# Molecular basis of mechanosensory transduction

**Peter G. Gillespie & Richard G. Walker**

*Oregon Hearing Research Center and Vollum Institute, Oregon Health & Science University, Portland, Oregon 97201, USA*
*(e-mail: gillespp@ohsu.edu)*

**Mechanotransduction — a cell's conversion of a mechanical stimulus into an electrical signal — reveals vital features of an organism's environment. From hair cells and skin mechanoreceptors in vertebrates, to bristle receptors in flies and touch receptors in worms, mechanically sensitive cells are essential in the life of an organism. The scarcity of these cells and the uniqueness of their transduction mechanisms have conspired to slow molecular characterization of the ensembles that carry out mechanotransduction. But recent progress in both invertebrates and vertebrates is beginning to reveal the identities of proteins essential for transduction.**

Mechanical forces impinge on us from all directions, transmitting valuable information about the external environment. Mechanosensory cells transduce these mechanical forces and transmit this sensory information to the brain. Hearing, touch, sense of acceleration — each informs us about what is nearby and how we are moving relative to our surroundings. An organism detects sensory information with a variety of cells that respond to force. Although different structurally, hair cells within our ear, cutaneous mechanoreceptors of our skin, and invertebrate mechanoreceptors share many mechanistic features; whether mutual molecular mechanisms underlie these similar transduction mechanisms remains to be determined. Here we review mechanisms of mechanosensory transduction by invertebrates and vertebrates, highlighting the increasing molecular understanding of transduction in each system.

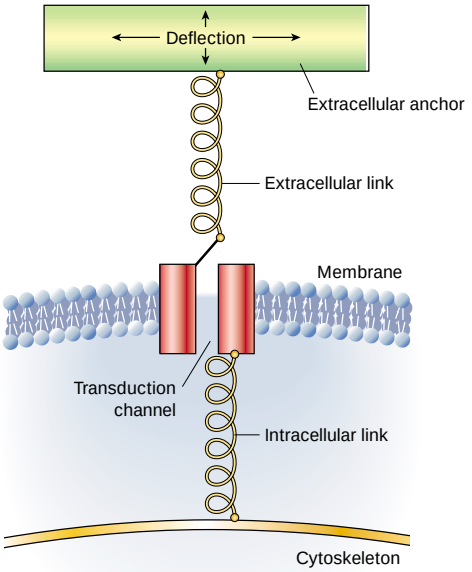



**Figure 1** General features of mechanosensory transduction. A transduction channel is anchored by intracellular and extracellular anchors to the cytoskeleton and to an extracellular structure to which forces are applied. The transduction channel responds to tension in the system, which is increased by net displacements between intracellular and extracellular structures.

## General features of mechanotransduction

As with most sensory systems, mechanosensory cells place a premium on speed and sensitivity. A common theme is for mechanical forces to be directed to specific ion channels, which can open rapidly and amplify the signal by permitting entry of large numbers of ions. Mechanical forces can also affect intracellular events in cells — such as gene transcription — directly through the cell surface and cytoskeleton, although such mechanisms typically are not used for rapid sensory transduction.

Speed requires that mechanical forces be funnelled directly to transduction channels, without intervening second messengers. Sensitivity requires that the maximal amount of stimulus energy be directed to the transduction channel. A general model — borrowed from worm touch receptors[1,2] and hair cells[3] — that applies to many mechanosensory transduction systems is illustrated in Fig. 1; its key feature is a transduction channel that detects deflection of an external structure relative to an internal structure, such as the cytoskeleton. Such a deflection could take the form of deformation of the skin, oscillation of a hair cell's hair bundle, or vibration of a fly's bristle. Deflection changes tension in all elements of the system, and the transduction channel responds by changing its open probability.

This general mechanism also explains other features that are common to mechanosensory cells, such as adaptation. Vestibular hair cells, for example, must reject the constant 1-*g* gravitational force to detect stimuli one-millionth that size[4]. During adaptation to a sustained stimulus, where there is a constant relationship between external and internal structures, tension applied to the transduction channel declines through a readjustment of the machinery. Adaptation could occur through deformation of the external coupling structure, lengthening of the external or internal anchors, slipping of the internal anchor relative to the cytoskeleton, or changes within the channel

