TRP channels in mechanosensation: direct or indirect activation?

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Abstract | Ion channels of the transient receptor potential (TRP) superfamily are involved in a wide variety of neural signalling processes, most prominently in sensory receptor cells. They are essential for mechanosensation in systems ranging from fruitfly hearing, to nematode touch, to mouse mechanical pain. However, it is unclear in many instances whether a TRP channel directly transduces the mechanical stimulus or is part of a downstream signalling pathway. Here, we propose criteria for establishing direct mechanical activation of ion channels and review these criteria in a number of mechanosensory systems in which TRP channels are involved.

Osmoregulation
A homeostatic mechanism by which cells maintain their volume despite changes in extracellular osmolarity.

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Mechanically sensitive ion channels mediate a vast array of different cellular and organismic sensations, ranging from the most basic that must occur in all living cells, such as osmoregulation, to the highly specialized, such as hearing and touch. The channels that mediate these sensations and how they convert a force stimulus into channel gating have remained largely a mystery. Members of the transient receptor potential (TRP) family of ion channels have been implicated in a wide variety of mechanical transduction processes in diverse organs and species.

There are 33 TRP channel genes in mammals (nearly 60 in zebrafish, 30 in sea squirts, 24 in nematodes, 16 in fruitflies and 1 in yeast)[1]. They are subdivided into seven subfamilies on the basis of sequence similarity[1] (FIG. 1). The channel subunits they encode have a molecular architecture similar to that of members of the voltage-gated ion channel superfamily: they all include at least six transmembrane domains, as well as a number of other domains (FIG. 2), and four subunits assemble to form a functional ion channel. We will use the subunit name to refer also to the channel it forms as a homotetramer. Some TRP channel subunits can form heterotetrameric channels as well, giving rise to a large variety of channels with different gating and permeability properties[2]. Most TRP channels are non-selective cation channels[3]. In addition to mechanosensation, TRP channels are involved in the transduction of a wide variety of other sensations, with roles in vision, olfaction, taste, chemosensation and thermosensation[4,5].

However, the specific function of these, and other, ion channels in the process of mechanosensation is not fully understood[6]. Is the ion channel the primary transducer — sensing force and passing the receptor current — or does it have a necessary, but indirect, supporting role? Here we review the current literature using a set of criteria designed to determine whether a candidate ion channel is directly activated by mechanical stimuli. Not all criteria can be met in all systems, so adequate proof in each system may comprise satisfying only a subset of these criteria. However, we hope that these criteria will help focus research in mechanosensation specifically for TRP channels and also, more generally, for all mechanically sensitive ion channels.

Criteria to establish direct mechanical activation

In evaluating a mechanosensory system, we need to ask whether the receptor current is carried by an ion channel that is directly activated by the stimulus, and whether a particular candidate protein participates in that channel.

Does mechanosensation involve direct activation of a channel? Several tests may distinguish between mechanically gated channels that act as force sensors themselves and mechanically sensitive channels that are activated by second messengers downstream of the true force sensors. First, the latency of the current elicited by the stimulus should be faster than known second-messenger systems, typically less than 5 milliseconds. Currently, many of the stimuli used to demonstrate mechanosensitivity, particularly osmotic stimuli, lack the rapid risetime needed to determine a latency that is this fast. Second, the kinetics of channel activation should depend on the amplitude of the stimulus — a larger mechanical force should result in faster channel opening. This is a simple consequence

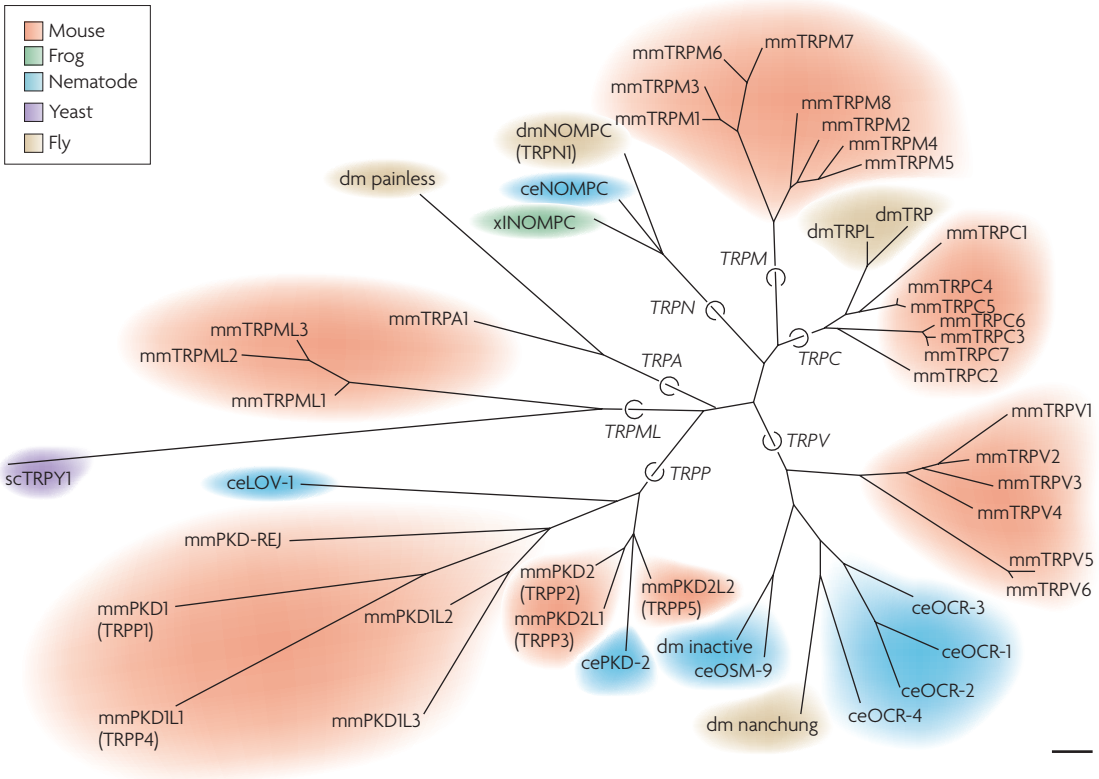


Figure 1 | **Phylogeny of representative TRP channels.** A phylogenic tree was generated in ClustalX by aligning the transmembrane domains of all 33 transient receptor potential (TRP) channels from mouse and some from other species. The seven main branches are denoted by circles at the branch roots. The letters and numbers following TRP denote TRP subfamily and member, respectively. Different species are indicated by colours and by prefixes. ce, *Caenorhabditis elegans*; dm, *Drosophila melanogaster*; mm, *Mus musculus*; sc, *Saccharomyces cerevisiae*; xl, *Xenopus laevis*. Scale bar represents 0.2 nucleotide substitutions per site.

of a larger force lowering the energy barrier to channel opening. Finally, there should be a mechanical correlate of channel gating, such as an observable movement or change in mechanical force, in the sensory cell or organ over the same range of stimuli as opens the channel. For force to open a channel, some part of the channel or an associated subunit that is closely coupled to the gating has to move in response to force and this movement can be detectable. This correlate will vary with differing gating mechanisms (BOX 1) and might be difficult to measure but, when present, it is strong evidence for direct gating. This criterion, of course, addresses only the mode of activation of the channel and not its molecular identity.

