PROFILE

Profile of David Julius and Ardem Patapoutian: 2021 Nobel Laureates in Physiology or Medicine

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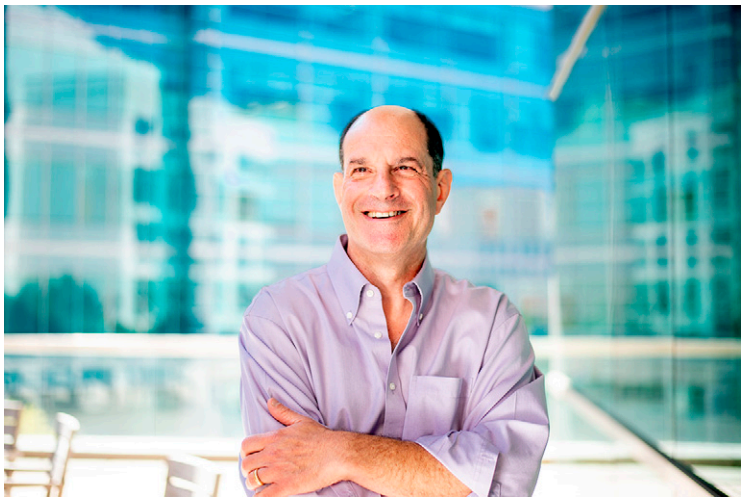
Three apparently unrelated findings led to the discovery of the temperature receptors: a better understanding of pain-sensing, the molecular nature of the channels in charge of touch and proprioception, and the 2021 Nobel Prize in Physiology or Medicine bestowed to David Julius and Ardem Patapoutian.

In 1953, state-of-the-art research in temperature sensing concluded that temperature afferent fibers came in two flavors, "cold" and "warm," characterized by discharging by a lowering in temperature and an increase in temperature, respectively (1). Hensel and Zotterman had previously described the sensitizing effect of menthol upon cold receptors (2). It is also an old observation that acute application of capsaicin, the active component of chili peppers, causes intense pain, and that its repeated administration renders animals insensitive to the action of this vanilloid compound (3). The fact that capsaicin induces a depolarization of ventral root neurons from the isolated rat spinal cord suggested to Theriault et al. (4) that capsaicin's acute and chronic effects on nociception could be explained by a direct action on primary sensory neurons. On the other hand, McCarter et al. (5) demonstrated the existence of mechanosensitive currents in rat dorsal root ganglion (DRG) cells, clearly pointing to the existence of mechanosensitive ion channels as being responsible for the sense of touch.

Since temperature is an "intensive" thermodynamic parameter (i.e., one that does not depend on the size of the system), it was difficult to think of specialized temperature receptors. However, the seminal 1997 *Nature* paper from Julius' laboratory demonstrated that living beings have evolved to transduce all stimuli coming from the external world using ion channels, including temperature (6). Caterina et al. (6) used an expression cloning strategy to search for the capsaicin receptor and found a single clone in which capsaicin induced a significant increase in cytoplasmic calcium. The isolated cDNA coded for a channel-forming protein, now known as TRPV1, belonging to the transient receptor potential (TRP) family and expressed in small- to medium-diameter neurons of



David Julius. Image credit: University of California, San Francisco.

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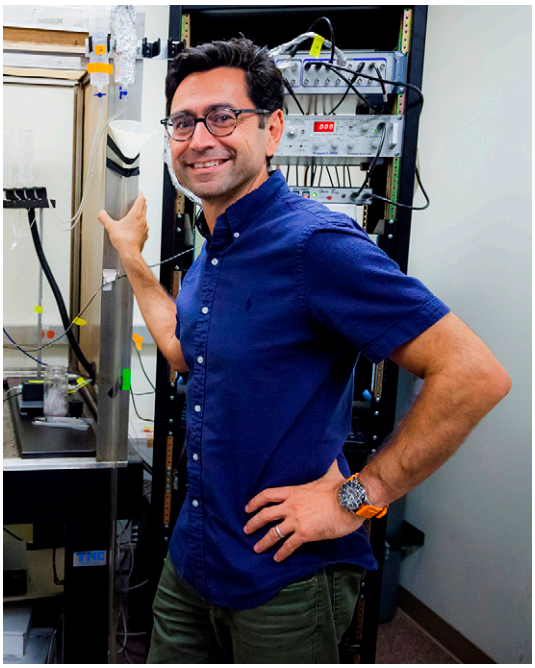

Ardem Patapoutian. Image credit: Kara Marshall (The Scripps Research Institute, San Diego, CA).

