Plug-N-Play: Mechanotransduction Goes Modular

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Mechanosensitive ion channels initiate sensory signals by converting mechanical information into electrochemical signals. In this issue of *Neuron* (Zhao et al., 2016), a data-rich structure-function study on mammalian mechanosensitive Piezo channels reveals a modular protein architecture that includes a central pore module surrounded by a force-sensing module.

Mechanosensation may be one of the most primitive senses and likely arose multiple times early in evolution (Martinac and Kloda, 2003). Imagine the first microbes navigating through the primordial seas. Microbes that could sense their physical surroundings and change course when they bumped into an immovable object or an osmotically unfavorable environment likely had a distinct evolutionary advantage over those that could not. Having arisen early, and perhaps on several occasions, implies that evolution has had eons to tweak mechanosensation and adapt it for a variety of higher-order functions (García-Añoveros and Corey, 1997). Indeed, our senses of touch, hearing, balance, and proprioception all depend on sophisticated and exquisitely sensitive mechanically gated ion channels, which function to transduce mechanical stimuli into electrical signals that are transmitted to the brain. In this regard, the number of senses that rely on mechanotransduction easily exceeds the number of senses that rely on more recent developments, phototransduction, for example. In addition to our fundamental senses, numerous internal physiological processes, such as blood pressure regulation, osmoregulation, muscle and tendon reflexes, bladder extension, and so forth, also depend on mechanotransduction. The identity and molecular mechanisms of these force sensors have long remained a mystery. In this issue of *Neuron*, an exciting new study from the lab of Bailong Xiao (Zhao et al., 2016), together with a number of recent structural and functional studies on force-sensing ion channels, is transforming the field of mechanobiology, providing provocative new insight into the molecular mechanisms of mechanosensation.

Piezo1 and Piezo2 are ion channels critical for a wide range of mechanosensitive processes including gentle touch, nociception, and vascular architecture development (Ranade et al., 2015). They have been shown to induce ion channel activity when reconstituted in lipid bilayers (Coste et al., 2012) and are gated by cellular membrane tension when expressed in heterologous cell lines (Lewis and Grandl, 2015). In a recent report (Ge et al., 2015), the authors of the current study described a cryo-EM (cryoelectron microscopy) structure for mouse Piezo1. The structure, albeit at a modest resolution of ~5Å, revealed that Piezo1 assembles as a trimer with 14 transmembrane (TM) domains in each subunit. The structure includes a central pore region and three peripheral domains with a "propeller-like" architecture. Interestingly, being trimers, the central pore module of Piezos display some structural resemblance to those of acid-sensing ion channels (ASICs) and ATP-gated P2X4 receptors, despite a lack of sequence homology. The three peripheral domains of Piezo1, which contain the extracellular "blades," the transmembrane "anchor-domains," and the intracellular "beam-domains," are absent in the other trimeric ion channels. These peripheral modules have been proposed to be responsible for mechanical gating.

For the current study, the authors investigated the functional role of the structurally defined Piezo1 domains. They established that Piezo1, despite its large size—with the highest number of TM domains among known ion channels—in fact contains an ion-conducting pore encoded only by the last ~360 residues (p.2,190–2,547). They used chimeras between mouse and fly Piezos, mutagenesis, and cysteine modification to examine the effects of manipulating various parts of the putative C-terminal ion conduction pathway on conductance, selectivity, and channel pharmacology.

The proposed pore module of Piezo1 is comprised of what the authors define as an outer helix (OH), a C-terminal extracellular domain (CED), an inner helix (IH), and an intracellular C-terminal domain (CTD; Ge et al., 2015). Together with the structural data, these new data suggest that the relatively large CED (~240 residues) forms an extracellular "cap" structure with fenestrations lined by negatively charged residues that select cation over anion entry into the pore. The authors reconstituted fragments of Piezo1 and demonstrated sufficiency of the putative pore lining IH and adjacent OH to form a pore, and they used cysteine modification to demonstrate accessibility to certain residues in the putative pore. Mutagenesis of two highly conserved, adjacent, negatively charged residues in the intracellular CTD domain (~70 residues) affected the calcium selectivity of the channel as well as block by the known Piezo blocker, ruthenium red, suggesting a binding site for calcium and charged blockers in the intracellular vestibule.

