A Nobel Prize for understanding the molecular basis of sensing pain and touch

The Nobel Prize for Physiology or Medicine for 2021 was awarded jointly to Prof. David J. Julius, University of California, San Francisco (UCSF), USA and Prof. Ardem Patapoutian, Scripps Research, San Diego, California, USA. The prize recognizes the decades long effort by the laboratories of both investigators to delve into the molecular underpinnings of sensing acute pain caused by chemical or physical stimuli, referred to as nociception and the sensation of touch, referred to as mechanosensation. As a prelude to winning the Nobel Prize, the two researchers were also awarded the Kavli Prize for Neuroscience in 2020. This note aims to capture the contributions, excitement and importance of both the Nobel laureates' long-standing research endeavours to study two remarkable and related phenomena of pain and touch.

David Julius grew up in Brooklyn, New York, USA, and attended the Abraham Lincoln High School. He did his undergraduate studies at MIT, where he spent some time to gain research experience in the laboratories of Joel Huberman and Alexander Rich. This was followed by graduate studies at the University of California, Berkeley with Jeremy Thorner and Randy Schekman, where he worked on the enzymes involved in the processing of pheromone, α-factor[1]. Julius subsequently did a postdoctoral fellowship with Richard Axel in Columbia University, where he was involved in the cloning and molecular characterization of the G-protein coupled serotonin receptor (5-HT1c)[2]. He joined as a faculty at UCSF in 1989 and has ever since been involved in deciphering the molecular mechanisms behind sensing heat, cold and pain through transient receptor potential (TRP) channels (Figure 1).

Ardem Patapoutian grew up in Beirut, Lebanon, where he did his early schooling and a year of undergraduate study at the American University of Beirut. He subsequently moved to Los Angeles, USA, and continued his undergraduate studies at the University of California, Los Angeles. During this time he got some exposure to molecular biology tools at Judy Lengyel's laboratory, where he was part of the team that identified the *tailless* gene in *Drosophila*[3]. He subsequently moved to Barbara Wold's laboratory at Caltech to study transcriptional regulation in the context of muscle differentiation. This was followed by a postdoctoral stint at Louis Reichardt's laboratory at UCSF, where he began research into the developmental biology of somatosensory neurons involved in touch and pain[4]. Patapoutian subsequently started his own research group in 2000 at Scripps Research, in a quest to identify the molecular players involved in sensing mechanical force, touch and pain (Figure 1).

The sense of pain is vital for the survival of any organism and underlies its ability to refrain from interacting with objects that could cause potential physical damage to the tissues. While pain could be classified into three types, including nociceptive/acute pain, neuropathic pain and inflammatory pain, this note delves into the contributions of the two Nobel laureates to nociceptive pain. The agents that cause acute pain could range from physical objects like pinpricks, extremes of temperature like heat and cold, to chemical irritants like capsaicin and allyl isothiocyanates (active compound in wasabi) that are natural compounds. The neural pathways for sensing pain are categorized as ascending and descending, representing the sensory and motor components of pain pathways respectively[5]. The neuronal fibres involved in sensing pain/touch extend from the peripheral tissues, face and jaws to the dorsal root ganglion of the spinal cord and trigeminal ganglion[6]. The sensory nerve fibres are divided into three major classes. The first class comprises Aδ fibres that retain medium-diameter, myelinated neurons which are known to have a rapid response to acute pain. These are further divided to type-I fibres that sense higher extremes of pain and type-II fibres that have a lower threshold for sensing pain. The second class of sensory neurons, referred to as the Aβ fibres comprises myelinated, large-diameter neurons that are involved in sensing touch. The third class of small diameter, unmyelinated neurons, also called the C-fibres, are involved in a slow response to pain and are considered to be responsible for diffuse and dull pain sensation[7]. The C-fibres also are heterogeneous and capable of responding to a wide range of stimuli, including gentle stroking, itch and chemical irritants. Most of these nerve fibres can also be distinguished based on the receptors/ion channels that are expressed on these neurons and are capable of perceiving stimuli and activating the neurons[8]. It is in the context of these ion channels/receptors that the two Nobel laureates have made seminal contributions to our understanding of pain physiology.