the DRG and trigeminal ganglia (TG). TRPV1 is a nonselective cation channel that is permeable to calcium, activated by capsaicin, and inhibited by capsazepine. TRPV1 is activated by noxious heat (>40 °C) and low pH and, as such, is closely involved in inflammatory pain. Experiments using mice lacking TRPV1 confirmed that this channel is vital for the transduction of the nociceptive and inflammatory effects of capsaicin (7). A Q_{10} of 40 makes TRPV1 one of the most efficient channels for transforming thermal into mechanical energy (the pore opening). The Caterina et al. (6) paper thus represents "a before and an after" in our understanding of how we sense temperature, and it is the beginning of a story that led to the discovery of an entire class of temperature-sensitive ion channels. Shortly after this 1997 publication, a second temperature-sensitive TRP channel, called TRPV2, was identified by Julius' group, which is expressed in sensory ganglia and other tissues (8). TRPV2 proved to have a high-temperature threshold (>52 °C) and to be capsaicin insensitive. However, the question remained as to whether other members of the TRP family were also involved in temperature sensing.

In 2002, TRPM8 was identified as a cold-sensing receptor (9, 10). The fact that TRPM8, like TRPV1, was also identified in a subpopulation of DRG and trigeminal neurons gave strong support to the hypothesis that TRP channels were temperature detectors in the mammalian peripheral nervous system. TRPM8 was also the target of menthol, a finding that identified the molecular source of the Hensel and Zotterman (2) sensitization effect of menthol on cold receptors. Temperatures below 28 °C activated the channel, but its involvement in the behavioral responses to cold was not demonstrated until the groups of Julius and Patapoutian showed that mice deficient in TRPM8 presented deficits in their responses to cold (11, 12). These studies and more recent research have shown that TRPM8 also contributes to the detection of noxious cold.

This fantastic saga that was the beginning of the unveiling of TRP channels as the molecular components of our sixth sense, temperature, was followed by the identification of TRPA1 as a sensor of noxious cold, which cohabitated in the same neurons as TRPV1 (13). TRPA1 is also present in *Drosophila melanogaster*, snakes, chickens, and frogs, where, unlike its mammalian counterpart, it is activated by heat. However, the phenotype of this channel in mammals became controversial when, in 2004, the group of Julius (14) reported that the rat TRPA1 was insensitive to cold but could be activated by mustard oils and cannabinoids. The activation by pungent compounds was also reported the same year by the group of Patapoutian (15); nevertheless, and in contrast to the findings from the Julius group, they found that both human and rat TRPA1 were activated by noxious cold. TRPA1 has become a case study to understand temperature sensing by TRP channels since Vandewauw et al. (16) showed that the sensing of acute noxious heat requires the presence of TRPV1, TRPA1, and TRPM3, suggesting that this channel participates in heat sensing. Nevertheless, TRPA1 sensitivity to cold is still an open question.

TRPA1 has also given an evolutionary advantage to certain dangerous animals. In 1937, Noble and Schmidt proposed that the pit organ of pit vipers serves to detect the body temperature of their prey (17). Based on this report, Bullock and Diecke went further to show that this organ contains a homogeneous population of warm-sensing fibers and, as such, behaves as an infrared receptor (18). Fifty-four years passed before we identified the molecular bases of infrared detection in pit-bearing snakes, when the group of Julius showed that TRPA1 was enriched 400-fold in their TG. In addition, they showed that the TRPA1 ortholog present in the neurons of the pit organ shows a lower temperature threshold and a higher Q_{10} compared to those of the TRPA1 expressed in the TG of rat snakes (19).

How do we fill the gap in temperature sensing between cold and noxious heat perception? Here again, TRP channels are involved and, paradoxically, the work of Yarmolinsky et al. (20) implied that detection of warmth is mediated by TRPV1. In contrast, Tan and McNaughton (21) argued that TRPM2 was involved instead, since mice lacking this channel show a deficit in their sensing of warm temperatures. On the other hand, the detailed study of Paricio-Montesinos et al. (22) revealed that mice lacking TRPV1, TRPA1, and TRPM3 could sense nonnoxious warmth. Thus, although TRPM2 plays a role in warm perception, it is not the only channel that can do so. In contrast, TRPM8 is absolutely required for warm perception since the results indicate that accurate detection of skin warming needs excitation of warm-excited and inhibition of cool-sensitive polymodal C-fibers (22).

The ion channel field has evolved extremely fast during the last 10 years, thanks to the power of cryoelectron microscopy (cryo-EM) in resolving ion channel structures quickly and with high resolution. Using this tool, the group of Julius revolutionized the whole field by giving us the structure of TRPV1 at <4-Å resolution (23, 24). Thus, there is no doubt that the discovery of a class of TRP channels with an exquisite temperature sensitivity opens a new world in our understanding of how we react, interpret, and survive the changes in the temperature of the external world.