The authors tested the intriguing hypothesis that the nonconducting, peripheral domain of Piezo1 (p.1–2,190) is the force-sensing module. For this experiment, the authors generated a chimera of the mouse Piezo1 by replacing the

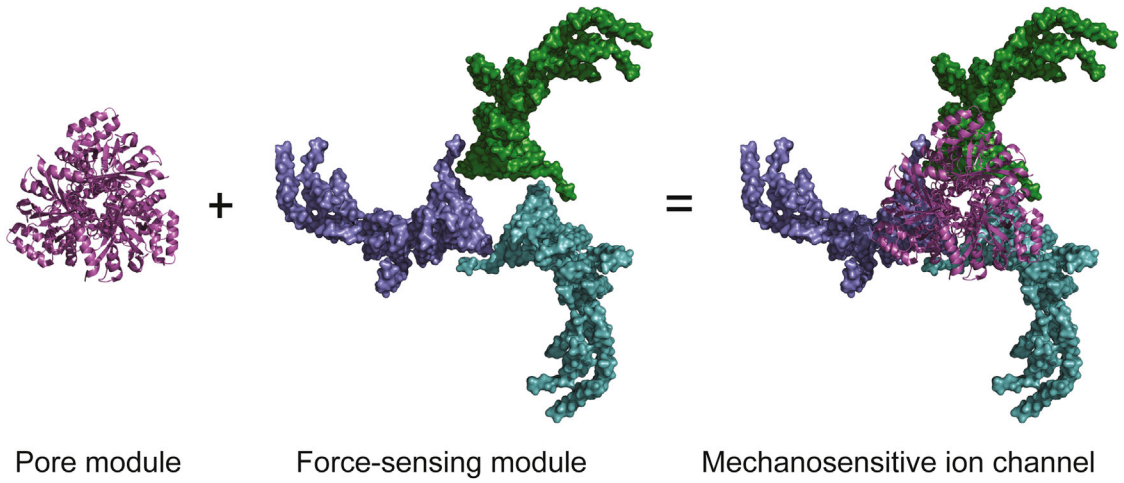

Figure 1. Modular Architecture of a Mechanosensitive Channel
The central pore module from either Piezo1 (shown) or from ASIC1, together with the peripheral force-sensing module from Piezo1, forms a mechanosensitive ion channel. Structural representations (Piezo1 PDB: 3JAC) were generated using the PyMOL molecular graphics system.

C-terminal pore region with the mechanoinsensitive ASIC1 channel. Remarkably, the resulting Piezo1-ASIC1 chimera was mechanosensitive, opening in response to membrane indentation in transfected HEK293 cells. These data support the hypothesis that the non-pore-forming region of Piezo1 is indeed a force-sensing module. As such, this study contributes to our understanding of not only the Piezo ion channel family but also how distinct modules within a channel complex can confer mechanosensitivity to structurally (but not genetically) related ion channel pores that are otherwise mechanoinsensitive.

Two models have been proposed to explain the ability of mechanosensitive channels to efficiently couple applied forces to channel gating. Both require an elastic element—a "gating spring"—such that tension in the element is reduced upon channel opening, making the open state energetically more favorable. According to the "tether" hypothesis, mechanosensitive channels are coupled to elastic elements, possibly accessory proteins, which focus macroscopic forces onto channel gates, as may be the case for inner ear hair cells. Protein motifs that exhibit the required elasticity, such as ankyrin repeat domains, have been proposed as gating springs (Howard and Bechstedt, 2004). Conversely, according to the membrane tension model, channel activation does not require accessory modules but is gated by disturbances in the surrounding lipid bilayer, such as membrane stretch when a cell is physically prodded or swells under osmotic tension. In this case, the lipid bilayer is the elastic element.