Does the candidate protein participate in mechanical transduction? To be a reasonable candidate for transducing the mechanosensory stimulus, a channel protein must be in the right place and must be necessary for mechanotransduction. Thus, the candidate channel gene must be expressed in the receptor cell by the time during development that the mechanically sensitive current is detected. The candidate channel protein should be located at the site of mechanical transduction within the cell. Finally, blocking candidate protein expression by knockdown or knockout should block the mechanically activated conductance.

Is the candidate protein mechanically sensitive? If a channel is directly activated by a mechanical stimulus then it should retain this mechanical gating property when expressed in other cells (as long as the force stimulus is applied in an appropriate manner). For example, if a channel operates by directly sensing lipid tension, then the recombinant protein should retain its ability to be opened by pressure stimuli when placed in liposomes. Furthermore, in a heterologous expression system the forces necessary to gate the candidate channel should be comparable to the physiological stimulus. However, it should be noted that, if force is conveyed to a channel by structural proteins that are present only in the receptor cell, or if required lipids or cofactors are present only in the receptor cell, activation in a heterologous expression system might be more difficult.

Is the candidate protein a pore-forming subunit? Even if expressed at the right time and place, a candidate protein could be an accessory protein for the channel but not part of the ion conduction pathway. If the candidate protein forms the ion channel pore, the pharmacology and permeation properties of the mechanically gated conductance in sensory cells should be similar to those of the heterologously expressed candidate.

Liposome
A lipid vesicle that is artificially formed by sonicating lipids in an aqueous solution.

Heterologous expression system
A system for studying the function of a protein in which a gene construct is transfected into suitable host cells such as bacteria or cultured mammalian cells that will produce the protein in a near-native environment.

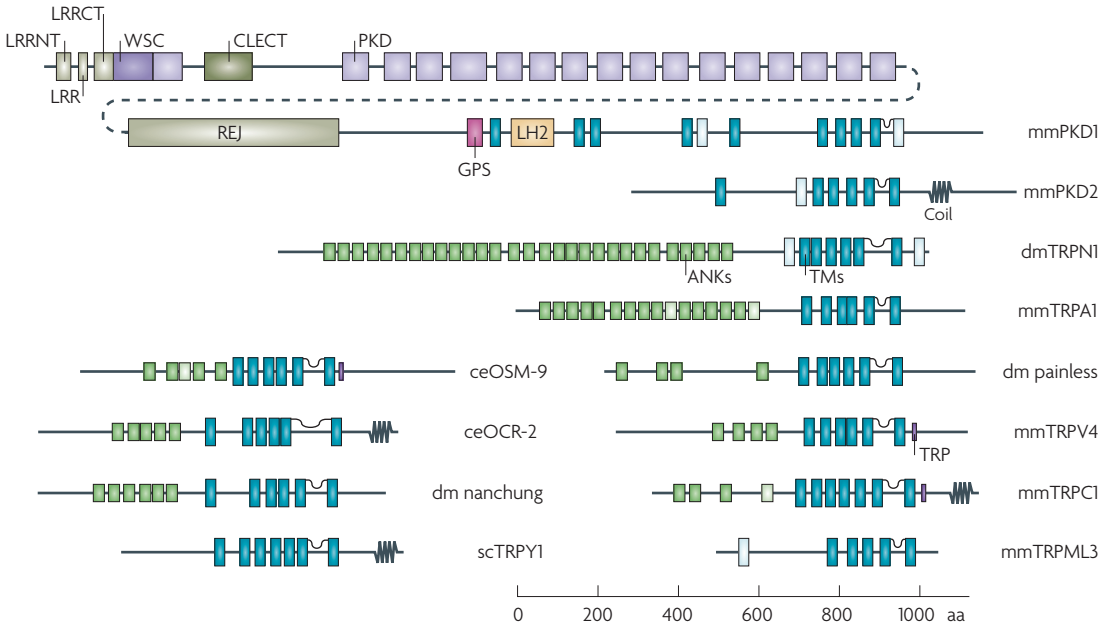


Figure 2 | **Domain structures of some TRP channels.** The polycystic kidney disease (PKD1) group have much longer N-termini (connected by dashed line) than other transient receptor potential (TRP) channels. Strongly predicted transmembrane domains are represented by blue boxes and possible transmembrane domains are represented in light blue. The letters and numbers following TRP denote TRP subfamily and member, respectively. ANKs, ankyrin repeat domains; CLECT, C-type lectin domain; coil, coiled-coil domain; GPS, G-protein-coupled-receptor proteolytic-site domain; LH2, lipoxygenase homology 2 domain; LRR, leucine-rich repeat; LRRCT, leucine-rich repeat C-terminal domain; LRRNT, leucine-rich repeat N-terminal domain; TMs, transmembrane domains; REJ, receptor for egg jelly domain; WSC, WSC domain. Species are indicated by prefixes: ce, *Caenorhabditis elegans*; dm, *Drosophila melanogaster*; mm, *Mus musculus*; sc, *Saccharomyces cerevisiae*; xl, *Xenopus laevis*. Scale shows length of primary structure in amino acids (aa).

A more definitive test is to ask whether the expression of a candidate channel protein with a mutation in the putative pore region alters the pharmacology or permeation properties of the native conductance in the sensory cell.

Is the candidate protein a force-sensing subunit?
In a heteromultimeric channel, it is possible that some subunits sense force and control gating, whereas others simply contribute to the pore. The best evidence that a candidate channel protein senses force is if the force sensitivity of the native conductance is altered when the candidate protein is mutated and overexpressed in the sensory cells.

With these criteria in mind, what can we say about the roles of TRP channels in mechanosensation?

Evidence from non-neural systems

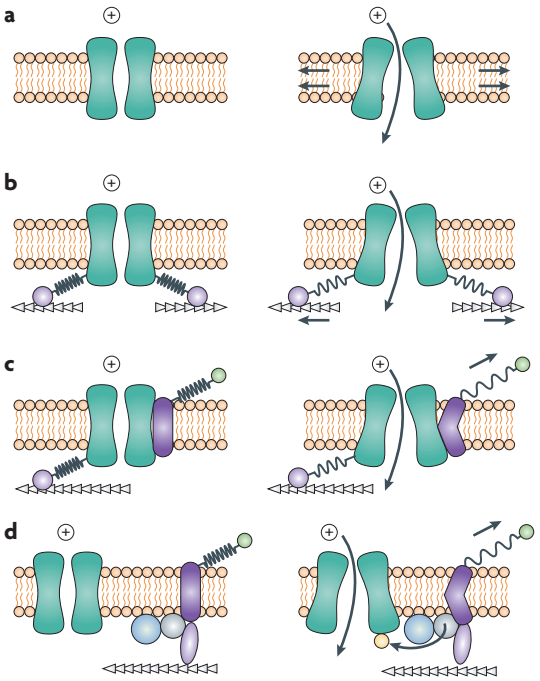
Much of the work on TRP channels in mechanosensation comes from non-neural systems, which we will review briefly. One of the first mechanically sensitive currents was found in *Xenopus laevis* oocytes[7]. The stretch-activated channel (SAC) underlying this current has a response latency of less than 5 milliseconds[8], meeting one element of the first criterion and suggesting that the channel is directly activated by stretch. Recent evidence suggests that this oocyte SAC contains TRPC1 (REF. 9).