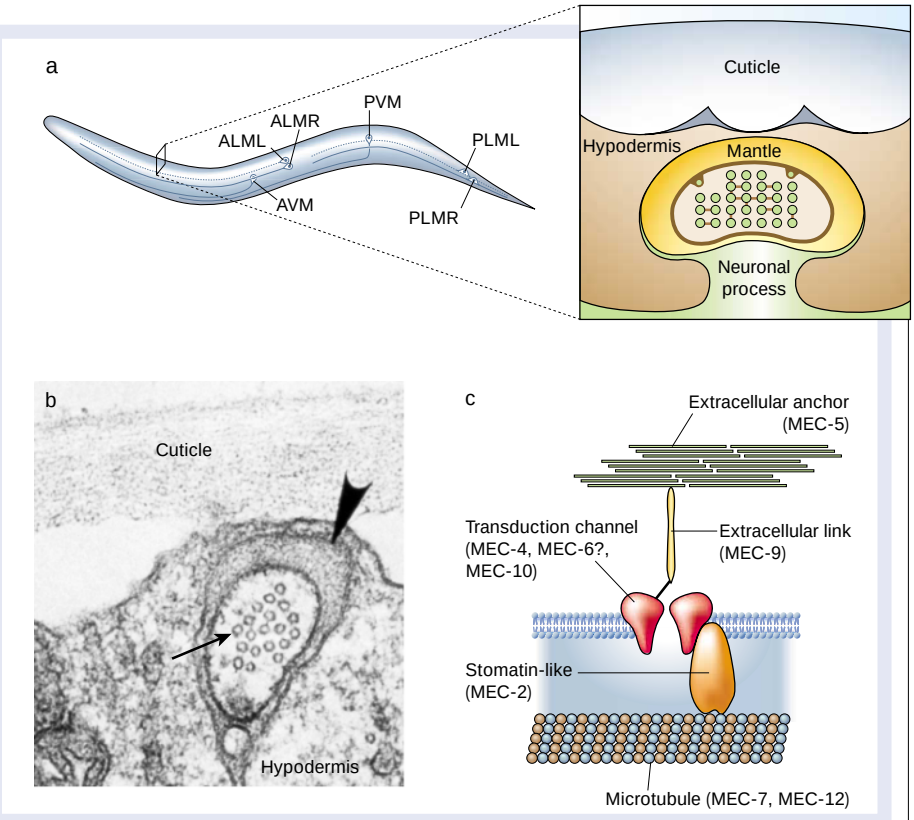



**Figure 2** *C. elegans* touch-receptor structure and transduction model. **a**, View of *C. elegans* showing positions of mechanoreceptors. AVM, anterior ventral microtubule cell; ALML/R, anterior lateral microtubule cell left/right; PVM, posterior ventral microtubule cell; PLML/R, posterior lateral microtubule cell left/right. **b**, Electron micrograph of a touch-receptor neuron process. Mechanotransduction may ensue with a net deflection of the microtubule array relative to the mantle, a deflection detected by the transduction channel. Arrow, 15-protofilament microtubules; arrowhead, mantle. Modified from ref. 3. **c**, Proposed molecular model for touch receptor. Hypothetical locations of *mec* proteins are indicated.

itself. Appropriately, all of these structures and interactions are amenable to molecular characterization.

## Invertebrate mechanoreceptor models

Interesting in their own right, invertebrate mechanoreceptors have also attracted considerable attention because they can be readily approached with genetics. The two most utilized invertebrate models — the nematode *Caenorhabditis elegans* and fruitfly *Drosophila melanogaster* — have yielded candidate transduction channels, as well as possible accessory molecules.

Other kingdoms possess mechanoreceptors too. Although not a mechanosensory transduction channel in the sense we discuss here, the cloned and reconstituted MscL channel of *Escherichia coli* responds unequivocally to membrane tension in the absence of other proteins[5]. The discovery of these channels in archaebacteria[6] highlights the ancient nature of mechanotransduction and its critical role in all cells. Although no MscL homologues have been identified in eukaryotes, the ability to determine the functional consequences of structural perturbations should permit an unparalleled view of a mechanically gated channel.

### Touch mechanotransduction in *C. elegans*

A simple and ingenious genetic screen identified *C. elegans* mutants (*mec* mutants) that were defective in mechanosensation[7,8]. Mutant worms that responded inappropriately or not at all to the simple touch of an eyelash were selected and most of the responsible genes have been identified. Although some of the gene products participate in development of the six mechanosensory touch neurons in the worm[9,10], most of the gene products seem to constitute a transduction machinery (Fig. 2). The identities and interactions of cloned mutant genes suggest a mechanoreceptive complex, with a mechanically sensitive ion channel connected to both intracellular cytoskeletal components and extracellular matrix proteins (including the mantle surmounting the touch-receptor process) to form a transduction apparatus (reviewed in refs 1, 2).

Consistent with the general model for a mechanotransduction apparatus (Fig. 1), which requires an intra- and extracellularly tethered channel, several of the *mec* genes encode components of the cytoskeleton, the extracellular matrix, or the links to them (Fig. 2). The genes *mec-7* and *mec-12* encode β- and α-tubulins[11,12], which constitute the 15-protofilament microtubules in sensory dendrites of touch neurons. Perhaps serving as a linker between these microtubules and a transduction channel, the MEC-2 protein is expressed exclusively by touch-receptor neurons and shows homology to stomatin, an integral membrane protein found in many cell types, which associates with the cytoskeleton and is involved in ionic homeostasis of the cell[13]. MEC-5 is a specialized collagen-like molecule secreted by the hypodermal cells that surround the receptor process and may be an extracellular anchor for the transduction apparatus[14]. The multidomain MEC-9 protein is expressed and secreted by the sensory neurons[14] and interacts genetically with MEC-5 and the proposed transduction channel MEC-4 (ref. 15), suggesting that they function together as part of the extracellular linkages of the transduction machinery.

### Transduction by DEG/ENaCs

The most intriguing set of genes to come from the screens for *mec* mutants were the degenerins (DEGs), which encode ion channels related to vertebrate epithelial sodium channels (ENaCs) responsible for $Na^+$ adsorption (reviewed in ref. 16; see Box 1). Most mutations in the *C. elegans* DEG genes *mec-4* and *mec-10* render the animal insensitive to light touch, whereas dominant gain-of-function mutations in *mec-4* and *mec-10*, as well as *unc-8* and *deg-1*, cause degeneration of the touch neurons (hence the familial name), probably by causing the channels to be open constantly[17]. Because of their critical role in the touch-receptor neurons, DEGs have been proposed to act as mechanosensory transduction channels or subunits thereof[18]. Attempts to elicit mechanically induced currents from heterologous cells expressing these channels have been unsuccessful[19], although suction-induced currents have been observed in patches containing the related vertebrate ENaC α-subunit[20]. It is possible that DEGs might require specific interactions with both intracellular and extracellular tethers and even other channel subunits for mechanical gating. Confirmation of these molecules as transduction

Box 1

DEG/ENaC channels

Members of the growing DEG/ENaC superfamily traverse the membrane twice and have intracellular N and C termini; a 33-residue portion of the N terminus is conserved among family members (reviewed in ref. 16). This domain, which shows similarity to thiol proteases, including the conservation of a catalytic histidine residue, is found near the first transmembrane region and may be important in subunit interactions. A large extracellular loop contains two or three cysteine-rich regions and is followed by the second transmembrane segment, which is thought to function as the channel's pore.

**Degenerins.** These include *mec-4* and *mec-10*, present in *C. elegans* touch receptors, as well as *unc-8*, *unc-105* and *deg-1*. Interacting genetically with type IV collagen in *C. elegans* muscle, *unc-105* might mediate stretch sensitivity in muscle[62]. Certain mutations in these genes cause dominant degenerative cell death, perhaps by constitutive ion-channel activation.

**Epithelial sodium channels (ENaCs).** Also called amiloride-sensitive sodium channels for their sensitivity to this drug, the ENaCs are responsible for $Na^+$ resorption in many epithelia, such as the kidney, distal colon, secretory glands and respiratory airways. In addition, the sense of salty taste is mediated by ENaC channels in the fungiform papillae of the tongue (see review in this issue by Lindemann, pages 219–225). The C terminus of ENaCs contains a proline-rich PY domain that mediates regulation of the channels by Nedd4, a ubiquitin-protein ligase that binds specifically to the PY domain. These molecules form heteromultimeric channels with some combination of α-, β-, γ- and δ-subunits likely in either a tetrameric or octomeric configuration.

