Quick guide

Piezo channels

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What are Piezo proteins? Piezo proteins constitute a family of excitatory ion channels directly gated by mechanical forces. These ion channels are involved in cell mechanotransduction — the conversion of mechanical forces into biological signals.

Is mechanotransduction important? Yes it is! All living organisms are subjected to mechanical forces from their environment and rely on mechanotransduction for their survival. For instance, our senses of touch, mechanical pain, proprioception, hearing and balance depend on mechanically-activated channels. And besides sensory systems, mechanotransduction is involved in diverse physiological functions, including vascular tone and blood flow regulation, bone and muscle homeostasis, and flow sensing in kidney and respiratory systems.

Does mechanotransduction rely exclusively on mechanosensitive channels? No, it does not. Cells integrate a variety of mechanical stimuli, such as shear stress, tension, torsion and compression, and translate them into short-term effects (i.e. changes in ion concentrations and voltage) and long-term effects via changes in gene expression. Many membrane-associated molecules are involved in mechanotransduction, including ion channels, specialized cytoskeletal proteins, cell junction molecules, G-protein-coupled receptors and kinases. The particularity of mechanosensitive ion channels is to convert mechanical forces into electrical signals within tens of microseconds. This function is particularly well suited to the fast signaling that occurs in specialized sensory cells involved in touch and hearing.

So, why is the discovery of Piezo channels important for mammalian physiology? Although some mechanically-activated ion channels were characterized decades ago in bacteria and invertebrate species, these channels either are not conserved in vertebrates or lost their mechanotransduction properties during vertebrate evolution. Therefore, the molecular identification of mammalian mechanotransduction channels has remained a long-standing question in the field of sensory functions. The discovery of Piezo proteins in 2010 has fueled mechanotransduction-related research, opening up the field for prolific work in a wide range of research areas over the past few years.

What is known about* Piezo *genes? There is no *piezo* gene in bacteria, but *Piezo* homologs are found in plants and animals, including protozoa. Most vertebrates have two *Piezo* genes — *Piezo1* and *Piezo2* — with the human genes encoding relatively large proteins of over 2,500 and 2,800 amino acids, respectively. The mammalian *Piezo* genes are expressed in a wide range of tissues, highlighting the potential contribution of Piezo channels to mechanotransduction in various organs.

What does the Piezo channel look like and how does it sense force? Piezo1 assembles as a 900 kDa homotrimeric complex to form an ion channel with a propeller-like structure surrounding a central pore module. Residues forming the ion-conducting region of this pore module are localized to the carboxy-terminal quarter of the Piezo protein. Piezo channels are functional in artificial membranes, demonstrating that the channel can detect changes in membrane tension in the absence of other cellular components, but the structures of the force sensor(s) and the transducer element(s) that gates the ionic pore remain to be determined.

What kind of stimulation leads to Piezo activation? Piezo channels can be activated by many mechanical stimuli *in vitro*. While some of these stimulations directly mimic physiological forces experienced by cells *in vivo*, such as shear stress applied in a microfluidic chamber, the most commonly used modes of stimulation consist of stretching the membrane by applying positive or negative pressure through a patch-clamp recording electrode or by poking the membrane using a blunt glass pipette. The main limitation of these techniques is that the amount of force required to gate Piezo channels cannot be accurately determined. Therefore, efforts are being made to develop other methods, such as stimulation with an atomic force microscopy cantilever to precisely quantify the force applied or the use of magnetic nanoparticles linked to the channel to apply localized pulling forces. Despite the limitations in pharmacological regulation of Piezo channels, a screen of more than three million compounds has now led to the identification of Yoda1, a synthetic small molecule that activates Piezo1 by lowering its mechanical sensitivity.

What type of ionic current is mediated by Piezo channels? Activation of Piezo channels generates cationic non-selective currents, i.e. these channels are permeant to monovalent cations, such as Na^+ and K^+, and to divalent cations, such as Ca^{2+} and Mg^{2+}. Therefore, Piezos are excitatory channels, with their activation producing membrane depolarization. Piezo channel openings lead to Ca^{2+} entry into the cell, potentially triggering intracellular Ca^{2+} signaling pathways (Figure 1). Piezo currents become inactivated during prolonged stimulations with relatively fast inactivation kinetics at negative potential (on a millisecond timescale) that tend to become slower as the membrane potential increases.