Julius and his colleagues identified the receptor for sensing heat produced by capsaicin (the pungent ingredient in chilli

David Julius Ardem Patapoutian

Figure 1. The Nobel Prize for Physiology or Medicine for 2021 was awarded to David Julius and Ardem Patapoutian for their seminal work on the molecular processes underlying the sensation of pain and touch (Image credits: Noah Berger, UCSF and Scripps Research).

peppers) from a cDNA library of the dorsal root ganglion. Pools of this cDNA library were expressed in mammalian cells and the team monitored cells that would display Ca^{2+} influx upon application of capsaicin. The process was captured by a simple, yet elegant screen of observing the fluorescence upon calcium entry into cells loaded with a calcium-sensitive dye, Fura2, in response to application of capsaicin. The screening led to the identification of the vanilloid receptor 1 (VR1) or the TRPV1 channel that was sensitive to capsaicin in a concentration-dependent manner[9]. TRPV1 was also observed to be responsive to bioactive lipids and tissue acidosis associated with inflammation in addition to capsaicin, thereby establishing its role as a major player in pain and heat sensation (Figure 2 *a* and *b*). The channel displays an activation threshold of around 41.5°C, beyond which it shows a steep, temperature-dependent activation[10]. The identification of TRPV1 was followed by the expansion of this family to multiple vanilloid receptor subtypes. For instance, TRPV2 was observed to sense a higher threshold of heat[11], TRPV3 to played a role in sensing itch[12], and TRPV5-6 was involved in calcium homeostasis[13]. In addition to the vanilloid receptors (TRPV), five other subfamilies of TRP channels were identified and categorized into TRPA (Ankyrin), TRPM (melastatin), TRPC (canonical), TRPML (mucolipin) and TRPP (polycystin)[14]. The function of TRPA1 was also characterized by Julius' laboratory as being responsive to chemical irritants and activated in response to pungent tastes of wasabi and garlic that comprise allyl isothiocynates and thiosulfinates[15].

In addition to sensing heat and chemical irritants, TRPM channels, particularly TRPM8, is involved in the sensing cold temperatures[16]. The studies were done independently in the laboratories of both Nobel laureates. The TRPM8 channel displays cold sensitivity that is in contrast to heat-induced activation of TRPV1. The channel displays activation at temperatures lower than 25°C. The TRPM8 channel also displays activation by menthol, a compound that is well known to provide cooling effect[16]. The laboratories of both Julius and Patapoutian identified this channel around the same time[16,17]. The functional roles of TRP channels extend to detection of warm-blooded prey in vampire bats[18] and also provide snakes with heat pits, like vipers and pythons, the ability to perform infrared detection of potential prey[19].

The ability to sense touch can span a wide range starting from a gentle touch or a hug to a more painful stimulus like an object hitting the skin. Chronic pain conditions like hyperalgesia tend to exacerbate the sensation of pain, which could be abnormally triggered even by a gentle touch. The phenomenon is ubiquitous in the physiology of an organism, since several organs and tissues respond to some form of mechanical stimulus. The ability to convert mechanical stimuli to electrochemical signals is referred to as mechanotransduction and although some of the TRP channels were indeed known to be mechanosensitive, their primary roles were in response to temperature and chemical stimuli. Therefore, an ion-channel that could primarily indulge in the process of mechanotransduction remained elusive until Patapoutian's laboratory discovered it. Bertrand Coste, a postdoc in Patapoutian's group along with other colleagues screened for a mechanosensitive channel in a neuroblastoma cell line (which displays mechanosensitive responses) by knocking down individual integral membrane protein genes using short interfering (si)RNA. They identified a set of genes in the family of sequence similarity 38 (Fam38) whose knockdown lead to a pronounced reduction of mechanosensitive currents. Their search yielded two genes within Fam38, *Piezo1* and *Piezo2* that were involved in depolarization via nonselective cation influx in response to application of mechanical force[20] (Figure 3 *a* and *b*). *Piezo1* showed activation at negative and positive pressures greater than 20 mM of Hg and *Piezo2* displayed activation only upon application of positive pressure[20,21]. The two Piezo isoforms populate diverse tissues with *Piezo1* found in the skin, colon, kidney, lung and bladder, whereas *Piezo2* is observed predominantly in the sensory neurons of the dorsal root ganglion and in the bladder, colon and lungs[20]. The two genes encode two of the largest ion channels, and comprise up to 38 transmembrane helices and have more than 2500 amino acid residues[22]. Mutations that alter the function of the Piezo channels are known to cause lymphatic dysplasia, haemolytic anemia, respiratory distress, deficits in joint proprioception and scoliosis[23,24].

