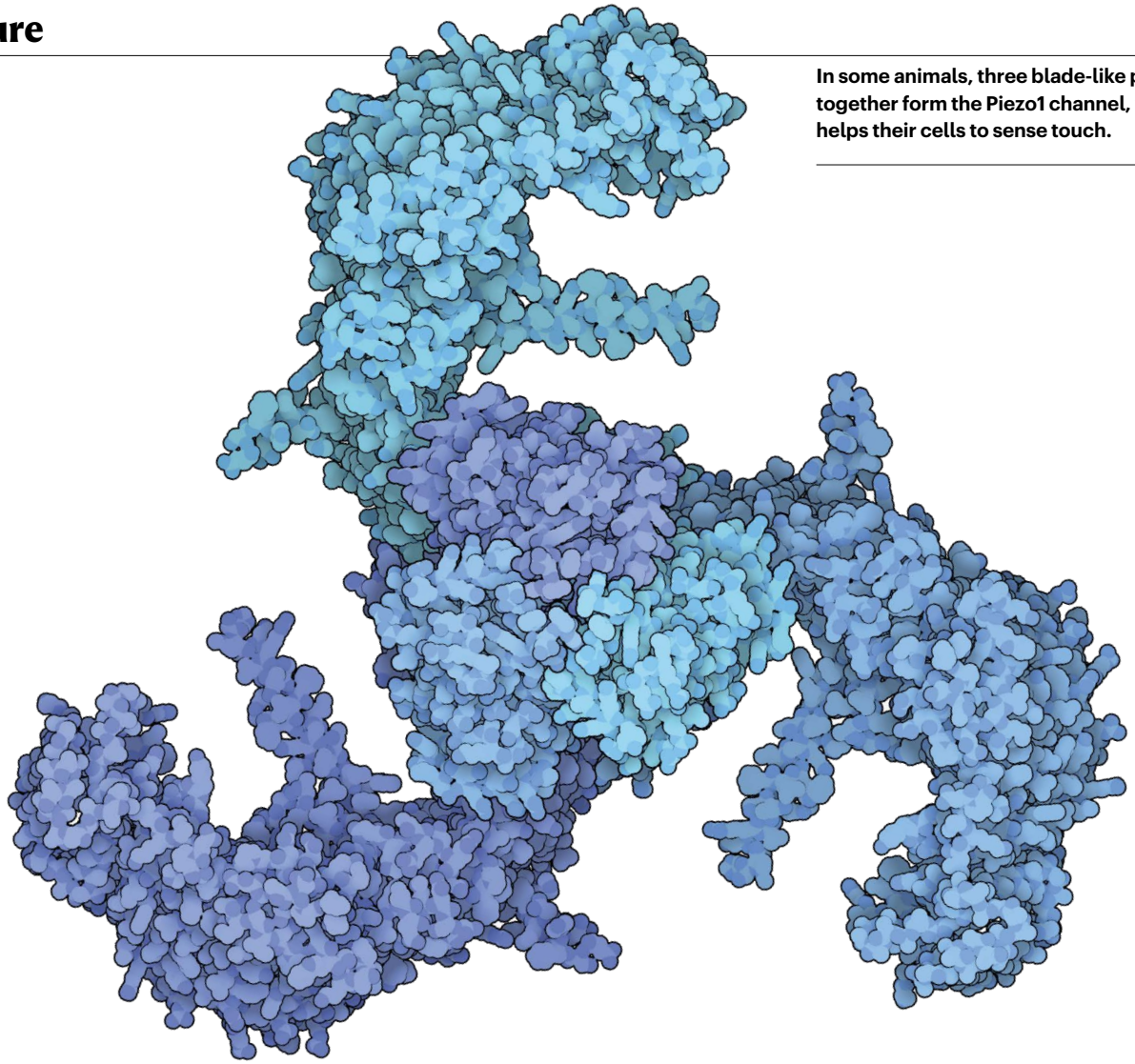


In some animals, three blade-like proteins together form the Piezo1 channel, which helps their cells to sense touch.

DAVID S. GOODSELL/RCSB PDB

FEEL THE FORCE

After decades of puzzling over how cells sense touch and pressure, scientists are zooming in on the proteins responsible. **By Amber Dance**

The girl tried hard to hold her arms and hands steady, but her fingers wriggled and writhed. If she closed her eyes, the squirming got worse. It wasn't that she lacked the strength to keep her limbs still — she just didn't seem to have control over them.

Carsten Bönnemann remembers examining the teenager at a hospital in Calgary, Canada, in 2013. As a paediatric neurologist with the US National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, he often travelled to weigh in on puzzling cases. But he had never seen anything like this.

If she wasn't looking at her limbs, the girl didn't seem to have any clue where they were. She lacked the sense of her body's position in space, a crucial ability known as proprioception. "This is something that just doesn't occur," says Bönnemann.