The membrane tension model was largely derived from studies of bacterial channels with small and large conductances (MscS and MscL), which are indeed activated by membrane tension in the absence of accessory proteins (Kung et al., 2010). Furthermore, recent work on two-pore domain potassium channels TRAAK and TREK1 demonstrated that these mammalian channels are capable of transducing a mechanical stimulus without attached tethers (Brohawn et al., 2014).

Although the tether hypothesis arose from observations of inner ear hair cells (Corey and Hudspeth, 1983), the most compelling evidence is from studies on NOMPC, a mechanosensitive channel from the TRP family, which underlies touch sensation and hearing in *Drosophila* (Yan et al., 2013; Effertz et al., 2012; Zhang et al., 2015). The NOMPC gating spring is now recognized as a dedicated module within the NOMPC amino acid sequence, a remarkable N-terminal module with 29 ankyrin repeats that tethers the channel to intracellular structures. When the NOMPC tether module was fused to mechanoinsensitive voltage-gated potassium channels, it rendered them mechanosensitive (Zhang et al., 2015).

The new work from Zhao et al. reveals a mechanistic picture for Piezo1 that is somewhere in between membrane stretch-sensitive MscS/MscL channels and tether-dependent NOMPC channels: a modular domain intrinsic to the amino acid sequence (like NOMPC) renders the complex sensitive to membrane stretch (like MscS/MscL), while a second, pore-forming module mediates conductance and ionic selectivity (Figure 1). This observation highlights that independence from accessory proteins is not a sufficient criterion to rule out the existence of a modular force sensor. On the other hand, such independence suggests that the origin of the gating force for Piezo1 may be lipid stretch and not necessarily a direct "pull" from tethers to extracellular or intracellular elements. Perhaps the peripheral force-sensing module of Piezo1 serves to gather membrane tension and focus it onto the gate of the central pore module. To test this hypothesis for mechanical activation and independence from accessory proteins, experiments will need to be performed on Piezo1 in a lipid bilayer, in the absence of other cellular components.

In a cellular environment, it has recently been shown that Piezo1 is highly sensitive to mechanical tension with half-maximal activation in the range of ~1.5 mN/m (Lewis and Grandl, 2015). This contrasts

with observations from the bacterial mechanosensitive channel, MscS, which opens in response to tension in the 5–8 mN/m range, and MscL, which opens at 10–14 mN/m, near lytic tensions for a bacterial cell (Kung et al., 2010). The biophysics of how these protein modules couple the applied mechanical force to channel gating and allow their specific range of sensitivity are unclear. In particular, given the new insight into Piezo1 structure and function, we can now ask what is the elasticity of the proposed force-sensing module and how does it trigger conformational changes in the pore module? Furthermore, since the mechanical activation properties of Piezos can be modulated by other proteins such as PKD2 and STOML3 (Effertz et al., 2012), it is now possible to consider how modulation by these proteins might confer a range of mechanical sensitivities in various cellular environments such as nociceptors and low-threshold mechanosensory neurons of the dorsal root ganglia.

It will also be interesting to investigate the applicability of this ''plug-n-play'' modular design to other mechanosensitive systems. For example, TMC1 and TMC2 membrane proteins have been proposed to underlie mechanotranduction in inner ear hair cells (Pan et al., 2013). However, it is unclear whether TMC1 or TMC2 include a pore-forming module, a mechanosensing module, a gating spring, or some combination of these. Is the modular configuration of Piezo1 conserved among other mechanosensitive channel complexes? Although the TMC amino acid sequences bear little resemblance to Piezos, they may share structural or functional similarities to some of the Piezo1 modules. As the recent work reveals, each system will need to be investigated independently. Nonetheless, there may be parallels in the mechanisms and modular domains that contribute to mechanosensitivity.

Evolution has made a habit of repurposing functional protein modules and adapting them to the advantage of their hosts. Indeed, the functional mechanotransduction modules identified by Zhao et al. may be the norm rather than the exception. If so, we predict that mechanotransduction in other systems, while molecularly diverse, may include modules that are functionally conserved, including force sensors, gating springs, and channel pores. The study by Zhao et al. raises the intriguing possibility that convergent evolutionary forces may have adapted these functional modules in a variety of ways to yield the diverse array of mechanosensitive systems that enable life on earth.

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