**Acid-sensing ion channels (ASICs).** Found in a variety of tissues, ASICs are activated by extracellular protons and may mediate pain induced by acidosis. ASIC1 is activated at relatively high pH levels and inactivates rapidly; there are two variants, ASIC1a and ASIC1b. ASIC2 also has two variants — ASIC2a, known additionally as BNC1 (brain sodium channel 1, sometimes abbreviated BNaC1), and ASIC2b, also known as BNC2 or ASIC-β. The BNCs have also been labelled MDEGs (mammalian degenerins), a phylogenetically inappropriate term. ASIC3 was originally named DRASIC (dorsal-root acid-sensing ion channel), although this channel is actually found in many tissues.

***Drosophila* family members.** Of the ~14 members of DEG/ENaC gene family in the fly genome, only two have been characterized: *ripped pocket* (*rpk*) and *pickpocket* (*ppk*). Because PPK is expressed in peripheral neurons thought to mediate mechanosensation, this channel is a strong transduction-channel candidate.

channels, however, awaits electrophysiological recording of receptor currents from mutant touch neurons.

Given the importance of DEG/ENaC family members in *C. elegans* mechanoreceptors, a role for these channels in the mechanical senses of other organisms seems likely. For example, there are at least 14 members of DEG/ENaC gene family in the fly genome, two of which, *ripped pocket* (*rpk*) and *pickpocket* (*ppk*), have been characterized[21]. There are currently no described mutants in either *rpk* or *ppk* with which to evaluate the role of these channels in the fly, although restricted expression of PPK to mechanosensory neurons is suggestive of a role in mechanosensation.

Vertebrates also have a large complement of DEG/ENaC channels (Box 1); the human genome encodes at least a dozen such genes. More recent research on these channels has implicated them in various mechanosensory modalities. For example, the γ-subunit of ENaC is present in sensory endings of baroreceptors, the mechanosensory neurons that sense blood pressure[22], and mechanically stimulated baroreceptor activity is blocked by benzamil, an amiloride analogue that also blocks ENaCs[22]. Another member of the DEG/ENaC superfamily, brain sodium channel 1α (BNC1α), a splice variant of BNC1, is expressed in dorsal root ganglia and transported to a number of cutaneous mechanosensory terminals[23]. BNC1α is found in most identified cutaneous mechanoreceptors, and not in endings specialized for other sensory modalities, indicating a particular role in touch.

To evaluate the role of BNC1 in touch sensation, mechanoreceptor responses were examined in mice whose BNC1 gene had been deleted[24]. In homozygous mutant mice, rapidly adapting neurons fired only about half the number of action potentials as wild-type mice in response to a 20-μm stimulus. Furthermore, slowly adapting neurons from BNC1-null mutants showed a modest decrease in sensitivity. One might expect that deleting a transduction channel or one of its subunits from the transduction complex might produce a more profound phenotype — BNC1-null neurons still responded to stimuli and there were no obvious behavioural deficits — but other members of this family may have compensated for the loss of BNC1. Indeed, the β- and γ-subunits of ENaC have been recently localized to mechanoreceptor terminals in the skin[25,26]. Unfortunately, mice bearing targeted disruptions of these two genes die of defects in electrolyte metabolism within a few days of birth (reviewed in ref. 27), making analysis of their mechanoreceptor function difficult.

### *Drosophila* mechanotransduction

When Kernan and colleagues screened for mechanosensory mutants in fruitflies using an adaptation of the *C. elegans* screen[28], they put *Drosophila* on the mechanosensory map. Renowned and increasingly powerful genetic tools coupled with the ability to record mechanosensory receptor potentials and currents from bristles make *Drosophila* a consummate model system for dissecting mechanosensation. The fly mechanosensory system comprises two sets of mechanoreceptors. Type I sensory organs have one to three sensory neurons, each with a single ciliated sensory dendrite, supported by accessory cells (Fig. 3), whereas type II mechanoreceptor neurons have multiple nonciliated dendrites and no accessory cells. Type I mechanoreceptors include bristle mechanoreceptors, chordotonal organs such as the Johnston's organ (the fly's antennal hearing apparatus) and campaniform sensilla, which are featured prominently in halteres, the club-shaped mechanoreceptive structures that relay information about wing beat. Because the large bristles are hollow and contain a conductive high-$K^+$ endolymph (similar to the endolymph of vertebrate hearing and vestibular organs) that also bathes the apical surface of the sensory epithelium, electrical access to the tiny sensory dendrite can be gained by snipping the bristle and placing a recording (and stimulation) electrode over its cut end[28,29]. It is possible to clamp the potential across this sensory epithelium and record mechanically gated transduction currents[29].

To test whether the larval mutants identified by the behavioural screen were defective in the transduction pathway, mechanoreceptor potentials were recorded from mutant adult sensory bristles and compared with those from the bristles of wild-type flies. A number of the larval touch-insensitive mutants developed into profoundly uncoordinated adults and showed either a reduced mechanoreceptor potential (*remp* mutants) or no mechanoreceptor potential (*nomp* mutants) at all. Evolution has apparently conserved the transduction

