Molecular Biology of Hearing and Balance

Peter G. Gillespie

GENERAL FEATURES OF MECHANORECEPTORS
Mechanotransduction is of great utility for all organisms. A unified model for mechanotransduction allows comparison of mechanoreceptors from many organisms and cell types.

MODEL SYSTEMS
Genes responsible for touch detection in *C. elegans* have been identified and suggest a model for mechanical transduction. Polymodal sensory neurons in *C. elegans* are also mechanoreceptors. *Drosophila* bristle receptors and chordotonal organs are surface mechanoreceptors. Insect mechanoreceptors and hair cells share evolutionary relationships.

HAIR CELLS
Hair cells are the sensory cells of the auditory and vestibular systems. Hair cells are exposed to unusual extracellular fluids and potentials. Mechanical transduction depends on activation of ion channels linked to extracellular and intracellular structures. Some of the molecules responsible for hair-cell transduction have been identified.

DEAFNESS
The 'deafness gene' approach has proven fruitful for finding genes important to the auditory system. A variety of genes have been identified, including a set of genes that apparently interact with each other in the auditory and visual systems. The surprise is that genes clearly involved in mechanotransduction have not yet been identified by the deafness-gene approach.

CONCLUSIONS
Hair cells are specialized mechanoreceptors located in the inner ear; these cells transduce mechanical forces transmitted by sound and head movement, and permit an organism to sense features of the external world. Well-characterized biophysically, a molecular description of hair-cell transduction has finally begun to emerge.

Although many key molecules remain to be identified, striking molecular and functional correspondences between vertebrate hair cells and invertebrate mechanoreceptors have indicated that some types of mechanoreceptors probably share a common ancestor. The continued application of genetic, molecular, biological and biophysical approaches should lead to a more thorough understanding of this critical sensory cell.

GENERAL FEATURES OF MECHANORECEPTORS

Mechanotransduction is of great utility for all organisms. Most multicellular organisms exploit mechanoreceptors — specialized cells that detect external mechanical forces — to help construct their internal view of the external world. The senses of hearing, balance and touch all rely on mechanoreceptors, as do the proprioceptive sensations that tell an organism how it is situated in the environment. Mechanoreception is probably one of the most ancient of the senses. A unified model for mechanotransduction allows comparison of mechanoreceptors from many organisms and cell types. Mechanoreceptors nearly universally use ion channels for transducing sensory information. Mechanoreceptors are either neurons or are neuroepithelial cells with synapses, and the currency of the nervous system is the membrane potential. Opening an ion channel allows a cell quickly and extensively to modulate its membrane potential, and hence neurotransmitter release, the final step in mechanoreception at the cellular level. Ion channels open or close upon relative movement of internal domains, movements that can be elicited by...
voltage, ligand binding, or force (see Ion Channels in Ch. 6). How can force influence domain movements within ion channels? In some cases, domains can be moved by tension within the plasma membrane. Bacterial osmosensors are thought to work this way; as membrane tension increases, the channels gate to reduce tension [1]. However, most mechanoreceptors apparently do not sense membrane tension. Instead, independent investigation of hair cells, worm touch receptors and fly bristles have converged on a single general model for how transduction channels are gated (Fig. 51-1). In this model, the ion channel is anchored on both sides of the membrane. The intracellular anchor is the cytoskeleton, actin in the case of hair cells and microtubules in the case of characterized mechanoreceptors in worms and flies. The extracellular anchor varies substantially from cell type to cell type. The critical feature of this model is that external forces cause a net displacement of the two anchors; the force is transmitted to the channel, and net domain movement is triggered [1].

MODEL SYSTEMS

Understanding the general features of mechanoreceptors has been greatly assisted by the use of model systems. Genetically tractable organisms like Caenorhabditis elegans and Drosophila melanogaster have permitted a much more thorough inventory of the parts required for mechanotransduction than is possible in most vertebrates; the recent ascent of the zebrafish Danio rerio as a model has extended the advantages of a genetic organism to vertebrate mechanotransduction.