Are Piezo channels involved in mechanosensation? Yes they are! Piezo2 is expressed in a subset of somatosensory neurons, the receptor cells that project throughout the whole body and are involved in the detection of touch, pain and proprioception. Since constitutive loss of *Piezo2* induces perinatal lethality, the physiological role of Piezo2 in mechanosensation has been characterized using conditional deletion of *Piezo2* specifically in sensory neurons. These studies demonstrated a crucial role for Piezo2 in light-touch sensing as well as in proprioception, while ruling out its involvement in mechanical pain. Moreover, Piezo2 in vagal and spinal sensory neurons that innervate the respiratory system

contributes to airway stretch sensing and mediates lung inflation-induced apnoea. Respiratory defects are therefore likely to be the cause of perinatal lethality in mice with constitutive loss of *Piezo2*.

… and in hearing? Auditory hair cells contain mechanosensitive channels in their stereocilia that detect sound-induced vibrations. A recent study has shown that Piezo2 is expressed in these hair cells. However, Piezo2 is localized in the apical membrane of hair cells and is responsible for a reverse-polarity current but not for the sensory-transduction current. Also, its specific deletion in these cells only induces a mild auditory defect in mice. These results highlight the presence of at least two molecularly distinct mechanosensitive channels in auditory hair cells, among which Piezo2 is not the 'hearing' channel. The precise function of Piezo2 in hair cells therefore requires further characterization.

What about Piezo1? Although Piezo1 has not been implicated so far in neuro-sensory functions, several studies have identified Piezo1 as a sensor of mechanical forces in endothelial, urothelial and renal epithelial cells. In particular, Piezo1 is involved in shear-stress sensing in blood vessel endothelial cells and is implicated in the developmental and physiological functions of the circulatory system, including the proper formation of blood vessels, regulation of vascular tone, and remodeling of small resistant arteries upon hypertension. The crucial role of Piezo1 in the development of the circulatory system explains the embryonic lethality of *Piezo1*-deficient mice.

In addition to its role in setting up and maintaining blood vessel integrity, Piezo1 is involved in red blood cell volume homeostasis. These cells experience significant mechanical forces while circulating in the bloodstream, and mechanosensitive Piezo1 channels act upstream of the calcium-activated potassium channel KCNN4 (also called the Gardos channel), which regulates intracellular cationic content and cell volume. Consequently, *Piezo1* deletion in mice leads to overhydration of red blood cells.

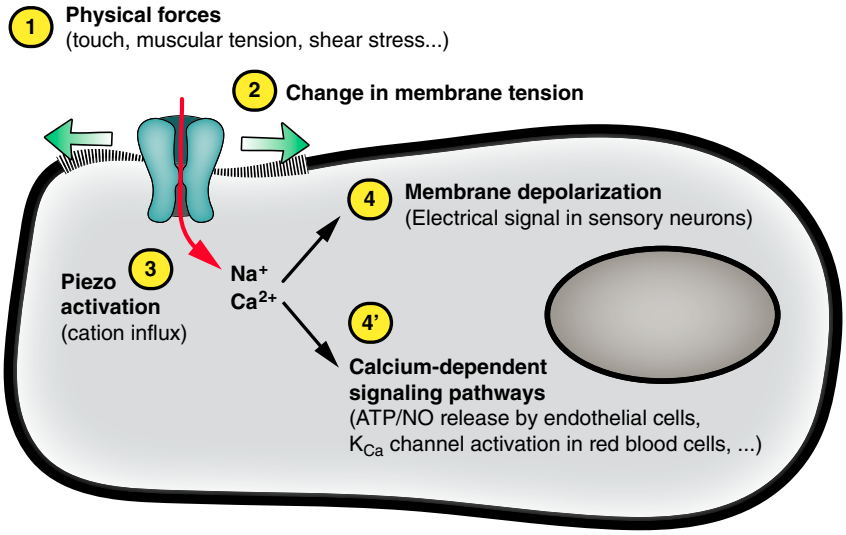

Figure 1. Piezo-dependent mechanotransduction.
Various mechanical stimuli exerted on cells induce changes in plasma membrane tension, eliciting Piezo channel opening. The resulting cation influx can trigger sensory neuron firing or activation of intracellular calcium signaling pathways.

Are there human diseases associated with PIEZO1 mutations? Yes, *PIEZO1* mutations have been linked to two main types of disorder in humans. Several autosomal dominant mutations, some of which have been characterized *in vitro* and lead to increased PIEZO1 signaling, are associated with dehydrated hereditary stomatocytosis (DHS). DHS is characterized by osmotically-driven dehydration of red blood cells that can lead to haemolytic anaemia. Therefore, *Piezo1* deletion in mice leads to overhydrated red blood cells, whereas 'gain of function' *PIEZO1* mutations associated with DHS in humans lead to dehydrated red blood cells, highlighting the crucial role of this channel in volume homeostasis in red blood cells.