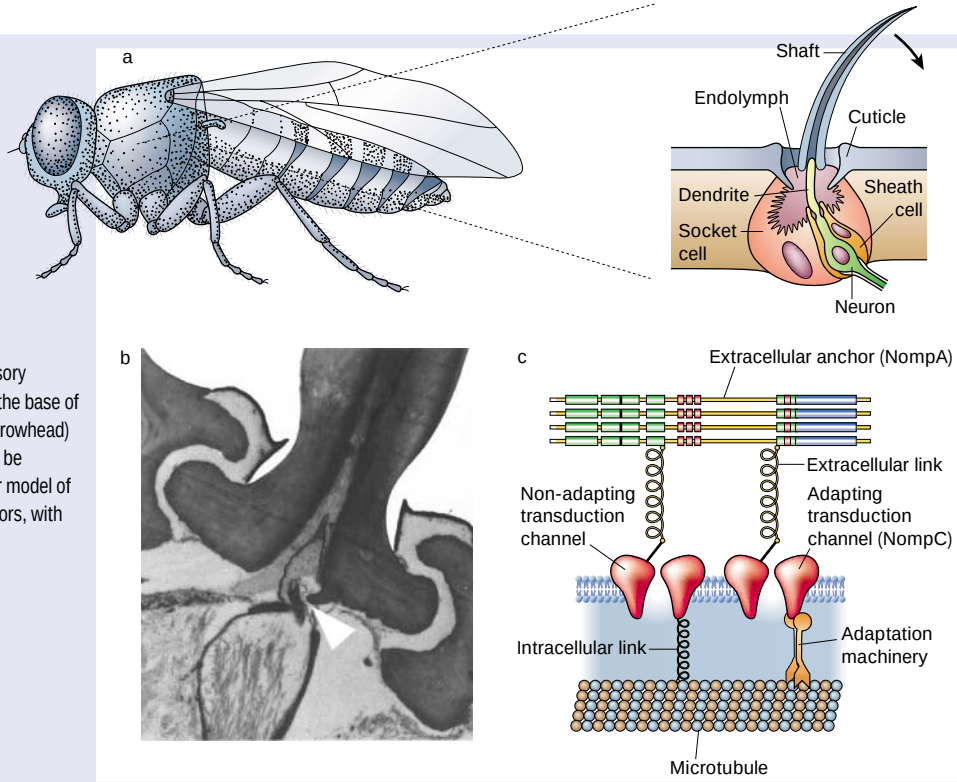



**Figure 3** *Drosophila* bristle-receptor model. **a**, Lateral view of *D. melanogaster* showing the hundreds of bristles that cover the fly's cuticle. The expanded view of a single bristle indicates the locations of the stereotypical set of cells and structures associated with each mechanosensory organ. Movement of the bristle towards the cuticle of the fly (arrow) displaces the dendrite and elicits an excitatory response in the mechanosensory neuron. **b**, Transmission electron micrograph of an insect mechanosensory bristle showing the insertion of the dendrite at the base of the bristle. The bristle contacts the dendrite (arrowhead) so that movement of the shaft of the bristle will be detected by the neuron. **c**, Proposed molecular model of transduction for ciliated insect mechanoreceptors, with the locations of NompC and NompA indicated.

mechanism of various mechanosensory modalities in flies, as almost all of the uncoordinated mutants identified in this screen are also deaf[30].

So far, only two of the mutant genes have been identified molecularly, *nompA*[31] and *nompC*[29]. Like the results from the *C. elegans* screen, the screen from flies has produced an extracellular-matrix protein and an ion channel (Fig. 3). The mechanosensory gene *nompA* encodes a modular protein, secreted by the supporting cells and localized to the extracellular dendritic cap-like structures of type I mechanoreceptors and some chemoreceptors. NompA probably serves as an extracellular mechanical link between the sensory dendrites of type I mechanoreceptors and their associated cuticular structures. It shows similarity to a number of proteins, including the tectorins[32], extracellular molecules that transmit mechanical stimuli to mechanoreceptive hair cells in the vertebrate auditory system.

The potency of coupling electrophysiology with traditional *Drosophila* genetics was demonstrated in the cloning of the mechanosensory gene *nompC*[29]. Although three alleles of *nompC* were severely uncoordinated and showed near-complete abrogation of transduction current, a fourth allele had near wild-type amplitude of response, but noticeably faster adaptation than controls. This indicated that the NompC protein would be intimately involved with the transduction and adaptation process.

The *Drosophila nompC* gene and a closely related *C. elegans* homologue encode a new and unusual member of the transient receptor potential (TRP) family of ion channels (see review in this issue by Hardie and Raghu, pages 186–193). The predicted protein of 1,619 amino acids can be divided into two regions: first, an unusual ~1,150-amino-acid amino terminus composed of 29 ankyrin repeats, and second, a carboxy-terminal segment with six transmembrane domains, a predicted pore loop, and sequence similarity to TRP channels. Ankyrin repeats are 33-residue motifs with a conserved backbone and variable residues that mediate specific protein–protein interactions[33]. Ankyrin repeats are found in numerous proteins of widely varying functions and subcellular locations[34]. Other members of the TRP channel family have between one and four ankyrin repeats of unknown function; that NompC bears 29 such domains suggests that it interacts either very strongly with a small number of partners or less avidly with many molecules.

Several lines of evidence led to the conclusion that NompC serves as a mechanosensory transduction channel. (1) NompC shows similarity with other sensory transduction channels in its primary sequence. (2) Loss-of-function *nompC* mutants show loss of almost all transduction current, whereas a point mutation in NompC changes the adaptation profile of the transduction current. (3) The *Drosophila nompC* gene is expressed specifically in mechanosensory organs. (4) A *C. elegans* NompC–GFP fusion construct is expressed in putative mechanosensory neurons at the site of transduction. (5) The N terminus of NompC is replete with ankyrin domains, a property one might expect of a mechanically tethered transduction channel.

Because opening of the transduction channels in fly mechanoreceptors takes place within 200 μs of a mechanical stimulus[29], fly mechanotransduction seems to take place by direct opening of a mechanically gated conductance without intervening second messengers. Although NompC accounts for ~90% of the transduction current, it is unclear whether the NompC channel is itself mechanically sensitive or instead takes its gating cue from the small mechanically activated current that can be observed in *nompC*-null traces. NompC would then be serving as an adapting amplifier of the true mechanically gated channel.

TRP channels and the closely related cyclic nucleotide-gated and vanilloid-receptor channels are involved or expressed in many sensory systems, including phototransduction, olfaction and taste, as well as in the perception of heat, acid and pain (see accompanying reviews in this issue). So the discovery that a member of this superfamily acts as or influences a transduction channel in mechanosensation should not be unexpected. Indeed, TRP family members have also been implicated in mechanotransduction's sister sense, osmosensation. A *C. elegans* TRP channel, OSM-9, is expressed in dendrites of some ciliated sensory neurons and is required for osmosensation and nose touch sensation[35]. In addition, a new mammalian member of the vanilloid-receptor family is expressed in osmotically sensitive cells and forms osmotically gated channels when expressed in cells[36].