For a long time, membrane protein structures were primarily deciphered using X-ray crystallography. This method, however, requires coaxing membrane proteins to form crystal lattices that must diffract to atomic resolution. Crystallizing membrane proteins is a painstaking exercise with very few laboratories succeeding in this endeavour. Technological advances in electron cryomicroscopy (cryo-EM) in the form of direct electron detectors and improved image-processing algorithms helped overcome this handicap and heralded a revolutionary improvement in the use of cryo-EM to obtain high-resolution structures of macromolecular complexes[25]. Cryo-EM is now the mainstay for determining

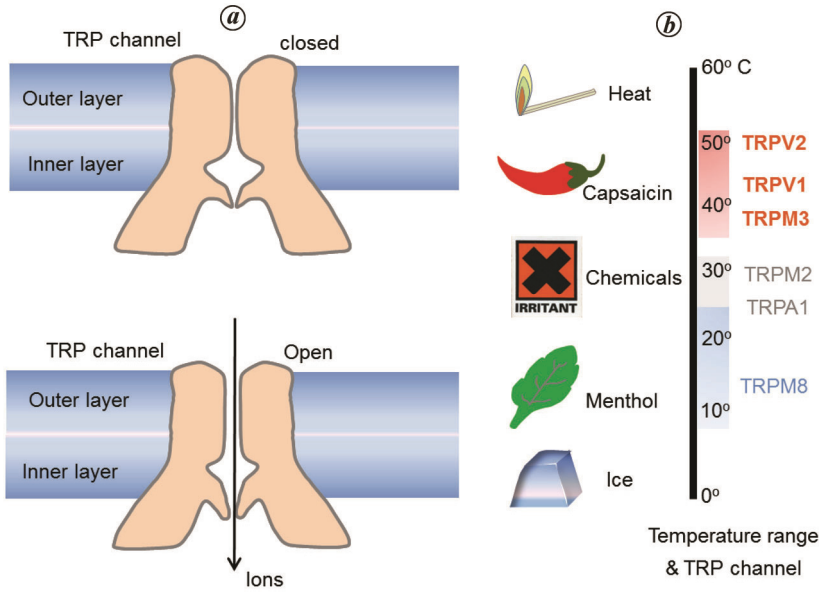

Figure 2. ***a***, TRP channels open in response to heat and natural compounds like capsaicin or wasabi. ***b***, Different sub-families of TRP channels respond to diverse stimuli, including heat, cold and chemical irritants.

high-resolution atomic structures of macromolecular complexes, including ion channels. In the area of membrane protein structural biology, the transition from crystallographic structures to cryo-EM structures was triggered by the elucidation of the TRPV1 structure through collaboration between Julius's laboratory and that of Yifan Cheng at UCSF (Figure 4 *a*). Erhu Cao and Maofu Liao, postdocs in the Julius and Cheng laboratories respectively, were involved in the cryo-EM reconstruction of the TRPV1 channel to around 3.4 Å (refs 26, 27). Prior to this, the only structural information that was available were the X-ray structures of cytosolic domains of some TRP channels[28] and a low-resolution reconstruction of the TRPV1 channel using electron microscopy (~15–20 Å)[29]. The structure of the TRPV1 channel in amphipols (an amphipathic polymer that mimics the lipid bilayer) revealed a homotrameric channel with each subunit comprising six transmembrane (TM) segments (referred to a 'S'), followed by a TRP domain at the C-terminus. The architecture broadly resembles the voltage-gated ion channels (VGICs). The general topology of the channels comprises ankyrin repeats at the N-terminus located in the cytosol, followed by helices S1 to S4 that are equivalent to the voltage-sensing domain (VSD) of VGICs and helices S5 and S6, and the intervening loop (also called pore loop) which lines the ion-permeation pore of the channel. The linker helix connecting S4 and S5 is parallel to the plane of the membrane and is vital to control the conformational transition of the pore-lining helices. The pore of the channel is lined by the pore loop that is comparatively wider than the highly K^+-selective ion channels, which may contribute to a corresponding reduction in ion selectivity to include Ca^{2+} and Na^+ as the permeating ions (Figure 4 *a*). The channel gating is mediated at two levels – at the entry of the pore through the pore loop and a constriction point at the cytosolic region by hydrophobic residues of helix S6. The TRPV1 structure was also determined in multiple conformations in complex with a spider toxin (double-knot toxin) and resiniferatoxin (and capsaicin), which suggested that channel activity could be modulated by interactions with diverse agents at discrete locations of TRPV1. The double-knot toxin interacts close to the pore loop region in the extracellular region in the channel and stabilizes an open state of the channel[27]. Resiniferatoxin (and capsaicin), on the other hand, interacts within the membrane plane in close proximity to helices S3 and S4 to facilitate movement of the S4–S5 linker helix that forces channel opening[30].