Other members of the TRP channel family, TRPC3 and TRPC6, along with TRPV2 and TRPM4, are implicated in mediating myogenic tone (the constriction of blood vessels under increasing pressure)[10]. Knockdown of these TRP channel genes in the smooth muscle cells of blood vessels decreases the mechanically responsive current that is observed in response to experimentally stretching these cells[11–14]. Some evidence suggests that the mechanically sensitive channel involved is activated by signalling pathways downstream of a G-protein-coupled receptor, but that it is not mechanically gated[15]. However, even in the presence of phospholipase C blockers, TRPC6 has been shown to respond to pressure stimuli in patches[16], arguing against second-messenger activation.

TRPM7 has also been implicated in mechanosensation in vascular smooth muscles at higher pressures. The mechanism of activation is controversial: one group reported that there was an increase in the insertion of TRPM7 in the plasma membrane with membrane stretch[17], whereas another found that stretching the membrane directly activated TRPM7 currents in cell-free patches and in cell-attached patches in the presence of a blocker of membrane trafficking from the Golgi[18,19].

A third member of the TRPM subfamily, TRPM3, was found to be activated by hyper-osmotic stimuli when it was first characterized[20]. Because of its localization in the

Box 1 | **Potential gating mechanisms for mechanically sensitive ion channels**



There are two general models of channel gating by mechanical stimuli (REFS 105,106). The membrane model supposes that force is delivered to the channel by surface tension or bending of the lipid bilayer, causing a hydrophobic mismatch that favours opening. Opening decreases the energy stored in surface tension (see figure, part **a**). The tether model supposes that specific accessory proteins such as intracellular cytoskeletal elements and/or extracellular matrix molecules are bound to channel proteins, and that the stimulus force is conveyed by these tethers to induce a conformational change (see figure, part **b**). Tethers can be direct or indirect; for instance, force might be conveyed to an accessory protein, inducing a conformational change that, in turn, communicates sterically with the pore-forming subunits (see figure, part **c**). Finally, the force-sensing protein might be more distant and communicate with the channel by generating a secondary signal such as a diffusible second-messenger molecule or activation of a kinase (see figure, part **d**). The channel might then be considered mechanically sensitive but not mechanically gated.

In these models the criteria for a mechanically gated channel are met in slightly different ways. In the tether model the stimulus usually results from movement between adjacent cells or between two parts of the same cell. Some part of the tether is elastic and may be termed the 'gating spring'. In favourable mechanosensory systems, gating of the channel produces a detectable movement of the tether[81,105]. In the membrane model the lipid is stretched by an applied force; as the channel opens (or closes) it causes an increase in the area of the membrane and thereby relaxes the lipid. The relaxation of lipid surface tension is the mechanical correlate of channel gating, although it might be difficult to measure. Figure modified with permission from REF. 6 © (2005) Elsevier Science.

kidney and its permeability to calcium it was suggested that TRPM3 has a role in calcium homeostasis in that organ; however, its apparent absence from the mouse kidney makes this less certain.

TRPV1 has recently been implicated in controlling the response of the bladder urothelium to stretch. TRPV1$^{-/-}$ mice have defects in bladder voiding[21], a process that is mediated by TRPV1 located in urothelial cells[22]. However, currents have not been recorded from urothelial cells in response to a mechanical stimulus. Therefore, it is still uncertain whether TRPV1 acts as a sensor of mechanical force in this system or, instead, detects the presence of molecules produced by stretch, which are either released into the extracellular space or produced in the cytoplasm.

Members of the TRP subfamily P (TRPP) are also thought to mediate mechanosensation in the primary cilium of kidney epithelial cells in the nephron[23]. When the primary cilia of kidney cells are experimentally displaced by a flow stimulus of the same magnitude as the flow in the nephron, intracellular calcium levels rise[24]. Mutations in two TRPP channel genes, PKD1 and PKD2 (also known as TRPP1 and TRPP2, respectively) cause polycystic kidney disease in humans. When these genes are disrupted in mice, the calcium response in kidney cells is abolished[25]. Because PKD2 can form an ion channel by itself, many have suggested that PKD1 acts as an accessory subunit; however, there is no good evidence that PKD1 does not participate in the ion conduction pathway. Moreover, a change in the membrane calcium conductance in response to cilia deflection has not been observed in these cells, indicating that the calcium may come entirely from intracellular stores.

Finally, TRPY1, the only member of the TRP family in yeast[26], has been implicated in the ability of yeast vacuoles to respond to osmotic stimuli. This function is similar to that of the bacterial ion channel mscL (BOX 2), which is the best understood mechanically gated ion channel. Both single-channel recording and calcium imaging show increases in TRPY1 activity when osmolarity is increased[27–29].

Although all of these TRP-channel functions in mechanosensation need further investigation, they suggest a general role for TRP channels as mechanically sensitive channels. We now turn to the role of TRP channels in mechanosensation in the nervous system, including osmosensation, body touch and hearing.

Systemic osmosensation

TRPV4 has been implicated in a wide variety of mechanosensory processes. Like many TRP channels, TRPV4 was discovered through homology screens of the mammalian genome and was initially named VR-OAC, OTRPC4, TRP12 and VRL-2 (REFS 30–33) by four independent groups. When expressed in heterologous expression systems, TRPV4 can be activated by

Box 2 | Osmotic swelling in bacteria

The best understood mechanically gated ion channel, mscL, mediates the response to osmotic shock in bacteria. Although not related to transient receptor potential channels, mscL offers a good example of mechanosensation research, and is the prototypical channel for activation by lipid stretch.

Osmotic swelling of bacteria increases membrane surface tension, opening large ion channels. mscL was discovered by fractionation and purification of proteins with channel activity from bacterial membranes[107]. Disruption of the *mscL* gene in bacteria eliminates channel activity, and overexpression increases channel number. mscL protein, reconstituted into liposomes, displays full activity[108,109].

It is difficult to apply tension quickly to bacterial membranes, so measures of speed and mechanical correlates of gating are lacking. mscL does demonstrate faster activation when larger pressure steps are applied[110]. Nevertheless, reconstitution of mechanically gated-channel activity with purified mscL protein leaves little doubt that the osmotically activated channel is directly activated by the mechanical stimulus, that mscL participates in mechanical transduction and that the candidate protein is activated by mechanical stimuli[108,109].

Whether mscL creates the pore of the mechanically activated channel has not been directly tested. Some of the properties of the reconstituted mscL channel pore have been determined, such as its size[111]. Although many mutants of mscL have been described, none affect the permeation properties and affinity of pore-blockers. Nevertheless, reconstitution of the conductance by mscL alone means it must form the conductive pore.

It is clear that mscL participates in force sensing. Both random and site-directed mutagenesis have explored regions of the channel controlling its force sensitivity. These have been well correlated with the crystal structure of the protein and they fit reasonably well with steered molecular dynamics simulations of gating[112–118].

hypo-osmotic stimuli, but not by hyper-osmotic or pressure stimuli[30,31]. Although a hypo-osmotic stimulus is easy to deliver, it is not well defined. It is both a chemical stimulus, in that ionic strength is decreased, and a mechanical stimulus, in that the cell swells, leading to both a stretch of the cell's cytoskeleton and an increase in membrane surface tension. Specific mechanical activation can in some cases be confirmed by alternative methods for delivering force.