Cilium-bearing mechanoreceptors in both *C. elegans* and *Drosophila* express NompC, whereas the non-ciliated touch-receptor

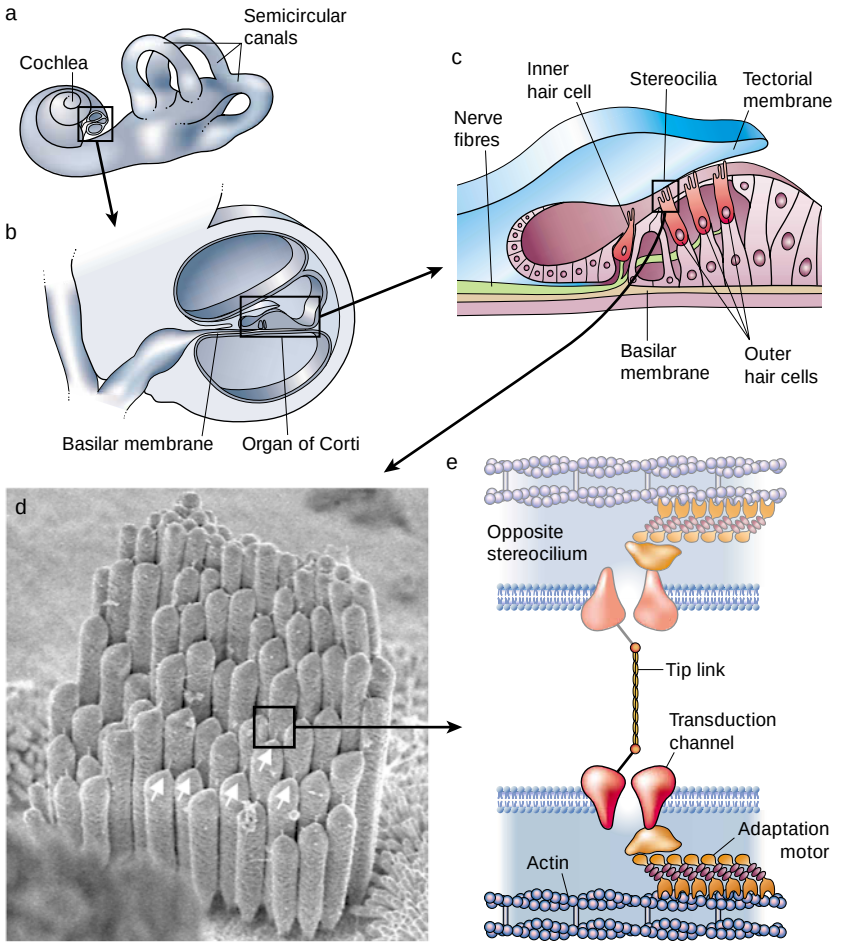



**Figure 4** Inner-ear structure and hair-cell transduction model. **a**, Gross view of part of the inner ear. Sound is transmitted through the external ear to the tympanic membrane; the stimulus is transmitted through the middle ear to the fluid-filled inner ear. Sound is transduced by the coiled cochlea. **b**, Cross-section through the cochlear duct. Hair cells are located in the organ of Corti, resting on the basilar membrane. **c**, Sound causes vibrations of the basilar membrane of the organ of Corti; because flexible hair-cell stereocilia are coupled to the overlying tectorial membrane, oscillations of the basilar membrane cause back-and-forth deflection of the hair bundles. **d**, Scanning electron micrograph of hair bundle (from chicken cochlea). Note tip links (arrows). **e**, Proposed molecular model for hair-cell transduction apparatus.

neurons in worms and the type II multiple dendritic neurons of flies instead express members of the DEG/ENaC family. This suggests not only a morphological schism, but also a mechanistic difference between these two kinds of mechanoreceptors. All vertebrate hair cells are endowed with a true cilium, or kinocilium, during the construction of the actin-based hair bundle. Given this odd remnant of ontogeny, it has been speculated that ciliated mechanoreceptors of invertebrates might be related to their once-ciliated vertebrate counterparts. Indeed this relationship extends further to the conservation of molecules that control the development of vertebrate and invertebrate mechanoreceptor organs. Lateral inhibition through signalling of the Notch receptor and its ligand Delta controls the cell-fate patterning of both *Drosophila* mechanoreceptor neurons[37] and vertebrate hair cells[38]. In addition, fly Atonal and its mammalian homologue Math1 also control the specification of cell type. *Drosophila atonal* mutants are devoid of chordotonal organs; similarly, *Math1*-knockout mice generate no hair cells during development of the sensory epithelium[39]. Their signalling is so similar that Math1 can substitute for Atonal in development of *Drosophila* mechanoreceptor organs[40]. Although both signalling modalities are found in other cell types, their control over vertebrate and invertebrate mechanoreceptors, as well as the sharing of specialized sensory structures, suggests that mechanoreceptors evolved prior to the common ancestor of invertebrates and vertebrates and that those mechanoreceptors might share more than just their developmental algorithms.

## Auditory and vestibular transduction by hair cells

Hair cells, the mechanoreceptors of the inner ear, transduce auditory and vestibular stimuli to allow us to hear and sense movements of our head. Jutting apically from a hair cell, the mechanically sensitive hair bundle bends back and forth in response to stimuli that are directed to it. Auditory stimuli induce a vibration of the structure on which the hair cells sit (the basilar membrane; Fig. 4). Vestibular stimuli cause displacement of acellular structures overlying the hair cells (otolithic membrane in the saccule and utricle, responsible for linear-acceleration detection; cupula in the semicircular canals, responsible for rotational detection), resulting in bundle deflection. An excitatory deflection of a hair bundle (Fig. 4) directly opens transduction channels, which admit cations and depolarize the hair cell. Inhibitory deflections close transduction channels and hyperpolarize the cell. These changes in membrane potential in turn increase (depolarization) or decrease (hyperpolarization) neurotransmitter release from graded synapses on basolateral surfaces of hair cells.