Other *PIEZO1* mutations resulting in attenuated or disrupted PIEZO1 function have been linked to autosomal recessive generalized lymphatic dysplasia, a congenital disease that causes persistent lymphoedema, revealing the involvement of PIEZO1 in the development of lymphatic structure. It should be noted that Piezo1 is a widely-expressed ion channel and the full extent to which it regulates development and physiology is yet to be elucidated.

… and with PIEZO2 mutations? Autosomal dominant *PIEZO2* mutations thought to cause a 'gain of function' effect have been linked to different forms of distal arthrogryposis and to Marden-Walker syndrome. These conditions are multi-symptomatic human disorders presenting overlapping phenotypic characteristics, including short stature, curved fingers with straight thumbs and contractures of hands and feet. The broad spectrum of clinical defects associated with these diseases suggests that PIEZO2 is involved in the development and function of various structures in the body. Furthermore, several cases of recessive mutations leading to truncated or malfunctioning PIEZO2 channels have been reported. Patients bearing these mutations display a progressive phenotype that is mostly distinct from that resulting from dominant mutations, although there is a partial overlap, with both types of mutation giving rise to short stature and contractures. In addition to scoliosis, myopathy and progressive respiratory failure, disruption of PIEZO2 function leads to impairments in proprioception and discriminative touch perception, in agreement with the critical role of

Piezo2 in light-touch sensing and proprioception in mice.

Where can I find out more?

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Primer

Platyhelminthes

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Platyhelminthes (flatworms) have captivated the imagination of biologists for centuries. Indeed, planarian flatworms were used as experimental models decades before *Caenorhabditis elegans* became known as 'the worm'. Although planarians experienced a brief fall from grace, with the advent of molecular tools, planarians, such as *Schmidtea mediterranea*, have emerged in recent years as powerful models to study the basis of stem cell regulation and tissue regeneration. Flatworms are not just everyone's favorite experimental subjects from high school biology — they also include some of nature's most successful parasites, many of which have plagued humans throughout our history. This Primer will focus on several aspects of the remarkable biology found throughout the phylum Platyhelminthes.

Basic flatworm biology

Platyhelminthes (*platy* = flat and *helminth* = worm), or simply 'flatworms', are dorsoventrally flattened and bilaterally symmetrical worms (Figure 1A,B). Often speculated in the classic literature to represent primitive basal bilaterians, modern molecular phylogenetic analyses place the Platyhelminthes within the Lophotrochozoa, a clade of invertebrate animals that includes annelids (segmented worms) and mollusks. Free-living members of the phylum, the so-called turbellaria, are largely restricted to marine and freshwater environments; however, some taxa, such as land planarians (Figure 1B), are capable of inhabiting warm humid terrestrial habitats. Turbellarian flatworms are almost entirely carnivorous, scavenging on the remains of dead animals or in some cases tracking, capturing, and killing their prey. Although these free-living taxa have evolved to thrive in a variety of habitats worldwide, the most successful group of flatworms without question belong to the Neodermata. This group of obligate parasites includes both flukes and tapeworms that together are responsible for a significant disease burden in livestock and humans throughout the world.

Given the diversity of flatworms it is difficult to make sweeping generalizations that unite all members of the phylum. However, unlike most bilaterians, flatworms lack a coelom and possess a 'blind gut' where food enters and exits via the same orifice (that is to say, they have no anus). Flatworms are also dorsoventrally flattened, ensuring the diffusion of oxygen and nutrients to their tissues. Despite this constraint, the absence of a cuticle, exoskeleton or shell has allowed these soft-bodied worms to adopt a dizzying array of shapes, body plans and sizes (Figure 1B–O). Many free-living flatworms are microscopic, but some can reach >10 centimeters in length (for example, land planarians; Figure 1B). These variations in body sizes and shapes are even more exaggerated among members of the Neodermata. Indeed, larval Neodermata rarely resemble the adult parasite (Figure 2), and tapeworms, arguably the most notorious of all Neodermata, can reach tens of meters in length growing inside their hosts (Figure 1K,L).

The outer surface of turbellarian flatworms is typically lined with a simple epidermis comprised of a single layer of columnar epithelium that sits on top of a basement membrane and several layers of muscles. This epithelium is usually ciliated, allowing these worms to swim through the water column or to 'glide' over the substrate. Flatworms lack a circulatory system but possess a primitive excretory system (protonephridia) and an array of secretory organs that aid in digestion, protection from predators, locomotion, prey capture and the prevention of desiccation in terrestrial environments. These worms also possess a well-organized central nervous system, consisting of an anteriorly positioned brain that interfaces with a peripheral nervous system and a variety of sensory organs, including pigmented 'eye-like' photoreceptors in many taxa (such as freshwater planarians; Figure 1C–E,I). Despite the seemingly