His team sequenced the girl's genes, and those of another girl with similar symptoms[1], and found mutations in a gene called *PIEZO2*. Their timing was fortunate: just a few years earlier, researchers looking for the mechanisms that cells use to sense touch had found that the gene encoded a pressure-sensitive protein[2].

The discovery of Piezo2 and a related protein, Piezo1, was a high point in a decades-long search for the mechanisms that control the sense of touch. The Piezos are ion channels — gates in the cell membrane that allow ions to pass through — that are sensitive to tension. "We've learned a lot about how cells communicate, and it's almost always been about chemical signalling," says Ardem Patapoutian, a molecular neurobiologist at Scripps Research in La Jolla, California, whose group identified the Piezos. "What we're realizing now is that mechanical sensation, this physical force, is also a signalling mechanism, and very little is known about it."

Touch underlies the functioning of almost every tissue and cell type, says Patapoutian. Organisms interpret forces to understand their world, to enjoy a caress and to avoid painful stimuli. In the body, cells sense blood flowing past, air inflating the lungs and the fullness of the stomach or bladder. Hearing is based on cells in the inner ear detecting the force of sound waves.

Over the past decade, the study of Piezos and other mechanosensitive ion channels has exploded. More than 300 papers have been published on the Piezos alone over the past three years. One of the biggest questions is how the proteins, situated in the cell membrane, sense and respond to force. Using cryo-electron microscopy (cryo-EM), scientists have made progress in unravelling the Piezo channels' bizarre, three-bladed structure, but a complete mechanism has been elusive. Researchers are also finding roles for Piezos beyond touch or proprioception. For example, Piezos might help to explain why certain people are resistant to malaria, and perhaps even why astronauts lose bone density while in orbit. Already, researchers are beginning to think about targeting force-sensing proteins with medicines to treat, for example, chronic pain.

"For a long time, we knew that cells did this, we had no idea how," says Miriam Goodman, a sensory physiologist at Stanford University in California. "Piezo really changed that."

Touch and go

Touch has long been a slippery sense. Other senses, such as sight or taste, are better understood, says Patapoutian: photons hitting the eye or chemicals infiltrating the nose and tongue all activate receptors in the same family. These receptors trigger ion channels to open and allow positive ions in. That depolarizes the cell, converting the stimulus into an electrical signal that the brain can decode.

Scientists suspected that in touch, proprioception and hearing, one protein acts as both the force sensor and the ion channel, because in hearing, the signalling happens fast – in microseconds. But the identity of these unified sensor-channel proteins remained mostly mysterious – at least in mammals. Researchers had found some mechanosensitive channels in bacteria, fruit flies and nematode worms.

So Patapoutian and his colleague Bertrand Coste hatched a plan. They would start with a type of mouse cell that they knew was capable of transforming a tiny poke from a pipette into a measurable electrical current. Then, Coste would knock out candidate ion-channel genes, a different one in each batch of cells, and look for a batch that suddenly lost its touch sensitivity. Coste started off confident, thinking it would take a few months, maybe even weeks, to find a hit.

It took the better part of a year. Then, shortly before the end of 2009, he saw something – or, rather, nothing. Coste poked with his pipette, and the cell did not respond. He must have eliminated a force-sensitive channel.

"It was a very nice day," recalls Coste, now at the French national research agency (CNRS) in Marseille. He and Patapoutian named the mouse gene *Piezo1*, from the Greek word for pressure, and soon identified *Piezo2*. Later, the team linked *Piezo2* directly to touch sensation in the sensory neurons and skin cells of mice[3].

Hidden blades

Researchers were abuzz with the result, Goodman recalls, particularly because the Piezo proteins were so large and complex. Made of more than 2,500 amino acids and weighing a hefty 300 kilodaltons, Piezo1's structure crosses the cell membrane a record-breaking 38 times. (For comparison, mammalian proteins typically contain closer to 500 amino acids.)

Unfortunately, that gargantuan size hindered researchers trying to answer the hottest questions in the Piezo field: how do the channels sense force? And how do they open and close? A protein structure is a big help with these kinds of question, says biophysicist Roderick MacKinnon at the Rockefeller University in New York City. "We don't know until we see it."

Structural techniques such as X-ray crystallography and nuclear magnetic resonance spectroscopy struggle to cope with big, complex proteins, says neuroscientist Bailong Xiao at Tsinghua University in Beijing, a former postdoctoral fellow in Patapoutian's lab.

Fortunately, as Xiao was setting up his lab in 2013, another option for obtaining high-resolution structures was coming online: cryo-EM. His group used the method to report[4] the first structure of Piezo1 in 2015, and since then, several higher-resolution versions have followed from Xiao's group, MacKinnon's, and Patapoutian's. Last September, Xiao followed up with a picture of Piezo2, which is similar to Piezo1 in size and shape. Xiao's picture of Piezo2 was the clearest view yet of the ends of the three blades, which move around and so are hard to capture[5].