### Transduction mechanism

The gating-spring model[41] successfully describes key biophysical features of mechanical transduction in hair cells. Transduction channels open so fast — within microseconds of a stimulus — that second-messenger cascades cannot have a central role[41]. Instead, elastic gating springs transmit external forces to transduction channels, and the channels' open probability depends on the tension in these springs. Thus, when tension is high, channels spend most of their time open; when tension is low, channels close. Although the original formation of the model indicated that the open probability of transduction channels in the absence of any gating-spring tension was <0.0001 (ref. 3), recent data from auditory hair cells suggest that the zero-tension open probability is 100-times larger[42]. This debate is relevant to channel identification; higher open probabilities for the channel in the

Box 2

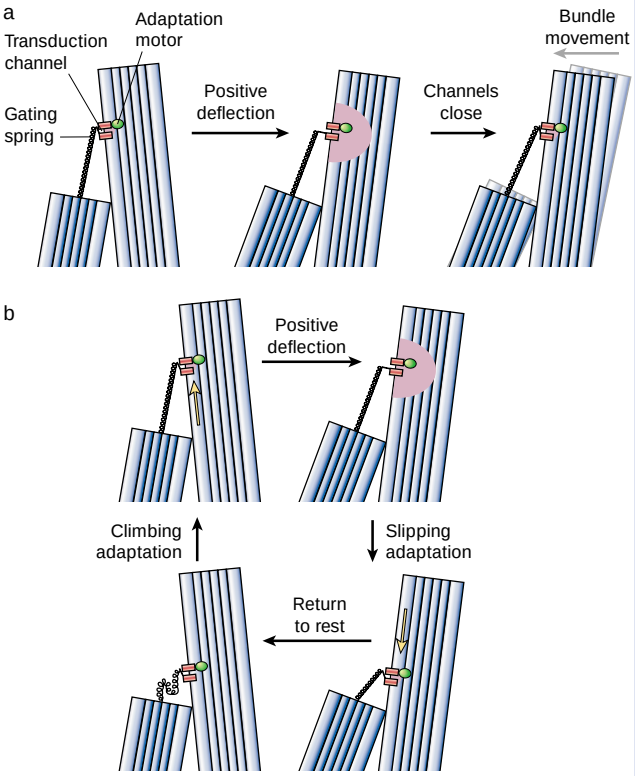
## Adaptation by hair cells

Two forms of adaptation are present in hair cells, each of which can modify the mechanical properties of the bundle (see figure opposite). Fast adaptation is remarkably quick (a millisecond or less in turtle auditory hair cells[63]), requires $Ca^{2+}$ (which enters through open transduction channels), and is tuned within individual hair cells, so that cells responding to high sound frequencies adapt more speedily than those tuned to lower frequencies[63].

Fast adaptation correlates with active hair-bundle movements[64,65]; hair bundles respond to a simple stimulus with a complex, active mechanical response. In hair cells of lower vertebrates, a step stimulus near threshold can initiate a bundle response in the opposite direction with sufficient force to move the bundle back to its original position.

In vestibular hair cells, a distinct mechanism — negative hair-bundle stiffness — permits oscillatory behaviour that is controlled by the mechanism responsible for slow adaptation, the adaptation motor[66]. A bundle with negative hair-bundle stiffness actually moves farther in response to a stimulus than the size of the stimulus itself. The adaptation motor continuously moves the hair bundle into the range of negative stiffness, triggering bundle oscillations that enable much more sensitive detection of small signals. $Ca^{2+}$-dependent channel closure could act synergistically with negative hair-bundle stiffness to power even larger active bundle movements.

Slow adaptation is thought to be mediated by an adaptation motor. Because the force producer of the adaptation motor may be a cluster of myosin molecules[67], the motor has attracted much attention. The strongest candidate is myosin 1c (Myo1c; formerly known as myosin Iβ), which is located near stereociliary tips at tip-link ends[67]. Myosin VIIA (Myo7a) may also be important in adaptation. In outer hair cells that are genetically deficient in this myosin, large displacements are required to begin to open channels, indicating that these cells lack the force generator that maintains resting tension[68].




**Box 2 Figure** Hair-cell transduction and adaptation. **a**, Transduction and fast adaptation. At rest (left panel), transduction channels spend ~5% of the time open, allowing a modest $Ca^{2+}$ entry (pink shading). A positive deflection (middle) stretches the gating spring (drawn here as the tip link); the increased tension propagates to the gate of the transduction channel, and channels open fully. The resulting $Ca^{2+}$ flowing in through the channels shifts the channels' open probability to favour channel closure (right). As the gates close, they increase force in the gating spring, which moves the bundle back in the direction of the original stimulus. **b**, Transduction and slow adaptation. Slow adaptation ensues when the motor (green oval) slides down the stereocilium (lower right), allowing channels to close. After the bundle is returned to rest (lower left), gating-spring tension is very low; adaptation re-establishes tension and returns the channel to the resting state.

absence of external force suggest that channel conductance might be detectable when expressed in a heterologous cell type.

The anatomical correlate of the gating spring has long been assumed to be the tip link, a fine extracellular filament that connects adjacent stereocilia along the sensitivity axis[43]. Tip links are in position to be stretched by excitatory stimuli and slackened by inhibitory ones (Fig. 4). But high-resolution structural characterization of the tip link has indicated that it might be too stiff to account for the gating spring. Instead, the elastic element within the bundle might reside elsewhere, such as the intracellular filaments at the basal insertion of the tip link[44].

### Transduction-channel properties

One approach to determining the identity of molecules required for transduction has been to extensively characterize transduction and adaptation, then suggest candidate molecules for the channel or motor. But the transduction channel, a nonselective cation channel[45], has few properties that distinguish it from other channels. Its conductance is large, ~100 pS (ref. 46), and its permeability to $Ca^{2+}$ is substantial[47]. The channel is blocked by relatively low concentrations of aminoglycoside antibiotics[48], amiloride and its derivatives[49], $La^{3+}$ (ref. 50), tubocurarine[51], and $Ca^{2+}$-channel antagonists such as D-600 (ref. 52) and nifedipine[53]. Unfortunately, this inhibition spectrum does not obviously match any other known channel type and the affinities of these blockers do not allow their use in biochemical isolation of transduction channels from inner-ear tissue.