TRPV4 appears in various tissues: *in situ* hybridization and RT-PCR revealed its expression in kidney, liver, heart, cochlea, sensory neurons, Merkel cells, keratinocytes, airway epithelia and the circumventricular organs of the brain, which are involved in osmoregulation[30,31,34]. The most studied mechanosensory role of TRPV4 in the mammalian nervous system is in the systemic response to changes in the osmolarity of the body. When serum is hyper-osmotic, osmosensitive neurons of the circumventricular organs, primarily the organum vasculosum lamina terminalus (OVLT) and the subfornical organ (SFO), activate the hypothalamus to mediate antidiuretic hormone (ADH) release and cause water reabsorption in the kidney and colon[35–37]. The circumventricular organs also have an important role in regulating water intake[35,36]. Both the OVLT and the SFO express TRPV4. Two independent lines of TRPV4 knockout mice, showed slight tendencies to drink less water and to become hyperosmolar, although they differed in secretion of ADH[38,39].

A pressing question is whether TRPV4 is directly gated or indirectly activated by mechanical stimuli. The kinetics and latency of the mechanically sensitive ion channel have not been studied in these osmosensitive systems; instead, the available evidence suggests that TRPV4 can be activated by second messengers. It was initially found that TRPV4 activation was downstream of a Src kinase that phosphorylated tyrosine 253 (REF. 40). However, a second group could not reproduce this finding and found that the activation of TRPV4 by hypo-osmotic stimuli depends on the breakdown of arachidonic acid to 5′,6′-epoxyeicosatrienoic acid (5′,6′-EET) by cytochrome P450 epoxygenase[41,42]. 5′,6′-EET was later shown to directly activate TRPV4 (REF. 43). Intriguingly, a recent study of TRPV4 in nociceptive osmosensation in mice found that activation of TRPV4 by both hypo- and hyper-osmotic stimuli depended on its phosphorylation by a Src family kinase[44]. The pathway leading to TRPV4 activation by osmotic stimuli remains unresolved.

So far most evidence suggests that TRPV4 lies downstream of an osmotic sensor and mediates the transduction of osmotic stimuli in a diverse range of cell types. A pore mutation that affects the permeation properties of TRPV4 and the affinity of blockers for this channel has been described[45,46], but it has not been used to investigate the physiological role of TRPV4.

A related channel, the heat-sensing TRPV1, has also been implicated in controlling the OVLT and the hypothalamic osmosensitive response of neurons to changes in osmolarity[47,48]. An isoform of TRPV1 is expressed in the supraoptic nucleus of the hypothalamus[47], and neurons from the OVLT and supraoptic nucleus in a TRPV1 knockout mouse did not respond to hyper-osmotic stimuli[48]. What this means for the interplay between TRPV4 and TRPV1 in these neurons has not been investigated yet.

Nose touch and osmosensation in nematodes

The sensation of touch in *Caenorhabditis elegans* is mediated by at least three distinct sets of neurons (FIG. 3). Touch to the body wall is detected by non-ciliated sensory neurons that express MEC-4 and MEC-10, which are members of the degenerin/epithelial sodium channel (DEG/ENaC) family of ion channels[49,50] that are unrelated to TRPs. These channels are probably directly activated by mechanical force (BOX 3). Nose touch is mediated by the ciliated ASH, FLP and OLQ neurons[51]. The ASH neuron is a polymodal nociceptive neuron that also mediates osmosensation and the response to a subset of aversive

Merkel cell
A specialized cell in the skin, often associated with sensory hairs, that is involved in cutaneous mechanosensation.

Circumventricular organ
A region of the brain that has a rich vascular plexus with a specialized arrangement of blood vessels. The junctions between the capillary endothelial cells are not tight in the blood vessels of these regions, allowing the diffusion of large molecules.

chemicals[52]. An invertebrate member of the TRPV family, OSM-9, is thought to mediate the response to nose touch; it is expressed in the cilia of nose touch neurons and many other chemosensory neurons[53]. Loss-of-function mutations in OSM-9 eliminate sensitivity to nose touch, to hyperosmolar solutions and to the attractive odorant diacetyl. Interestingly, when TRPV4 is expressed in the place of OSM-9 it can rescue the sensitivity to nose touch and hyperosmolarity[45]. Rescue only requires the pore region of TRPV4 as constructs that had both the N- and C-termini truncated were still able to rescue OSM-9 mutants but those with a mutation in the pore region of TRPV4 were not. This suggests that OSM-9 makes up part of the conduction pathway for this mechanosensitive channel but is not required for gating.

Four OSM-9 homologues, named OSM-9/capsaicin-receptor-related (OCR) proteins, have been identified[54] (FIG. 1). Two of these, OCR-2 and OCR-4, are expressed in ASH and OLQ neurons, respectively. Abolishing OCR-2 function reduces sensitivity to nose touch and osmotic sensation. Moreover, OSM-9 and OCR-2 are each necessary and sufficient for the localization of the other to the cilia plasma membrane[54], suggesting that they form a complex, perhaps as subunits of a heteromeric channel. Animals with a mutation in OCR-2 were not rescued by expression of TRPV4, suggesting that TRPV4 is less similar to OCR-2 than to OSM-9[45].

The three modalities of nociception mediated by ASH neurons — mechanosensation, osmosensation and chemosensation — use distinct signalling pathways, but all converge on OSM-9 (REF. 53). The cytosolic protein OSM-10 is also necessary for the response to hyperosmolarity, but not for the response to nose touch[55]. Sensitivity to nose touch requires the activity of neurons downstream of ASH that express the glutamate receptor GLR-1, but osmosensation remains intact when GLR-1 is mutated[56,57]. Both osmosensation and nose touch responses require the function of the G-protein ODR-3 (REF. 58), and both are impaired in mutants that are defective in polyunsaturated fatty acid (PUFA) production[59]. These observations suggest that OSM-9 and OCR-2 are not directly activated by mechanical stimuli, but that they lie downstream of distinct signalling mechanisms for each stimulus.

Vulva location in nematodes

Another process in the nematode that is thought to involve mechanosensation is the male-specific behaviour of locating the vulva in hermaphrodites. Male nematodes use sensory rays on their tail to locate the vulva[60] (FIG. 3). Invertebrate TRPP homologues have been implicated in this process: both LOV-1, a TRPP1 homologue, and PKD2, a TRPP2 homologue, are expressed in the cilia of sensory neurons innervating the male sex organ[61]. Mutation of either or both of these proteins impairs the ability of the male to locate