The images were striking. Three Piezo proteins come together in a trimer that straddles the plasma membrane (see 'Pressure sensors'). From the central pore, three arms spiral out like the blades on a propeller. They curve up and out, creating a deep divot in the surface of the cell.

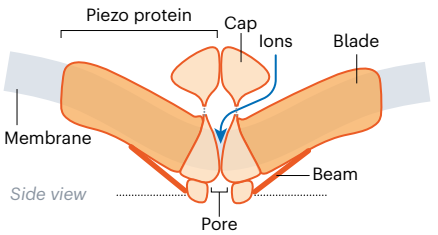
Patapoutian and Xiao think that when a force hits the membrane, the blades move protein 'beams' on the interior of the channel, which somehow drag the pore open. To MacKinnon, the unusual way in which the Piezos' blades pucker the membrane suggests a different mechanism: if a push or pull increases the tension of the membrane, the curved channel

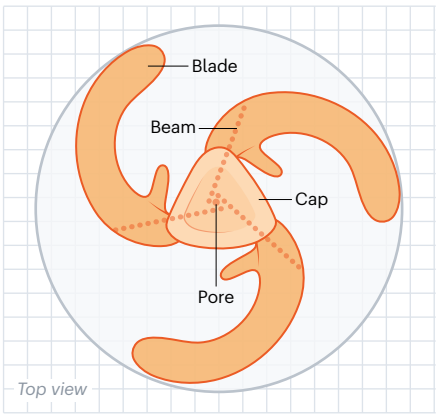
PRESSURE SENSORS

Cells have specialized proteins that help them to sense force. Piezo proteins are some of the best characterized in mammals, and they form channels that ferry ions into and out of cells. Researchers are trying to determine how the channels open.

Closure close-up

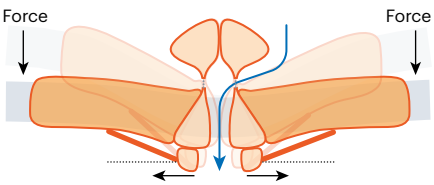
Images of closed channels reveal that they are built from three Piezo proteins, each contributing a blade and beam arranged around a central pore. The channel puckers the membrane in which it sits, forming a divot.



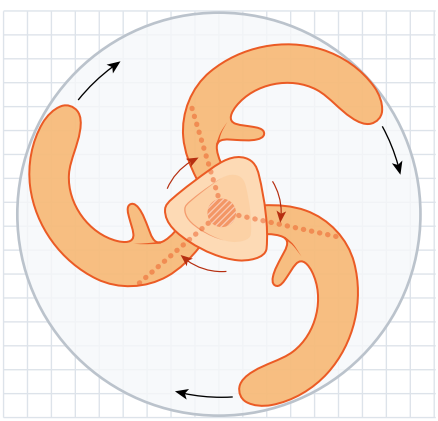


Opening gambits

There are no pictures of open Piezo channels, but researchers have a few theories about how they might unlock. The curved blades could flatten to pop open the pore, or a force could move the blades, somehow acting on the beams to open the channel.



The blades might rotate and twist the pore open. Some work suggests that each blade can move independently.



might flatten out, opening the pore.

The hypotheses can't be tested yet, because researchers have been able to study only isolated Piezo proteins, separate from their membrane and in the closed conformation. Snapping a picture of a Piezo in a membrane and open should help scientists to understand its secrets. "We want to see it in its natural environment," says Patapoutian.

Several labs are trying to image an open Piezo. Patapoutian's group is using a Piezo1-activating compound that it named Yoda1 after the diminutive, green, force-wielding Jedi master in Star Wars. Patapoutian hopes that with Yoda1 present, Piezo1 might open up for a picture. He is also interested in plugging Piezo proteins into artificial membranes called nanodiscs, which might help to stabilize the open conformation. Xiao, meanwhile, is working with cryo-electron tomography, which involves imaging the sample at different tilt angles, and might help to clarify the structure in a native or artificial membrane.

Sore spot

In parallel with the structural studies, scientists are finding that Piezo proteins have diverse roles in the body.

In 2014, neuroscientist Alex Chesler had just joined the National Center for Complementary and Integrative Health in Bethesda. Inspired by Coste's discovery, he was creating mice lacking Piezo2 to investigate the channel's role in touch. Then, one day, he received an e-mail from Bönnemann, who worked in his building, about the girls who lacked proprioception.

Chesler ran straight upstairs to Bönnemann's office. Panting, he announced, "You have no idea what you have." Chesler couldn't directly ask mice lacking Piezo2 what they felt, or rather didn't feel – but he could ask people.