Early hopes that the transduction channel might incorporate the α-subunit of the ENaC family were dashed by the persistence of normal transduction in mice lacking this subunit[54]. But the DEG/ENaC family is large, so the hair-cell transduction channel may yet be identified from this family. An alternative candidate is the $P2X_2$ receptor, a purinergic-receptor channel that is located in hair bundles[55], although expression of $P2X_2$ is low[56] at a time when hair cells transduce vigorously[57]. Furthermore, the transduction channel and hair-cell $P2X_2$ receptor show distinct patterns of blockade by inhibitors[51]. Another possible source for the transduction channel is the TRP family, which includes at least two channels definitely involved in mechanosensation (NompC in flies and OSM-9 in worms); the conductance properties of this family certainly could accommodate those of the hair-cell transduction channel[58].

Box 3

## Essential molecules for hair cells

Hair-bundle proteins identified by genetics or other means can be assembled into an incomplete molecular model (see figure). Hair bundles contain more than 30 major proteins[69], however, indicating many more molecules remain to be identified.

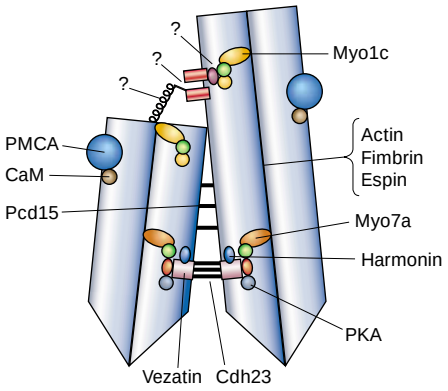
**Usher genes.** At least ten different genes can cause Usher syndrome, with varying clinical impact. Usher 1, caused by mutations at six or more loci, is the most clinically severe form of the disease. The first of these genes identified, that responsible for Usher 1B, was *MYO7A*[70]. Hair bundles are disarrayed when *Myo7a* from mouse[71] or zebrafish[72] is mutated, which supports a role for Myo7a in anchoring ankle links, a class of stereociliary crosslinks near the base of the stereocilia[73]. A new member of the cadherin family of $Ca^{2+}$-dependent cell-adhesion molecules, Cdh23, is responsible for Usher 1D[74]; because stereocilia adhesion is disrupted in the corresponding mouse mutant, *waltzer*, Cdh23 probably forms one of the classes of stereociliary crosslinks[75]. The gene mutated in Usher 1F[76,77] and the mouse deafness model *Ames waltzer*[78] encodes another member of the cadherin family, protocadherin-15 (Pcd15). *Ames waltzer* mice have disorganized stereocilia, suggesting that Pcd15 might crosslink adjacent stereocilia. Mutations in the genes encoding Myo7a, Pcd15 and Cdh23 produce an identical clinical phenotype, indicating that these proteins may be part of the ankle-link complex. A new protein — harmonin or USH1C — is mutated in Usher 1C; its multiple PDZ domains indicate a role in assembling a complex of proteins, although it might also assemble with ankle links. Usher 2 has three identified loci and is characterized by a modest hearing loss, normal balance and late-onset retinitis pigmentosa. USH2A, a molecule with multiple domains shared by extracellular matrix/adhesion molecules, is mutated in Usher 2A[79].

**Myo7a-interacting proteins.** Two proteins have been identified by two-hybrid screening with Myo7a bait. One is the RII subunit of the cyclic AMP-dependent protein kinase[80], which coincides with an earlier observation that the resting open probability of the transduction channel declines in response to agents that raise cAMP levels[63]. The other is a new protein named vezatin[81], which localizes in hair cells to the ankle-link region and, in tissue-culture cells, interacts with cadherin-based cellular junctions. This protein most likely forms the transmembrane link between Myo7a and the ankle links.

**Other myosin isozymes.** Myo6 and Myo15 may participate in hair-bundle formation and maintenance. Myo6, a negative-end-directed motor, localizes to the cuticular plate and basal insertions of the stereocilia; it may provide outward force on stereocilia or anchor the stereociliary membrane[73,82]. Mice lacking Myo15 have abnormally short stereocilia, indicating that this isozyme might participate in actin-filament elongation (as do yeast myosin I isozymes[83]).

**$Ca^{2+}$ control in stereocilia.** Because $Ca^{2+}$ entry through open transduction channels is essential for fast and slow adaptation and may regulate tip-link assembly[84], proteins that control $Ca^{2+}$ levels in stereocilia should be essential for hair-cell function. A conventional knockout approach[85], as well as identification of the mouse deafness mutants *deafwaddler* (*dfw*)[86] and *Wriggle mouse sagami* (*wri*)[87], showed that mice require isoform 2 of the plasma-membrane $Ca^{2+}$-ATPase (PMCA) for proper auditory and vestibular function. In all hair cells, the only isoform found in the bundle is PMCA2a[88], which can be present at the remarkably high density of 2,000 molecules per $\mu m^2$ (ref. 89). Calmodulin is present in hair bundles at high concentrations[90,91], where it regulates PMCA, myosins and presumably other bundle molecules[92]. A gene that interacts genetically with *dfw*, *modifier of deafwaddler* (*mdfw*)[93], seems to be a *Cdh23* allele[75]. Mice heterozygous for *dfw* become deaf only when homozygous for *mdfw*; a possible explanation is that the level of intracellular $Ca^{2+}$ in the stereocilia — presumably higher when only one PMCA2 allele is present — affects the function of this candidate *Cdh23* allele. Furthermore, a gene that imparts age-related hearing loss, *ahl1*, also seems to be a *Cdh23* allele.

**Structural proteins.** A properly formed hair bundle is essential for mechanotransduction. Actin has long been known to be the main cytoskeletal element of hair bundles[94], and fimbrin was identified as an important crosslinker over a decade ago[95]. More recently, espin was identified as a second crosslinker of stereocilia, responsible for the *jerker* mouse deafness mutation[96]. In addition, a human homologue of the fly protein diaphanous is mutated in the nonsyndromic deafness DFNA1 (ref. 97). Diaphanous is a ligand for the actin-binding protein profilin and is a target for regulation by Rho, which regulates cytoskeletal assembly in many cell types.




**Box 3 Figure** Schematic illustration of identified hair-bundle proteins showing hypothetical locations of molecules implicated in stereocilia function. Myo7a, vezatin, Cdh23 and PKA may form the ankle-link complex. Pcd15 presumably also interconnects stereocilia. Myo1c may carry out adaptation, whereas PMCA maintains a low $Ca^{2+}$ concentration. Actin, fimbrin and espin have structural roles; not shown is DFNA1, which may help form the cytoskeleton. Calmodulin regulates several enzymes within the bundle, including PMCA, Myo1c and Myo7a.