Genes responsible for touch detection in C. elegans have been identified and suggest a model for mechanical transduction. Worms like C. elegans respond to a gentle touch on the nose by turning away. Mechanical deformation of the worm's cuticle stimulates specific cells (six body-touch sensory neurons), which transmit sensory information to neural circuits that control locomotion. Genetic experiments have revealed ~12 genes involved in this response, and the identities of these genes have suggested a specific molecular model for the transduction apparatus (reviewed in [2]). Extracellular matrix molecules (MEC-5, MEC-9) presumably are mechanically connected to ion channels formed by MEC-4 and MEC-10 subunits, which belong to the DEG/ENaC family of channels. MEC-6 also contributes to this channel complex. These ion channels are connected (probably by MEC-2) to an underlying microtubule network, which consists of unique 15-protofilament microtubules (tubulin subunits encoded by the mec-7 and mec-12 genes). Movement of the cuticle relative to the microtubule...
network mechanically stimulates the transduction channel, which admits cations that depolarize the cell. Recent work has shown that excitation of the mechanosensory neurons leads rapidly to Ca\(^{2+}\) influx, apparently through MEA-4-type channels, which can be detected in live worms subject to touch stimuli [3]. Although direct mechanical gating of the DEG/ENaC channels has not been observed in intact neurons or cultured cells, the preponderance of the evidence supports this model.

Is this system relevant for hair cells? It does not appear so. Indeed, there appear to be at least two broad classes of mechanoreceptors, one like those of C. elegans touch neurons that rely on DEG/ENaC channels, and another class that apparently relies on transient receptor potential (TRP) channels. As we will see later, circumstantial evidence suggests that hair cells use TRP channels. Other mechanoreceptors in vertebrates may be related to C. elegans touch cells.

**Polymodal sensory neurons in C. elegans are also mechanoreceptors.** Polymodal sensory neurons in C. elegans seem to fall into the TRP class of mechanoreceptors as they apparently rely on several TRP channels for mechanosensation [4]. These sensory neurons respond to touch, hyperosmolarity and volatile repellents by triggering a backward response in the worm; the OSM-9 and OCR-2 channels apparently mediate these responses, and the pathways differ for each sensory modality. However, little is known about the transduction mechanism in these cells.

**Drosophila bristle receptors and chordotonal organs are surface mechanoreceptors.** These surface mechanoreceptors on insects have been studied for many years. Progress on identifying molecules responsible for insect mechanoreception received a big boost in 1994, when Kernan et al. developed a screen for mechanoreceptor mutants in Drosophila [5]. They identified several dozen mutants that were deficient in mechanoreception and, with the assistance of their colleagues, have begun to identify them.

The most important molecules so far identified from this screen include a likely transduction channel, an extracellular molecule that could gate channels, and several molecules known to be important for axonemal structure and function. Although the set of molecules is less complete than that identified for C. elegans touch receptors, the diversity of mechanotransduction in Drosophila and the apparent similarity of these receptors to those in vertebrates, including hair cells (see below), demonstrates the significance of this model system.

The protein NompC is a TRP channel and appears to be the major transduction channel in fly bristles [6]. Bristle mechanotransduction resembles hair-cell transduction remarkably in its speed, polarity and adaptation [6], suggesting the possibility of a close evolutionary relationship between these mechanoreceptors. NompC is not the only bristle transduction channel, however, as a residual transduction current remains in nompC null flies. Moreover, NompC plays a relatively small role in fly hearing [7], where the TRP channel nanchung is essential [8]. These results have intensified the search for TRP channels that could mediate transduction in hair cells, as discussed below.

The gene nompA encodes a multi-domain extracellular protein that is expressed by support cells that cradle the mechanically sensitive neuron [9]. NompA is localized to the dendritic cap, which is covers the mechanically sensitive outer segment of the neuron. NompA is a good candidate for a protein that couples mechanically sensitive channels to external stimuli.

**nompB** is the gene for a protein involved in intraflagellar transport, the basic mechanism by which axonemal structures like mechanoreceptor dendrites are maintained by the cell [10]. We certainly expect that this screen will identify genes like nompB just as deafness screens have identified proteins thought to be involved in hair-cell structural integrity.

**Insect mechanoreceptors and hair cells share evolutionary relationships.** In flies, the proneural gene atonal controls development of mechanoreceptors; Math1 plays a similar role in mice. Indeed, atonal can fully substitute for Math1 in mice and Math1 can fully substitute for atonal in flies [11], suggesting that these two disparate systems might share a common genetic program used for development. As another example, as we will discuss later, Drosophila bristle mechanoreceptors and zebrafish hair cells each rely on the TRP channel NompC for mechanotransduction.

**HAIR CELLS**

**Hair cells are the sensory cells of the auditory and vestibular systems.** Hair cells are the sensory cells of the internal ear, essential for the senses of sound and balance. The hair cell's transduction apparatus, the molecular machinery that converts forces and displacements into electrical responses, can respond to mechanical stimuli of less than 1 nm in amplitude, and of tens or even hundreds of kilohertz in frequency. Indeed, our hearing is ultimately limited by Brownian motion of water molecules impinging on the transduction apparatus.