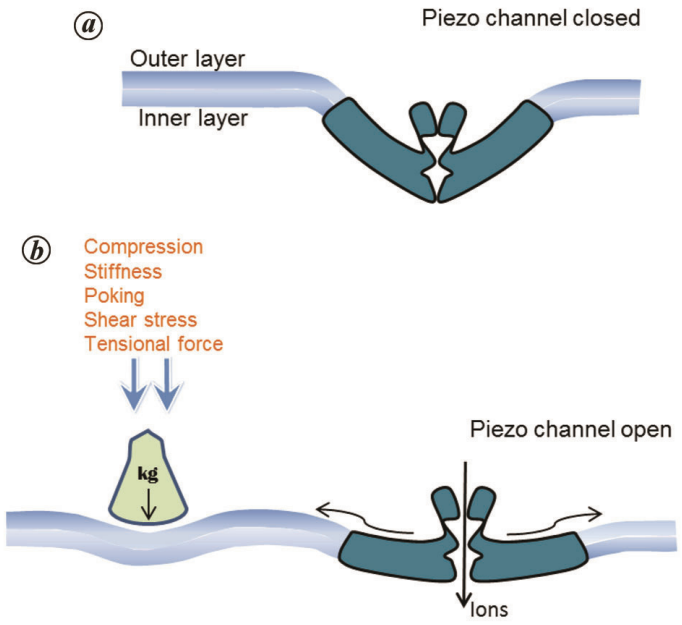

Figure 3 ***a***, ***b***. Schematic depicting the activation of mechanosensitive piezo channels in response to mechanical forces that induce channel opening.

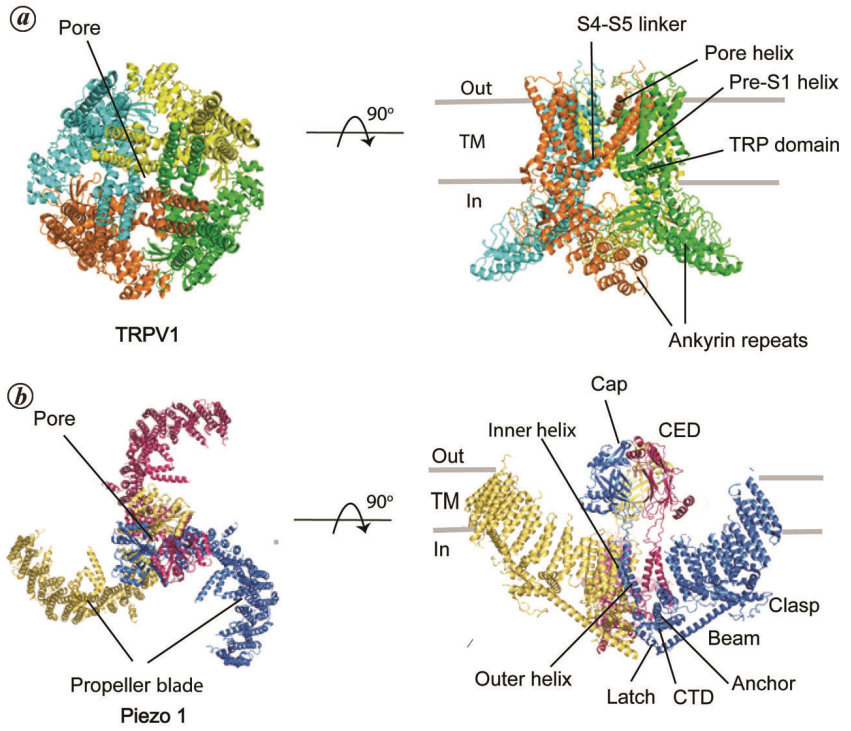

Figure 4. Cryo-EM structures of (***a***) TRPV1 and (***b***) *Piezo1* displaying individual protomers in distinct colours. Regions of the channels are labelled for clarity.

The partial cryo-EM structures of Piezo channels display homotrimeric organization that forms a propeller-shaped architecture with 38 TM segments[22,31]. A specialized structure consists of a long α-helix (beam) followed by a coil (latch) and several helices (the clasp) that connect the cytosolic domain with repeats 7 and 8 of the blade. The

three blades are profoundly curved and together form a nanodome that protrudes into the cell. The pore is formed by two pore-forming helices (TMs 37 and 38), wherein helix 38 is in continuity with the intracellular CTD (C-terminal domain) and extracellular CTD (CED) (Figure 4 *b*). The pore is connected to the blade through an anchor region made of three semi-transmembrane helices. CED covers the pore by forming a dome. The structures available are of closed conformation. Structure-guided mutagenesis experiments showed that during force transduction, the blade, cytosolic regions, anchor and CTD may move in a controlled manner to activate the channel. The activated channel can conduct the ions through an open cavity at the centre of the CTD or through the gap between the CTD and the membrane; this needs to be explored. The nanodome architecture at the TM region is favourable for inducing local distortion of the lipid bilayer[31]. Reconstitution of the channel into vesicles and atomic force microscopy studies suggest that external force leads to reversible flattening of the dome[32]. This is further supported by symmetry-free 3D classification of the protein where some portion of particles show blades of the protein to be slightly flattened and twisted, suggesting that activated channels may undergo similar conformations[33].