### Adaptation by hair cells

Like all other sensory receptors, hair cells respond to sustained stimuli by adapting, which restores their sensitivity to threshold deflections. The mechanisms responsible for adaptation also set the resting tension in gating springs; the resulting open probability enables maximal sensitivity and high-frequency signal transmission[59]. Properties of adaptation vary substantially from preparation to preparation, and recent data suggest that this arises from differences in hair cells in different organs. Two distinct $Ca^{2+}$-dependent forms of adaptation operate simultaneously in hair cells that have been examined closely; one form is fast and involves the transduction channel directly, whereas the other is slower and uses an adaptation motor (Box 2). Much attention has been paid to the molecular characterization of adaptation, as this approach should elicit strong candidates for members of the transduction apparatus.

Fast adaptation occurs on a millisecond to sub-millisecond timescale, and probably ensues when $Ca^{2+}$ enters an open transduction channel, binds to a site near the channel, and causes the channel to close (Box 2). In slow adaptation, which requires tens to hundreds of milliseconds for completion, a motor molecule is hypothesized to

move up and down the actin core of a stereocilium to restore gating-spring tension towards its resting value[60]. Slow adaptation may be mediated by either myosin 1c (Myo1c or myosin Iβ) or by myosin VIIA (Myo7a) (Box 2).

### Essential molecules for transduction

Traditional biochemical and molecular-biological methods for identification of molecules essential for hair-cell function have been largely thwarted by hair cells' scarcity. The identification of 'deafness genes' by genetic screens has been the most productive approach used so far. Mutation of at least 100 human genes and 50 mouse genes lead to deafness associated with other dysfunctions (syndromic deafness) or deafness alone (nonsyndromic deafness).

But protein products of deafness genes generally do not have direct roles in mechanotransduction. Although unfortunate for those interested in transduction, this observation is unsurprising: the inner ear is a complicated structure, and sound detection relies on middle- and inner-ear formation, hair-bundle assembly, ionic homeostasis, synaptic transmission and a host of other events. Many of these deafness genes instead encode proteins required for development of the auditory or vestibular systems, for maintenance of the unusual extracellular fluid (endolymph), or for other essential functions not directly related to mechanical transduction[61].

A few of the identified molecules do seem to carry out their critical roles in stereocilia. Molecules essential for hair bundles fall readily into several classes of molecules (described in Box 3): cytoskeletal components like actin, espin, Myo6, Myo7a, Myo15 and the mammalian homologue of diaphanous; cell-adhesion or junctional molecules like Cdh23, protocadherin-15 (Pcd15) and vezatin; and the calcium pump PMCA2. These molecules and others prominent in hair bundles like fimbrin and calmodulin can be assembled into a speculative and incomplete picture (Box 3). An important question is whether the already-identified molecules form a transduction complex or instead are important for other hair-bundle functions, such as maintaining structural integrity of the bundle. Loss of Myo7a, Pcd15 and Cdh23, at least, leads to disarrayed hair bundles, suggesting that these molecules help hold stereocilia together.

## Future directions for mechanotransduction research

Because of the difficulties in working with tiny, inaccessible touch cells, the genetic scheme of *C. elegans* touch transduction has yet to be confirmed by electrophysiological or biochemical experiments. Perhaps the model will be more easily approached in a vertebrate mechanoreceptive cell that is more amenable to such approaches; the relevance of transduction in such a cell type will depend on the molecular makeup of the transduction apparatus in various mechanoreceptive cells. Alternatively, the challenges of measuring mechanically sensitive currents in *C. elegans* may yet be overcome.

Characterization of mechanotransduction in *Drosophila* has just begun. Although there is strong evidence indicating that NompC contributes to *Drosophila* mechanotransduction, residual mechanically sensitive current remaining in the absence of NompC indicates that other transduction channels are present. In addition, little is known about the ultrastructure of the transduction apparatus — where exactly is it located and how do mechanical stimuli lead to channel opening? The combination of electrophysiology and powerful genetics afforded by the sequenced fly genome suggests that we can expect important developments over the coming few years.

A crucial question for the field is the generality of the results from invertebrate model systems. The touch-receptor transduction apparatus of *C. elegans* seems to be used by some vertebrate mechanoreceptors. Does conservation through the vertebrates also hold for the distinct *Drosophila* transduction apparatus? In addition to shared developmental-control molecules and intriguing structural similarities during development, *Drosophila* mechanotransduction exhibits several surprising physiological similarities to vertebrate transduction by hair cells. These similarities include a high-$K^+$-receptor endolymph made by supporting cells, directional selectivity, adaptation to sustained mechanical stimuli that is voltage- and size-dependent, microsecond response latencies, and sensitivity to nanometre-scale stimuli[29]. Although no obvious homologues of *nompC* are present in the human genome, the presence of dozens of orphan members of the broader TRP family suggests that a vertebrate transduction channel could still come from this family. Identification of more of the *remp* and *nomp* genes should assist in determining the generality of this transduction system.

Many questions remain about hair-cell transduction. The rough draft of the transduction apparatus comprising of the known proteins of the hair bundle is, to say the least, incomplete. Although two strong candidates have been advanced for the adaptation motor, we do not know how the motor assembles and how it interacts with other parts of the transduction apparatus. Although Cdh23 and Pcd15 each probably contribute to stereociliary crosslinks, which crosslinks contain these molecules remains unknown. Equally mysterious are the identities of two of the most interesting parts of the transduction apparatus, the transduction channel and tip link. Furthermore, the known human and mouse deafness genes do not constitute a saturating search of the genomes, and only a fraction of these have been cloned. Although the genetic approach is powerful, other important transduction molecules that are essential elsewhere in the organism will be missed. Screening for proteins that interact with essential hair-bundle proteins — such as those interacting with Myo7a — will continue to be a powerful method for discovering new bundle proteins, including those involved in transduction. The complete sequencing of the human and mouse genomes will no doubt spur more powerful approaches. The transduction channel and other important molecules are there — someone has probably already seen their sequences — but we still do not know how to recognize them. The next five years will undoubtedly be an exciting time, as the molecular clues that have begun to emerge will stimulate the right experiments to find the rest of the transduction apparatus. □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