II mediated internal forces [78],

optical tweezers [79], nanoparticles [80], and laminar shear stress [81,82]. There are several recent works devoted to the study of the gating mechanisms of Piezo channels. All these works report a direct activation of Piezo channels with membrane indentation, explained by the force-from-lipid model [70,71,74,75,83–85] (Figure 3D). Moreover, an important contribution to Piezo gating comes from the actin cytoskeleton [86,87]. Works by Cox et al. [86] and Retailleau et al. [87] suggest that it plays a mechanoprotective role, regulating the activation of the channels and the membrane tension [86–88]. In addition, Zheng et al. [85] have recently shown that Piezo-1 and Piezo-2 differ in the activation mechanisms. Indeed, while Piezo-1 channels are extremely sensitive to cold-induced membrane stiffness alterations, Piezo-2 channels seem to be unaffected by such changes, suggesting that the force-from-lipid is not the principal activation pathway for Piezo-2 [85].

2.4. Anoctamin Superfamily

The Anoctamin proteins superfamily includes cation and anion channels and lipid scramblases [89]. The superfamily is divided into seven families: the Anoctamin (ANO), the Transmembrane Channel (TMC), the Ca^{2+}-permeable Stress-gated Cation Channel (CSC), the Anoctamin-Like (ANO-L), the Transmembrane Channel-Like (TMC-Like), and the Ca^{2+}-permeable Stress-gated Cation Channel-Like (CSC-L1 and CSC-L2) families [89]. The last four families (ANO-L, TMC-L, CSC-L1, and CSC-L2) are still functionally uncharacterized; while other channels belonging to this superfamily have been reported to be mechanosensitive, such as the plat OSCA channels, the TMC channels, and the TMEM63 channels [89].

TMC channels are expressed both in vertebrates and invertebrates, and they play a critical role in mechanotransduction [55,90]. The TMC family is composed of two channels, TMC-1 and TMC-2, that are essential for hearing in different organisms. In mammals, TMC-1 and TMC-2 are expressed in the stereocilia of the inner ear hair cells, where they are part of an MeT complex (Figure 1) [91,92]. TMC channels are important also for *Drosophila* proprioception and locomotion. In *Drosophila* larvae, DmTMC-1 and DmTMC-2 are expressed in the sensory neurons that provide sensory feedback for locomotion, such as the class I and class II dendritic arborization neurons and bipolar dendrites neurons [93,94]. TMC channels are critical also for *Danio rerio* water motion detection and hearing, with a prominent role of TMC-2 instead of TMC-1 [8,95]. *C. elegans* possesses two TMC genes: *tmc-1* and *tmc-2* that are expressed in mechanosensory neurons [9,96]. Recent studies, based on cryo-EM and size-exclusion chromatography, confirmed that both TMCs are pore-forming subunits that assemble in dimers [10,90,97]. However, TMC channels alone are not sufficient to transduce mechanical stimuli into electrical currents and couple with other proteins forming MeT complexes [6,8–10] (Figure 1).

2.5. TRP Superfamily

Transient Receptor Potential channels are a superfamily of channels firstly discovered in *Drosophila*. TRP channels are found in yeast, fungi, and animals, where they are involved in processing a wide variety of sensory stimuli, such as temperature, sound, light, chemicals, osmotic changes, and mechanical forces [98].

Based on structural homology, TRP channels are divided into nine subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin), TRPN (no mechanoreceptor potential C, NOMPC), TRPS (soromelastin), and TRPY (yeast) [99,100]. Except for TRPN and TRPS, which are exclusively expressed in invertebrates, at least one member per subfamily is expressed in both vertebrates and invertebrates [99,101]. Most TRP channels are non-selective cation channels, structurally similar to voltage-gated channels (VGCs) [100,101]. The channels have 6 transmembrane domains (TM), a pore loop domain between TM5 and TM6, a cytosolic (N), and a carboxy (C) termini. In addition, some classes of TRP channels (i.e., TRPN, TRPC, TRPA) possess ankyrin repeats that may be important to define their mechanosensitive properties [102] (Figure 4). TRP channels assemble in homo- or hetero-tetramers [3,101] in which the