In his treatise *De Anima* [On the Soul], Aristotle stated 2,400 years ago that without touch, the rational nature of men is not possible, as it endows them with the sense of inquisitiveness that builds the foundations of knowledge.

The first studies on cutaneous sensitivity were carried out in 1834 by Ernst Weber, one of the founders of experimental psychology (25). Sir Charles Sherrington used the term "interoreceptor" for the first time to describe the nerve terminals that reacted to mechanical stimuli arising from within the body (26).

In 1987, Martinac et al. (27) identified the first mechanosensitive ion channel, MscS, in bacteria. Mechanotransducers were also found in *D. melanogaster* (28, 29). Unfortunately, following several failed tests of candidate genes, the search for the molecular basis of the sense of touch in vertebrates was not having the same success as the one enjoyed by temperature sensing. However, the situation was about to change radically.

In 2010, after screening several cell lines, the group of Patapoutian found one (N2A) that caused the expression of a nonselective, fast inactivating, and mechanoactivated (MA) cationic current. The currents were like those recorded from DRG cells after poking the membrane with a glass probe or applying negative pressure (30). Coste et al. (30) made a short list of 73 candidate genes, which were abundantly expressed in N2A cells and included at least two putative transmembrane (TM) segments. Patapoutian's group suppressed their expression one by one, eventually finding that only silencing the Fam38A gene severely impaired the MA-induced currents. The proteins coded by the Fam38A and Fam38B genes were dubbed Piezo1 and Piezo2, respectively and showed to be large membrane proteins, predicted to contain >24 TM segments. Piezo1 and Piezo2 generated MA currents in several cell types (31). Piezo channels would continue to amaze us when their atomic coordinates were determined by cryo-EM. These studies coming from several groups, including Patapoutian's, showed a trimeric propeller-like structure, with each subunit having 38 TM segments (32, 33). With an extracellular cap right above the ion conduction pathway, resembling P2X and acid-sensing ion channels, the blades of the propeller curved the plasma membrane, challenging the models we know for mechanosensitive channel activation. However, and despite knowing the structural intimacies of the channel, still pending was finding out the physiological roles of these novel molecular mechanotransducers.

Somatosensory receptors in the skin detect a variety of mechanical stimuli, including touch, texture, and vibrations. While studying the responses of cat hair cells, Iggo and Muir (34) recognized the Merkel cells and their sensory afferent as the mechanosensory complex responsible for gentle touch. However, conflicting experimental evidence and the lack of molecular targets made the contribution of these cells to the mechanosensory response unclear for decades.

Patapoutian asked whether Piezo2 was the molecular mechanotransducer in Merkel cells using a skin-specific Piezo2 knockout (KO) mouse (35). Unlike wild-type mice, Merkel cells from these genetically engineered animals exhibited no response to mechanical stimulus, as well as a moderate decrease in gentle touch response. These findings tipped the balance in a decades-long discussion, supporting a two-receptor site model in which both Merkel cells and their afferent neurons are mechanosensitive elements.

Proprioception, or the sense of the position of the body and the limbs, is acquired by specific proprioceptive neurons with cell bodies in DRGs. They receive inputs from two different mechanoreceptor classes expressed in skeletal muscles that sense muscular fiber length and tone. After generating a conditional Piezo2-KO mouse targeting different populations of proprioceptors, Woo et al. demonstrated that Piezo2 is required for proprioceptive mechanotransduction (36), thereby providing the molecular basis for Sherrington's studies on sensorimotor control in the early 1920s.

Patapoutian's work expanded the frontiers of mechanobiology by addressing the role of Piezo channels in many physiological responses. The lethality of Piezo1-KO mice led to the discovery of its role in vascular remodeling by allowing the endothelial cells to sense the shear stress of blood flow (37). By generating conditional Piezo1-KO mice lacking Piezo1 channels in their red blood cells, Cahalan et al. (38) showed that Piezo1 plays a role in red blood cell volume control by mechanically induced calcium entry and downstream signaling through the KCa3.1 channel. Nonomura et al. (39) used an engineered mouse lacking Piezo1 in endothelial cells to show that this channel is critical for lymphatic valve formation. Use of Piezo1 and Piezo2 that were genetically modified to show impaired expression in the nodose and petrosal sensory ganglia revealed that they are required for arterial baroreflex activity (40). Additionally, the work by Song et al. (41) showed that Piezo1 inhibits axon regeneration in *Drosophila* and mice, which opened a novel avenue for the search of therapies aimed at reversing spinal cord injury.

By discovering the molecular transducers underlying the senses of temperature and touch, the 2021 Nobel laureates in Physiology or Medicine increased greatly our understanding of how the nervous system codes sensory information.

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