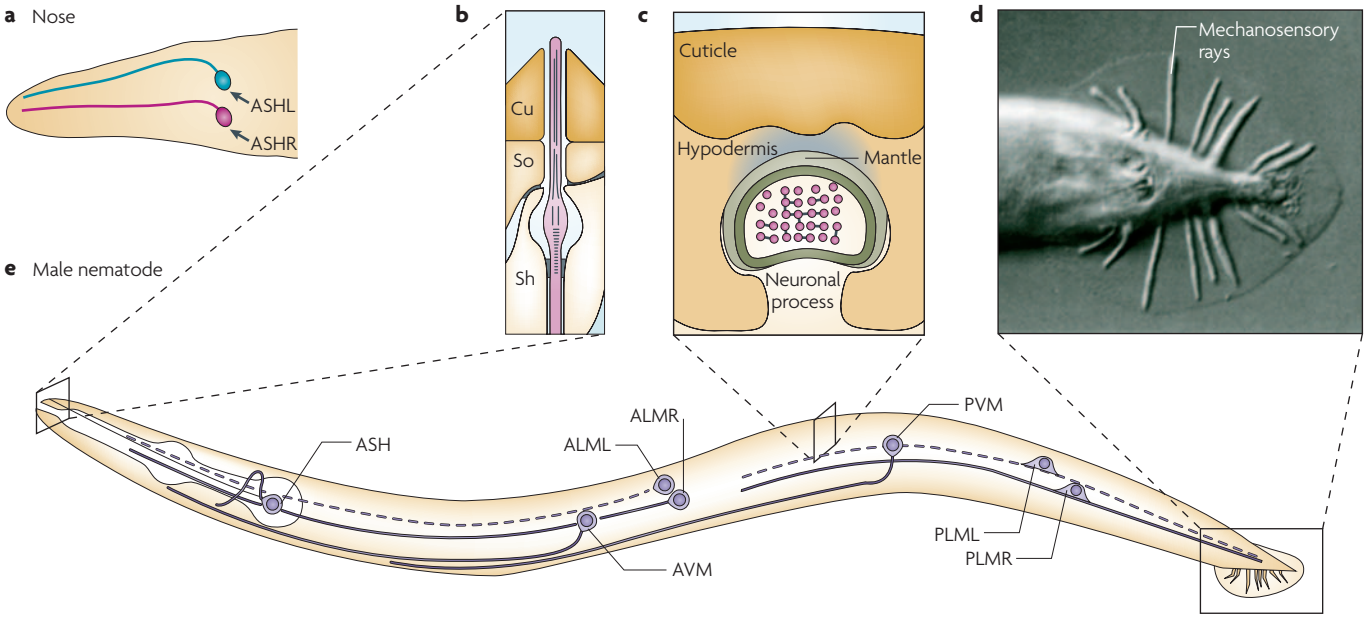


Figure 3 | **Touch sensation in nematodes. a** | ASH neurons (left and right) send processes rostrally to sense touch and chemical stimuli at the nose. These neurons use the transient receptor potential (TRP) channels OSM-9 and OCR-2. **b** | ASH neuron endings pass through the amphid sensilla sheath to sense the external environment. **c** | Lateral sensory neurons (AVM, ALMs, PVM and PLMs) send processes within the hypodermis in close proximity to the cuticle to sense touch to the body wall. These neurons use mechanically sensitive ion channels containing MEC-4 and MEC-10, unrelated to TRP channels. **d** | Male nematode tails have nine pairs of mechanosensory rays; they require the TRP channels LOV-1 and PKD2 for vulva location. **e** | Position of sensory structures in a male nematode. Cu, cuticle; Sh, sheath; So, socket. Panel **a** was reproduced with permission from REF 55 © (1999) Society for Neuroscience. Panel **b** was reproduced with permission from REF 131 © (2003) Annual Reviews. Panel **d** was reproduced with permission from REF 132 © (1995) Elsevier Science.

Johnston's organ
The hearing organ in insects, formed by a collection of mechanosensory neurons in the second antennal segment that respond to sound-induced rotation of the third antennal segment.

Arista
A feathery appendage of the insect antenna that is moved by acoustic stimuli.

Box 3 | Body touch in nematodes: MEC-4 and MEC-10

Perhaps the first group of ion channels to be implicated in mechanosensitivity in eukaryotes was the DEG/ENaC (degenerin/epithelial sodium channel) superfamily. Channel proteins of this family have two transmembrane domains with a large extracellular loop and assemble as heteromultimers of 4–6 subunits[119,120]. Evidence that some of these ion channels could be activated by mechanical stimuli came from a mutation screen in *Caenorhabditis elegans* for animals that were insensitive to light touch[121]. Two of the twelve known *mec* genes, *mec-4* and *mec-10*, encode members of the DEG/ENaC superfamily[122,123]. Others encode structural proteins that might form a tether system. Transcripts of *mec-4* and *mec-10* are expressed in the six mechanosensitive neurons that innervate the body of the worm (FIG. 3), and the MEC-4 and MEC-10 proteins are appropriately located in punctae along the length of these neurons[124,125].

Mechanotransduction is probably direct: the response latency can be as short as 0.7 milliseconds and it decreases with increasing stimulus amplitude[50]. A mechanical correlate of gating has not been described and will be difficult to observe. MEC-4 and MEC-10 by themselves do not form mechanically gated ion channels in heterologous expression systems; however, potential tether proteins were not co-expressed[126].

A pore mutation that affects selectivity of the channel has not been identified. The physiological conductance of MEC-4 and MEC-10 channels is similarly inhibited by amiloride, and both channels are similarly permeable to sodium, potassium and *N*-methyl D-glucamine[50]. No other putative channel proteins were found in this saturated screen.

the vulva[61,62]. Although this process is thought to involve mechanosensation, mechanical responses of cilia deflection have not yet been investigated and therefore, the possibility remains that the process involves chemosensation.

Nociceptive mechanosensation in fruitflies

Larvae of the fruitfly *Drosophila melanogaster* respond to a painful stimulus (heat or pinching) by stopping their movement and rolling away from the stimulus; this response is mediated by multidendritic neurons in the skin[63]. A behavioural screen for *D. melanogaster* larvae defective in this pain response isolated a mutant line, dubbed *painless*, that carries an insertion in a gene encoding a fly member of the TRP subfamily A (TRPA). The *painless* mutant also showed decreased heat-stimulated activation of multidendritic neuron firing. Antibodies to the painless protein labelled sensory dendrites of multidendritic neurons, implying a direct sensory role for this TRPA channel.

The role of mammalian TRPA1 in mechanical pain sensation (see below) is consistent with the direct mechanical activation of the *D. melanogaster* TRPA; however, the latency of painless activation by noxious stimuli is not known. It will also be important to determine the role of *painless* in adult flies.

Bristle touch in fruitflies

In adult *D. melanogaster* most touch sensitivity is mediated by sensory bristles that cover the body (FIG. 4a). Although the development of these bristles has been extensively studied, how their deflection is transduced into neuronal firing is less well understood. The neuron that mediates this transduction projects a dendritic tip into the base of the hollow bristle (FIG. 4b). The channel that senses bristle deflection and carries the receptor current is probably directly gated by the mechanical stimulus because the latency between the stimulus and receptor current is short (less than 200 μs)[64]. A mechanical correlate of channel gating has not been observed, but it might be difficult to find given the intrinsic stiffness of the bristle.

A mutational screen uncovered one channel gene, a TRP channel originally called NOMPC (no mechanoreceptor potential C, also known as TRPN1), that is required for bristle touch sensitivity[64]. Three out of four mutant fly alleles nearly abolished the transient current in response to bristle deflection, although a small, steady current remained (FIG. 4c). The fourth mutation, located in the extracellular loop of TRPN1 between the third and fourth transmembrane domains, caused a change in the kinetics of adaptation to maintained deflection, which is consistent with an intimate association between TRPN1 and the transduction apparatus. However, the remaining non-adapting current raises the possibility that TRPN1 simply functions as an amplification mechanism for another true mechanically gated ion channel. Furthermore, TRPN1 expression has been reported in the bristle complex, possibly in the sensory neuron itself, but finer localization studies have not been done. Thus, we still do not know if TRPN1 is localized in the correct place to mediate mechanotransduction. TRPN1 in fly bristles has the potential to be one of the best systems for understanding force activation of a TRP channel, but more direct evidence for its role has been elusive.