He and Bönnemann invited the girls to Bethesda to evaluate their condition more extensively. Both girls could compensate for their disability remarkably well, using vision to help them walk in a line or touch a target. But blindfolded, they struggled. Similarly, they could sense the vibration of a tuning fork against their skin because they could hear it. While wearing noise-cancelling headphones, they didn't notice the vibration at all[1].

Patapoutian found the same phenomenon in mice: without Piezo2 in nerves that supply muscles and tendons, they lacked proprioception and were uncoordinated[6]. His team also found a role for Piezo2 in pain-sensing neurons in allodynia, a specific type of pain sensation in which even a gentle caress feels like being pricked with needles. Some people with neuropathic pain experience this hypersensitivity all the time.

Mice normally show allodynia when injected with capsaicin – the spicy molecule found in hot chilli peppers – or after nerve injury, but not if they were missing the Piezo2 gene[7]. Chesler and Bönnemann reported similar changes to pain perception among people with *PIEZO2* mutations[8].

"Chronic pain has such a debilitating effect," says Swetha Murthy, who led one of the allodynia studies while a postdoc with Patapoutian. "I think we can start looking at drug targets for Piezo2 for these neuropathies." Both Patapoutian and Chesler are on the hunt for compounds that would block Piezo2 activity at a pain site, without interfering with the protein's other roles throughout the body. "There is huge potential for Piezo-channel drug discovery," says Xiao.

It's not only neurons that need to sense touch; almost every cell is subject to some kind of force. Take red blood cells, which deform to squeeze through tiny capillaries. Mutations that hyperactivate Piezo1 cause blood cells to shrivel, and that can provoke anaemia in people with a rare condition known as dehydrated hereditary stomatocytosis.

Those scrunched-up blood cells reminded Patapoutian of sickle-cell anaemia. The sickle-cell gene mutation has persisted in many people of African descent because it protects against malaria, and Patapoutian wondered whether *PIEZO1* mutations might do the same.

"There is huge potential for Piezo-channel drug discovery."

If so, there should be a relatively high rate of such mutations in people of African descent. Database searches revealed that Patapoutian was right: in fact, one particular *PIEZO1* variant appeared in one-third of people in the database with African ancestry[9]. A separate team has reported that carriers of this *PIEZO1* mutation are resistant to severe malaria[10].

Piezo1 also has a job in bone formation and maintenance, according to work from Xiao's lab. When his team knocked out *Piezo1* in mouse osteoblasts – bone-making cells – the animals grew up shorter and skinnier than normal. The long bones that support body weight were lighter, thinner and weaker than in control mice.

Moreover, wild-type mice that are partially suspended in the air – so they don't have to support their full body weight – have lower levels of *Piezo1* expression and bone mass[11]. It's a phenomenon much like what happens to people with osteoporosis, those who are bedridden and astronauts and cosmonauts aboard the International Space Station, says Xiao.

Pressure points

"The discovery of Piezos was a huge step forward for the whole field," says Kate Poole, a biologist at the University of New South Wales in Sydney, Australia, but "it is also clear that the story is not just Piezos."

Scientists interested in hearing have been chasing the relevant channel for four decades. "There have been a lot of false leads along the way," says Jeffrey Holt, a neuroscientist at Boston Children's Hospital in Massachusetts. "Now we think we've got a pretty solid handle on it."

The key channel protein is called TMC1. When Holt altered amino acids in TMC1, the procedure changed the ability of inner-ear cells to translate mechanical signals into electrical ones[12]. Another report showed that purified TMC1 is able to create a mechanosensitive ion channel in artificial membranous bubbles[13]. The structure of TMC1 is still a mystery, however, because the protein has been hard to purify in sufficient quantities to get good cryo-EM images.

Patapoutian's team, meanwhile, is looking for entirely new channel families. In 2018, he, Murthy and Scripps structural biologist Andrew Ward reported what they think could be the largest group of mechanically activated channels. They knew of a protein family that helps plants to sense osmotic pressure – the OSCA proteins – and reasoned that they might sense force more generally. In human kidney cells, OSCAs did indeed respond to Murthy's stretching of the cell membrane[14].

The researchers also knew from previous studies that the OSCA proteins were closely related to another family of proteins in mammals, the TMEM63 proteins. TMEM63 channels from mice, humans and even fruit flies responded to stretch in Murthy's assays, too, so OSCA and TMEM63 proteins make up a large family of force sensors that is common to many living things.

The channels discovered so far cannot explain all instances of cellular mechanosensitivity, says Murthy, now a biophysicist and neuroscientist at Oregon Health & Science University in Portland. More mechanosensors must be out there.

And those sensors probably have more jobs than are known today, says Patapoutian. "We've just barely scraped the surface."

Amber Dance is a freelance writer in Los Angeles, California.

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