Even though well-characterized at a biophysical level, the mechanical transduction mechanism of hair cells is still not fully understood in molecular terms. This discrepancy is in part due to the extreme scarcity of hair cells; instead of the millions or even hundreds of millions of receptor cells that the olfactory and visual systems possess, only a few tens of thousands of hair cells are found in the internal ears of most vertebrate species. The small number of hair cells and the direct transduction mechanism has greatly impeded molecular biological and
biochemical characterization. Consequently, molecular
description of hair-cell transduction has lagged well
behind description of vision and olfaction.

Hair cells are neuroepithelial cells; their large basolat-
eral surface includes synaptic contacts with afferent and
efferent nerve fibers, while the mechanically sensitive hair
bundle is located on their apical surface. The hair bundle
is an ensemble of 30–300 actin-filled stereocilia and a
single axonemal kinocilium (Fig. 51-2). The kinocilium,
present during development in all hair cells, degenerates
in those cells sensitive to high auditory frequencies.
Stereocilia range in height from 1 μm, for very high
frequency auditory detection, to 50 μm or more, in some
vestibular systems. Stereocilia contain hundreds of cross-
linked actin filaments throughout their length; as the
stereocilium approaches the apical surface of the hair cell,
that number systematically declines to a dozen or two.
Stereocilia are thus mechanically stiff throughout most of
their length, but are flexible at the insertion point.
Stereocilia do not flex independently, however, as they are
cross-linked together by a variety of linkages, including
ankle links, lateral links and tip links.

The consequence of the mechanical properties of the
stereocilium and their interconnection by flexible linkages
is that, when the bundle is deflected by a mechanical stimu-
lus, the bundle moves as a whole. Moreover, individual
stereocilia slide with respect to each other, a movement
that underlies mechanical transduction (Fig. 51-3).

**Hair cells are exposed to unusual extracellular fluids
and potentials.** The apical surfaces of hair cells are
exposed to an unusual extracellular fluid called endol-
ymph. Endolymph is relatively similar in ionic compo-
sition to cytoplasm: it is high in K⁺ (~150 mM), low in
Na⁺ (~2 mM), and relatively low in Ca²⁺ (~100 μM).
Endolymph may have evolved to separate from hair cells
the energy expenditure required to remove ions from hair
cells. Normally, an excitatory cell like a neuron must pump
out all of the ions that entered during an excitatory
response (see Ch. 5). The ear separates this pumping task
away from the hair cell. K⁺, the major current-carrying
ion, enters hair cells down an electrical gradient (hair cells
are typically ~60 mV) through transduction channels;
when channels are closed, however, K⁺ readily leaves
through basolateral K⁺ channels down the normal K⁺
electrochemical gradient. The energy expenditure required
for transduction is thus passed on to the stria vascularis
cells, which, via the Na/K-ATPase, establish the high K⁺
concentration in the endolymph.

The endolymphatic compartment of the auditory
system is at an elevated potential (about +80 mV); this
endocochlear potential increases the driving force on
K⁺ yet more, producing additional transduction current.
Some mutations that cause deafness affect either K⁺ levels
in the endolymph or the endolymphatic potential itself.

**Mechanical transduction depends on activation of ion
channels linked to extracellular and intracellular struc-
tures.** A comprehensive model for hair-cell transduction
has emerged, derived primarily from biophysical and
morphological investigations. Residing in the mechanore-
ceptive organelle of a hair cell, the hair bundle, the trans-
duction apparatus consists of at least three components:
the transduction channel, a mechanically gated ion chan-
nel; the tip link, an extracellular filament that transmits
force to the channel’s gate; and the adaptation motor,
a mechanism that maintains an optimal tension in the tip link so that the channel can respond to displacements of atomic dimensions (Fig. 51-3). The tip link is probably connected in series with the gating spring, an elastic element through which stimulus energy can affect the transduction channel. Although highly specialized for the internal ear, the channel, gating spring, and adaptation mechanism are likely to be general requirements for any mechanical transduction apparatus; detailed characterization of the transduction apparatus of the hair cell may therefore eventually illuminate other transduction systems.