The expression of TRPN1 has also been described in *C. elegans*, where it appears in the ciliated mechanosensory neurons CEPV, CEPD and ADE, which innervate the head of the worm[65], as well as in two interneurons, DVA and DVC. Recent work suggests that TRPN1 functions as a stretch-activated channel in the DVA to modulate body bending[66].

Hearing in fruitflies

D. melanogaster detect sound, such as courtship songs, using Johnston's organs. The fly uses the arista of the antenna (FIG. 4a) to capture incoming sound. Sound waves make the arista vibrate, causing a rotational movement of antennal segment 3 (A3) relative to antennal segment 2 (A2) (FIG. 4d). A3 contains a stalk and hook that project into A2 and that contact the ciliated neurons in Johnston's organ. Thus, the rotational

Chordotonal organ
A sensory organ in insects that detects mechanical and sound vibrations.

Stereocilia
Elongated microvilli emanating from the apical surfaces of hair cells, composed of a dense core of crosslinked actin filaments surrounded by the cell membrane.

force of A3 is translated into stretching of the neurons in Johnston's organ[67]. The molecular mechanisms that underlie fly hearing are beginning to be uncovered. Conveniently, many of the mutations affecting bristle touch in the fly also cause defects in hearing[68].

The latency between the acoustic stimulus and the receptor current is less than 1.2 milliseconds (perhaps much less)[61], which implies the presence of a directly activated channel. Moreover, vibration of the aristae is actively amplified in live flies, which is consistent with the transduction process pumping energy back into antenna movement[69–71]. If the transduction channel itself generates the amplification force, as a mechanical correlate of gating, this system would meet one of the criteria for a directly-gated channel. A similar process occurs in the vertebrate cochlea and is suggested to result from force production by transduction channel gating[72–74].

There are currently three candidates for ion channels to mediate mechanotransduction in fly hearing. The first is TRPN1, which is also implicated in bristle touch. In TRPN1 mutants, auditory nerve firing was reduced by approximately 50% and antennal vibration showed no active amplification[68], which is consistent with a deficit in the transduction process itself[70]. The other two candidates are *D. melanogaster* members of the TRPV family — nanchung (Nan) and inactive (Iav), which are homologues of the worm OCR channels. When the function of either Nan or Iav is abolished, auditory nerve responses completely cease[75,76]. Nan and Iav are both located in the ciliary outer segment of the chordotonal organ, as expected for a channel mediating auditory transduction. Correct subcellular localization of either Nan or Iav depends on the other, which suggests that these two channel proteins form a heteromultimeric channel. Both Iav and Nan are activated by hypo-osmotic stimulation in heterologous expression systems, supporting the idea that they are mechanically sensitive. Nan and Iav mutants show defective gain control in the active amplification process, but the amplification depends more critically on TRPN1 (REF. 77). More work is needed to verify that Nan and Iav are functioning as the mechanically gated ion channel in fly hearing, rather than as modulators of TRPN1. Establishing the subcellular localization of TRPN1 in both Johnston's organ and bristle sensory neurons will be important for determining its function.

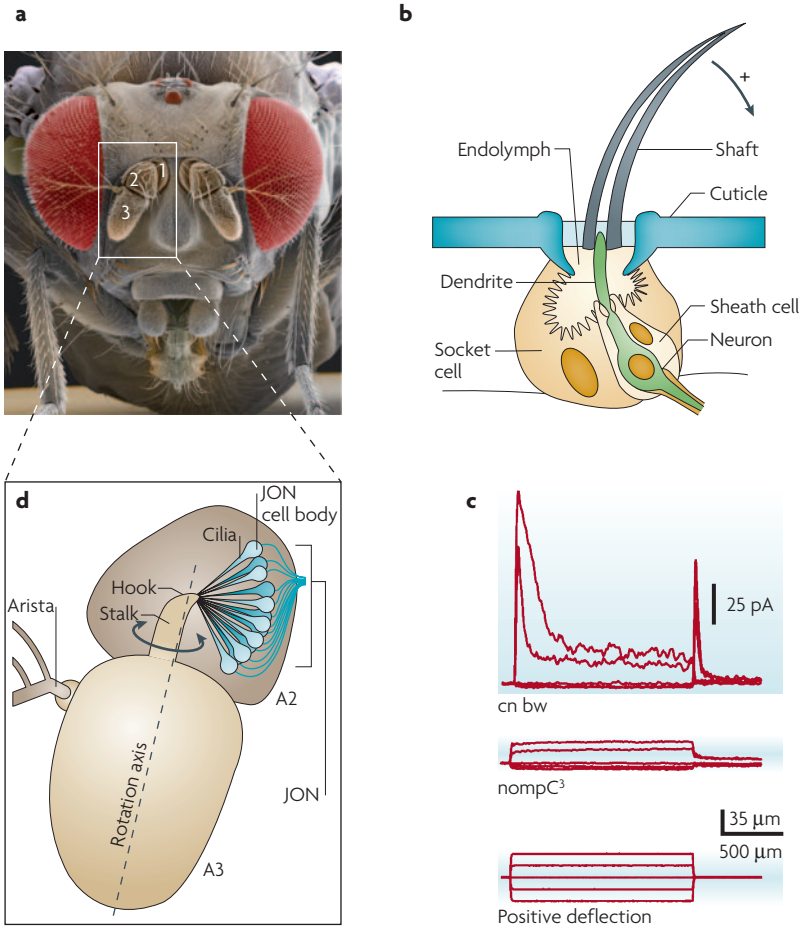


Figure 4 | **Mechanosensory organs in the fruitfly. a** | The *Drosophila melanogaster* head bears both sensory bristles and segmented antennae (segment numbers 1–3 indicated). **b** | One or two neurons innervate each bristle organ on the body surface and are stimulated by positive deflection of the bristle shaft (+). Bristle neurons, like Johnston's organ (JON) neurons, have a sensory cilium that projects into the moving structure to receive the stimulus. **c** | A receptor potential can be measured from the bristle if its tip is cut and an electrode is slipped over the hollow shaft (top trace). Positive bristle deflection (bottom trace) elicits a fast, phasic response from wild-type flies (cn bw), but only a small, tonic response from flies with a mutation in no mechanoreceptor potential C, also known as TRPN1 (nompC[3]) **d** | Antennal segment 3 (A3), bearing feathery arista. The associated stalk and hook vibrate rotationally in response to sound waves that impinge on the arista, thereby stimulating neurons of JON within segment 2 (A2). Panel **a** from Andrew Syred, Science Photo Library. Panels **b** and **c** were reproduced with permission from REF. 64 © (2000) American Association for the Advancement of Science. Panel **d** was reproduced with permission from REF. 133 © (2006) Wiley-Liss.

Hearing in vertebrates

The most intensely studied mechanoreceptor is the hair cell of the vertebrate inner ear. The organs of hearing and balance use hair cells to transduce acoustic vibrations and head movements into neuronal signals. Each hair cell has a bundle of actin-cored stereocilia that projects from its apical surface and that displays a characteristic staircase gradation of heights (FIG. 5a,b). When the bundle is deflected in the direction of the tallest stereocilia, a non-selective cation conductance is activated. *In vivo*, where the stereocilia face the unusual ionic environment of endolymph, channel opening allows the influx of potassium and calcium ions (FIG. 5c,d). The biophysics, permeation and pharmacology of the channels that mediate this conductance have been extensively characterized, but their molecular identity has remained elusive. However, the non-selective permeability, the large conductance (100–300 pS) and the pharmacology of the channels suggest that members of the TRP superfamily are the most likely candidates for this transduction channel.