In conclusion, the Nobel Prize aptly recognizes the exceptional contributions of Julius and Patapoutian towards understanding the ion channels involved in the sense of heat, cold, pain and touch. The insights into the pharmacology and mechanisms of natural compounds like toxins, capsaicin and menthol on TRP channels and mechanosensitive Piezo channels, gained from their research, will likely provide a roadmap for pharmacological interventions to treat abnormal pain conditions.

1. Julius, D., Schekman, R. and Thorner, J., *Cell*, 1984, **36**, 309–318.
2. Julius, D., MacDermott, A. B., Axel, R. and Jessell, T. M., *Science*, 1988, **241**, 558–564.
3. Pignoni, F. *et al*., *Cell*, 1990, **62**, 151–163.
4. Moqrich, A. *et al*., *Nature Neurosci*., 2004, **7**, 812–818.
5. Yam, M. F. *et al*., *Int. J. Mol. Sci*., 2018, **19**, 2164.
6. Dubin, A. E. and Patapoutian, A., *J. Clin. Invest*., 2010, **120**, 3760–3772.
7. Julius, D. and Basbaum, A. I., *Nature*, 2001, **413**, 203–210.
8. Basbaum, A. I., Bautista, D. M., Scherrer, G. and Julius, D., *Cell*, 2009, **139**, 267–284.
9. Caterina, M. J. *et al*., *Nature*, 1997, **389**, 816–824.
10. Cao, E., Cordero-Morales, J. F., Liu, B., Qin, F. and Julius, D., *Neuron*, 2013, **77**, 667–679.
11. Caterina, M. J., Rosen, T. A., Tominaga, M., Brake, A. J. and Julius, D., *Nature*, 1999, **398**, 436–441.
12. Steinhoff, M. and Biro, T., *J. Invest. Dermatol*., 2009, **129**, 531–535.
13. van Goor, M. K. C., Hoenderop, J. G. J. and van der Wijst, J., *Biochim. Biophys. Acta Mol. Cell. Res*., 2017, **1864**, 883–893.
14. Zhao, Y., McVeigh, B. M. and Moiseenkova-Bell, V. Y., *J. Mol. Biol.*, 2021, **433**, 166914.
15. Jordt, S. E. *et al*., *Nature*, 2004, **427**, 260–265.
16. Bautista, D. M. *et al*., *Nature*, 2007, **448**, 204–208.
17. Dhaka, A. *et al*., *Neuron*, 2007, **54**, 371–378.
18. Gracheva, E. O. *et al*., *Nature*, 2011, **476**, 88–91.
19. Gracheva, E. O. *et al*., *Nature*, 2010, **464**, 1006–1011.
20. Coste, B. *et al*., *Science*, 2010, **330**, 55–60.
21. Shin, K. C. *et al*., *Sci. Rep*., 2019, **9**, 6446.
22. Saotome, K. *et al*., *Nature*, 2018, **554**, 481–486.
23. Alper, S. L., *Curr. Top. Membr*., 2017, **79**, 97–134.
24. Assaraf, E. *et al.*, *Nat. Commun*., 2020, **11**, 3168.
25. Kuhlbrandt, W., *Science*, 2014, **343**, 1443–1444.
26. Liao, M., Cao, E., Julius, D. and Cheng, Y., *Nature*, 2013, **504**, 107–112.
27. Cao, E., Liao, M., Cheng, Y. and Julius, D., *Nature*, 2013, **504**, 113–118.
28. Phelps, C. B., Huang, R. J., Lishko, P. V., Wang, R. R. and Gaudet, R., *Biochemistry*, 2008, **47**, 2476–2484.
29. Moiseenkova-Bell, V. Y., Stanciu, L. A., Serysheva, II, Tobe, B. J. and Wensel, T. G., *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 7451–7455.
30. Kwon, D. H. *et al*., *Nature Struct. Mol. Biol*., 2021, **28**, 554–563.
31. Wang, L. *et al.*, *Nature*, 2019, **573**, 225–229.
32. Lin, Y. C. *et al.*, *Nature*, 2019, **573**, 230–234.
33. Ge, J. *et al*., *Nature*, 2015, **527**, 64–69.

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