EPPENDORF

The Cellular Feeling of Pressure

Bertrand Coste

Mechanotransduction is the conversion of mechanical forces into biological signals. It allows our body to detect various forms of mechanical stimuli such as gentle stroking for touch, harmful pinches for pain, or sound vibrations for hearing. Mechanotransduction is not restricted to sensory systems; it is involved in many other fundamental biological processes, including blood pressure regulation, sensing the flow of kidney fluid, and bone homeostasis. In the late 1970s, Corey and Hudspeth observed, using bullfrog auditory hair cells in vitro, that the latency between mechanical stimulation and response was too fast for involvement of second-messenger signaling; they predicted the existence of ion channels directly activated by mechanical stimulation (*1*). This postulate was validated several years later by the first patch-clamp recordings of stretch-activated channels (*2*). Despite the molecular identification of invertebrate mechanotransduction channels, the identity of these mechanical biosensors in mammals has remained elusive (*3*). Therefore, mammalian mechanotransduction remains one of the most fascinating and challenging problems in the field of signal transduction.

I first became interested in mechanotransduction during my graduate studies, when I recorded from endogenous mechanically activated (MA) currents present in sensory neurons of the dorsal root ganglia (DRG) that are responsible for sensing innocuous touch and noxious pressure (*4*). Recording MA currents quickly led me to the question that has motivated physiologists for more than 30 years: What is the identity of ion channels that respond to a physical stimulus such as pressure? As a postdoctoral fellow in the Patapoutian lab at The Scripps Research Institute, I set out to answer this question.

DRG neurons heterogeneously express three distinct types of MA currents, and my first idea entailed comparing gene expression profiles of these distinct mechanosensitive neurons to generate a list of candidate genes that could be tested by small interfering RNA (siRNA) inhibition in a follow-up screen. But I quickly realized the challenges of this approach. Indeed, patch-clamp recording of MA currents is time-consuming and would make the low-throughput siRNA screen in transfected primary cultures of heterogeneous DRG neuron populations difficult to perform efficiently. Because mechanotransduction is relevant for various biological processes and many cell types express MA currents, the critical idea was to use immortalized mammalian cell lines instead, providing unlimited and easy-to-handle material. Among several cell lines that I tested, I found that the mouse neuroblastoma neuro2A cells expressed the most robust MA currents. I then put together a list of transcripts enriched in these cells and further narrowed the list, focusing on plausible candidates by utilizing bioinformatics analyses, such as the number of predicted transmembrane domains (≥2), public database expression profiles, and the present understanding of these candidates (whether or not any could be a novel cation channel). The resulting list was still long for patch-clamp screening, and it took me about 10 months to test each candidate separately by siRNA knockdown in Neuro2A cells. But the assay was robust and gave rise to very few false-positives. Indeed, I immediately felt both a big relief and great excitement the day I tested the 72nd candidate and observed nearly complete knock-down of the mechano-response (*5*).

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This candidate was a little-known gene called *Fam38A*, for member A of the family with sequence similarity 38, which had one homolog in mammals, *Fam38B*. Remarkably, overexpression of both genes in heterologous expression systems led to expression of large MA currents, and we chose to rename these genes *piezo1* and *2* from the Greek "píesi" meaning pressure. Our first study identified Piezo proteins as components of mechanotransduction channel complexes but did not fully establish their molecular nature. We showed in a follow-up study, in collaboration with the Montal lab, that these genes form a new family of ion channels (*6*). Indeed, reconstitution of purified Piezo proteins in artificial bilayers leads to ion channel activity, and overexpression of Piezos from different species induces distinct MA channels. A striking characteristic of Piezo proteins resides in their unique structure, as they do not share any homology with any known ion channels or proteins, and they are predicted to span the membrane over 30 times; potentially Piezo1 and 2 have more transmembrane domains than any other mammalian protein. They associate at least as homotetramers, giving rise to a large ion channel complex of about ~1.2 million daltons.

An interesting feature of Piezo MA channels is their conservation among animals, plants, and some unicellular organ-

Bertrand Coste, for his essay "The cellular feeling of pressure." Dr. Coste is a CNRS Research Scientist at the Research Center of Neurobiology-Neurophysiology of Marseilles, France. He received his Ph.D. in Neurosciences from the University of the Mediterranean Aix-Marseille II. In his doctoral work, he worked on pain sensitivity and investigated the modulation of nociceptive neuron excitability during inflammation. After receiving his Ph.D., he moved to The Scripps Research Institute in La Jolla, California, USA, where he was a postdoctoral fellow from 2007 to 2012. His work focused on the identification of molecular components involved in the transduction of mechanical forces into biological signals and led to the identification of a new family of ion channels.

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isms, suggesting a role in mechanotransduction that may have appeared in the first eukaryotic species. But what are the physiological functions of Piezos? In mice, Piezo1 and 2 are broadly expressed in various tissues such as lungs, bladder, skin, kidney, and colon, which suggests that they may be involved in mechanotransduction signaling in these tissues. It is noteworthy that only Piezo2 is highly detected in DRG neurons, where it accounts for rapidly adapting MA currents, one of the three types of MA currents described in sensory neurons. Costaining experiments show that Piezo2 is present in both populations of nociceptive and innocuous sensory neurons; this suggests that it may play a role in pain and touch signaling. In line with this, we have shown, in collaboration with the Cook lab, that the single Piezo family member in the fruit fly is involved in mechanical pain sensing at the larval stage (*7*). Therefore, Piezo channels are physiologically relevant for neuronal sensory functions, and their broad expression suggests involvement in a variety of biological processes in mammals. Indeed, two recent studies have discovered nonsensory roles of Piezo1 in maintaining homeostatic epithelial cell numbers (*8*) and erythrocyte volume homeostasis (*9*).

I believe that this discovery of a novel family of elusive MA ion channels will enhance our understanding of mechanotransduction. Future studies will explore the structure-function relation of Piezos and their roles in a variety of specific tissues and cellular processes. This will provide insights into mammalian mechanotransduction at molecular, cellular, tissue, and organ levels.

References and Notes

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