The evidence that the vertebrate hair-cell transduction channel is directly activated by mechanical force is quite convincing. The latency of the response is less than 40 µs in the bullfrog saccule (FIG. 5e) and less than 20 µs in the rat cochlea[78,79]. In both cases, channel activation becomes faster with larger deflections, as

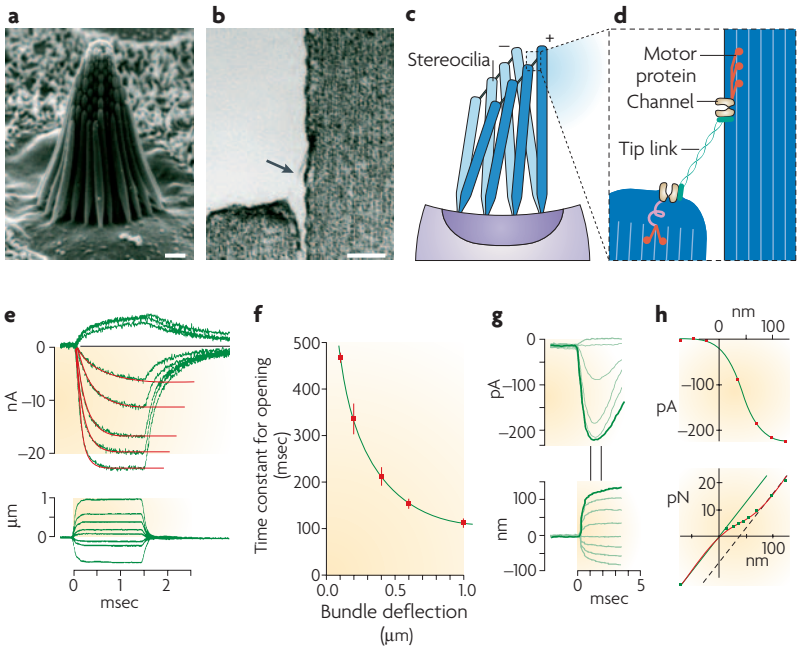


Figure 5 | **Mechanotransduction in vertebrate hair cells. a** | The hair bundle emanating from a hair cell shows a progressive increase in stereocilia heights (bar represents 1 μm). Deflections towards the tallest stereocilia open transduction channels near their tips, and are excitatory. **b** | A 170 nm tip link (arrow) extends from the tip of each stereocilium to the side of its tallest neighbour, along the sensitive axis (bar represents 100 nm). **c** | Stereocilia remain touching during deflections, so excitatory deflections increase tension in the tip links. **d** | The current model for transduction supposes that ion channels at both ends of a tip link are pulled open by tip-link tension. Channels are linked intracellularly to the actin cores of stereocilia (pale blue lines) by motor proteins and part of the linkage is elastic (pink). **e** | Hair-cell transduction channels (here, a group of approximately 400 from bullfrog saccule cooled to 4 °C) open within microseconds of bundle deflection. Top trace shows receptor current and lower trace shows size of deflection **f** | The time constant for the exponential opening is faster for larger stimuli, consistent with direct coupling. **g** | Receptor currents from a single bullfrog hair cell (top) in response to bundle deflection (bottom) show a fast activation and the beginning of an adaptation. **h** | The activation curve of peak receptor current compared with deflection (top), measured between the lines in panel **g**, range of 100–200 nm; the relation of stimulus force (in pN) to deflection (in nm) shows a slope or stiffness decrease over the same range of deflections, providing a mechanical correlate of transduction channel gating (bottom). Panels **e** and **f** were reproduced with permission from REF 80 © (1983) Society for Neuroscience. Panels **g** and **h** were reproduced with permission from REF 74 © (2005) The Biophysical Society.

expected for a channel gated by force[79,80] (FIG. 5f). A mechanical correlate of channel gating has also been well defined[74,81]. Force produces a linearly proportional bundle deflection in the extreme ranges, where channels are all closed or all open. The stiffness measured in this way is the sum of the pivoting stiffness of stereocilia and of an unidentified elastic element in series with each channel called the 'gating spring' (BOX 4). However, deflection produced by force is larger in the range at which channels open, a phenomenon termed 'gating compliance', which suggests that channel opening relieves tension to allow more movement (FIG. 5 g,h). Pharmacologically blocking channel opening blocks the gating compliance[81].

There are four potential candidates within the TRP superfamily for the vertebrate hair-cell transduction channel. The first is TRPN1, for which there is mixed evidence. In zebrafish lateral line hair cells, knockdown of TRPN1 using morpholinos decreased receptor potentials and loading of FM1-43 (a fluorescent dye known to pass through these transduction channels)[82], consistent with the involvement of TRPN1 in transduction[83]. TRPN1 is also expressed in hair cells of the zebrafish inner ear, as well as in inner-ear hair cells of *X. laevis*. However, antibody localization of TRPN1 in *X. laevis* showed the presence of the protein in the kinocilia, but not in the stereocilia, as would be expected for the hair-cell transduction channel[84]. Moreover, the *TRPN1* gene occurs in the genomes of invertebrates and of some lower vertebrates[83,84] but not in mammalian genomes. It seems likely that the hair-cell transduction channel is the same in all vertebrates, so the absence of TRPN1 in mammals is puzzling.

Another candidate is TRPML3 (TRP, subfamily ML, member 3). TRPML3 is mutated in the varitint-waddler mouse[85], named for its variegated coat colour, which is born deaf and shows degenerative hair-cell loss. TRPML3 is located in vesicular cytoplasmic organelles in hair cells and possibly also in stereocilia. Further evidence for a role for TRPML3 in transduction, such as single-cell electrophysiology from the varitint-waddler mice, is lacking thus far.

A third candidate for the vertebrate hair-cell transduction channel is TRPV4, which as discussed previously is a vertebrate osmosensor that might be expressed in inner-ear hair cells[30]. The pharmacology of TRPV4 has some overlap with the hair-cell transduction channel, but the ionic permeability of the hair-cell transduction channel is different from that of TRPV4 (REFS 46,86). Importantly, TRPV4$^{-/-}$ mice do not have gross hearing or vestibular defects[30]. Their delayed-onset hearing loss[87] is perhaps more consistent with defects in endolymphatic ion transport as TRPV4 is also expressed in the transporting epithelium that produces endolymph.

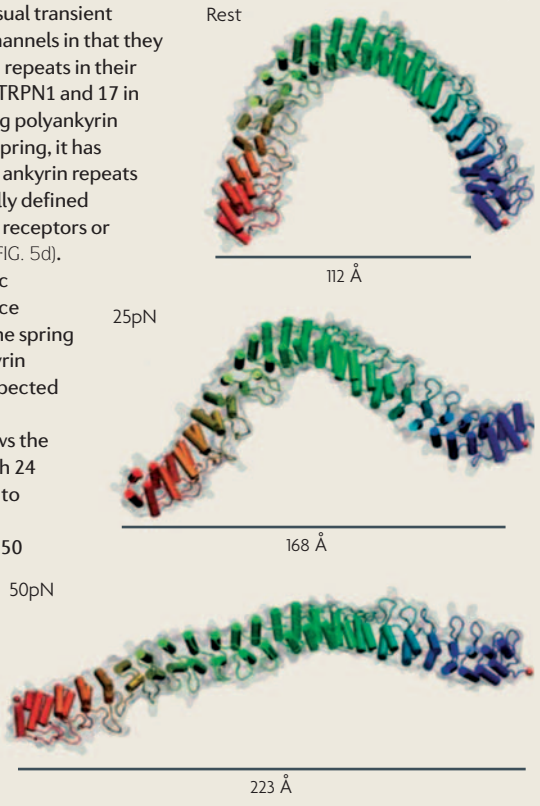
The final candidate for the transduction channel is TRPA1. TRPA1 was first described as a sensor of painful cold stimuli in peripheral sensory nerves[88], and it is activated by noxious chemicals[89–92]. TRPA1 initially appeared to be a strong candidate for the hair-cell transduction channel. It is weakly expressed in the hair cells of both the cochlea and vestibular system in mice[93]. It first appears in the utricle at embryonic day 17, matching the onset of mechanotransduction, and was located by immunolabelling in the distal stereocilia in both mice and bullfrogs, as expected for the hair-cell transduction channel. Morpholino-mediated knockdown of TRPA1 in zebrafish decreased inner ear microphonic potentials and FM1-43 accumulation in both inner ear and lateral line hair cells. Knocking down TRPA1 expression in mouse hair cells decreased transduction currents by approximately 80%. The pharmacology of the hair-cell transduction channel and TRPA1 are qualitatively similar, although there are certain discrepancies[94–97]. For instance, TRPA1's order of preference for alkali cations is opposite to that of the transduction channel[88]. Importantly, two

Endolymph
The fluid filling the scala media of the cochlea and the lumen of the vestibular organs. Endolymph has an unusual ion composition with high potassium and low sodium concentrations.

Morpholinos
Antisense oligonucleotides that block gene expression by interfering with the translation initiation complex or with RNA splicing.

Box 4 | **A built-in gating spring**

TRPN1 and TRPA1 are unusual transient receptor potential (TRP) channels in that they both contain many ankyrin repeats in their extended N-termini (29 in TRPN1 and 17 in TRPA1; FIG. 2). Because long polyankyrin domains are curved like a spring, it has been suggested that these ankyrin repeats might form the biophysically defined 'gating spring' of fly bristle receptors or vertebrate hair cells[127–129] (FIG. 5d). Steered molecular dynamic simulations and atomic force microscopy showed that the spring constant of these polyankyrin domains is close to that expected for a physiological gating spring[129,130].The figure shows the elongation of a protein with 24 ankyrin repeats, predicted to occur in about 10 nsec in response to forces of 25 or 50 pN[129]. It will be important to test this for *Drosophila melanogaster* bristles, perhaps by altering the number of ankyrin repeats in the channel protein to see if the apparent spring constant is changed.



Kinocilia
A single true cilium containing microtubules that emanates from the apical surfaces of hair cells, adjacent to the tallest stereocilia.

Utricle
One of three types of vertebrate vestibular organs (along with the saccule and semicircular canals) that is sensitive to linear acceleration.

Microphonic potential
An extracellular receptor potential from inner ear organs caused by current flowing through receptor cells. Like a microphone, the cochlea produces a small voltage in response to acoustic stimuli.

Slowly adapting neuron
Sensory neuron that maintains firing for the duration of a stimulus.

Rapidly adapting neuron
Sensory neuron that fires at the start of a sensory stimulus but shows a decay, or adaptation, of firing during maintained stimuli.

independent TRPA1 knockout mouse lines do not have hearing or vestibular deficits[98,99], and hair-cell transduction in the knockout is indistinguishable from that of wild-type mice[98]. Thus, TRPA1 is not necessary to form the hair cell transduction channel, and it is not clear what role, if any, it has in mechanotransduction. It may have a more general function in regulating sensitivity to sound.

In short, there is not convincing evidence for a role of any of these four candidates in hair-cell transduction, leaving room for still more TRP channels to be considered as candidates in this sensitive and fast mechanosensory cell.

Touch in vertebrates

The sensation of touch in vertebrates is mediated by sensory neurons that have cell bodies located in the trigeminal ganglia and dorsal root ganglia (DRG), which send processes to the skin. The mechanically sensitive subset of these neurons can be broadly divided into low threshold mechanoreceptors and high threshold nociceptors. Each group contains both slowly adapting and rapidly adapting neurons[100]. In culture, the cell bodies of some DRG neurons have a mechanically activated current in response to stiff probe stimulation of the cell body[101–103]. The latency of the response is less than 1 millisecond, indicating that the channels that mediate this current are directly activated[103]. Further experiments showed that, in a subset of slowly adapting neurons, the current reverses at approximately 0 mV and is blocked by Ruthenium Red (a commonly used blocker of TRP channels that also blocks mitochondrial calcium influx), suggesting the involvement of TRP ion channels in the process[101,103].

Like the *D. melanogaster* TRPA channel painless, mammalian TRPA1 channels seem to be involved in mechanical pain. TRPA1 is expressed primarily in small-diameter nociceptive neurons and to a lesser extent in large-diameter neurons that sense light touch. In one line of mice lacking TRPA1 (REF. 99) (but not in another[98]) the sensitivity to painful mechanical stimuli was diminished. This may represent a direct role of TRPA1 in transduction of the mechanical stimulus, or a broader role in the regulation of the general excitability of nociceptive neurons. Another candidate for a touch-sensing channel is TRPV4. Knockout of TRPV4 was also reported to diminish sensitivity to nociceptive mechanical stimuli[30,104]. However the same caveats as for TRPA1 apply here as well. Presently there is no good candidate for a channel mediating sensitivity to light, non-painful touch.

Conclusion

TRP ion channels are emerging as candidate transduction channels in a wide variety of sensory systems, particularly those involved in sensing mechanical stimuli. For these TRP channels, and indeed for all mechanically gated channel candidates, it remains essential to differentiate between channels that are directly gated by mechanical forces and those that are downstream of a force sensor. It is also vital to demonstrate the involvement of these candidate proteins with experiments that test a number of specific criteria. For some systems — such as bristle touch and hearing in fruitflies, vertebrate hair-cell transduction and mechanosensitivity of DRG neuronal cell bodies — channel activation is almost certainly direct. In others, such as osmosensation by TRPV4, the channel appears to be downstream of the initial force sensor. Some TRP channel proteins have a key role in mechanosensation — including Nan and Iav in fruitfly hearing, TRPN1 in fruitfly bristle touch, painless in fruitfly body touch and OSM-9 and OCR-2 in nematode nose touch — but none has been demonstrated to be mechanically activated. The next few years should bring direct evidence to replace the circumstantial implication in many of these systems.

Once direct activation is established, an even more intriguing endeavour is to figure out exactly how mechanical force opens an ion channel. We are now beginning to appreciate how voltage exerts force on the charged S4 helix of voltage-gated potassium channels, and how that force is coupled to S6 to open the channel. A similar understanding is beginning to emerge for the binding of ligand to acetylcholine receptor channels. It will be interesting to dissect the molecular chain conveying force to the transmembrane domains of a TRP channel, and to understand how that force causes a conformational change to open the pore. However, we still have a long way to go.

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Competing interests statement
The authors declare no competing financial interests.

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