In this model, deflection of the hair bundle in the excitatory direction—towards the tallest stereocilia—causes a shorter stereocilium to slide down its neighbor's side, stretching the gating spring. Tension in the gating spring is transmitted to the transduction channel, which responds by increasing the frequency and the duration of the openings.

Transduction channels do not remain open, even if a deflection is maintained; using two independent processes, the hair cell adapts to a sustained mechanical stimulus [12]. Rapid channel reclosure (sometimes called fast adaptation) occurs on a time-scale of a few milliseconds or less; Ca\(^{2+}\) entering open transduction channels binds to a site on or nearby the channel, causing the channel to close (Fig. 51-4). Because closing channels exert a negative-directed force on the hair bundle, the fast adaptation mechanism can mechanically amplify an input; if the negative force occurs during the negative phase of a sinusoidal stimulus, the forces add and bundle movement is enhanced.

A slower mechanism, operating on a time-scale of tens of milliseconds, adjusts the transduction apparatus so that the open probability at rest is optimal and fast adaptation is enhanced. Slow adaptation is mediated by a cluster of myosin molecules [13]; the myosins exert a resting tension, but the oppositely directed force in a stimulus overcomes the resting tension, dragging the myosin complex down the stereocilium (Fig. 51-5). As the motors descend the stereocilium, tension in the gating spring is reduced, and the transduction channels close. When the bundle is moved in the negative direction, reducing tension in the gating spring and hence tension applied to the motors, the adaptation motor climbs the actin of the stereocilium until the motors generate enough tension in the gating spring to stall themselves (Fig. 51-5).
Some of the molecules responsible for hair-cell transduction have been identified. A few key molecules, some already described, have been identified as part of the transduction complex (Fig. 51-6). Myosin molecules clearly play essential roles, and hair cells express a variety of myosin isoforms. Because it is located at the tips of the stereocilia, near the tip-link anchors, myosin-1c is the best candidate for the adaptation motor. Selective inhibition of a sensitized myosin-1c mutant with an ADP analog proved that this myosin participates in adaptation [14], although contribution by other myosins has yet to be ruled out. For example, mice with near-null mutations in myosin-7a have defects in auditory transduction that are consistent with alterations in the adaptation machinery, suggesting a central role for this myosin too [15].

Although mice with mutations in the myosin-6, -7a, and 15a genes are deaf, these isoforms apparently mediate essential hair-cell functions besides adaptation. Myosin-7a may play an important role in bundle integrity, as mice and fish with no functional myosin-7a protein have highly disheveled bundles.

Myosin-6 is an unusual isoform as it moves in the opposite direction along actin filaments, as compared to all other well-characterized myosins. Myosin-6 is located near the base of the hair bundle, and could play a role in anchoring the apical membrane to prevent fusion along the stereocilia shafts [16] or it may affect the forces applied to stereocilia actin filaments [17].

Myosin-15a is located at stereocilia tips and apparently controls the rate of actin filament growth [18]; stereocilia with more myosin-15a grow faster than those with lesser amounts of the myosin. Hair cells with no functional myosin-15a protein grow stereocilia that are very short and stubby, indicating that myosin-15a is important for actin filament elongation but not in the specification of the positions of the stereocilia.

Recent experiments have suggested that the tip link, a complex of two or three braided glycoprotein filaments [19], may be made in part from cadherin-23, a Ca$^{2+}$-dependent cell-adhesion molecule [20]. Moreover, cadherin-23 can interact directly or indirectly with myosin-1c, suggesting that these two molecules form part of the transduction complex in hair cells [20].
Although the transduction channel has attracted much attention, it too has yet to be identified. This channel passes cations, including Ca\(^{2+}\), and can be inhibited by aminoglycoside antibiotics and amiloride derivatives. Unless controlled by tension applied through the gating spring, the channel remains shut. Most researchers in the field feel that the transduction channel belongs to the TRP channel family. TRP channels are remarkably diverse in structure and function and include many channels central for sensory function, including those involved in vision, pheromone sensing, temperature detection, and mechanical responsiveness. Indeed, TRP channels have been shown to be important in insects for mechanotransduction. In the fly, the TRP channel NompC plays a predominant role in the bristles and a lesser role in the auditory system. At least one TRP channel of the TRPV family, *nanchung*, is essential for fly hearing.

Are any of these TRP channels found in hair cells? The answer is yes for NompC, although the details are unexpected. NompC is found in zebrafish hair cells, where it has been shown to be essential for transduction [21]. Surprisingly enough, however, NompC has yet to be identified in mammalian genomes. This suggests the possibility that NompC is just one of several channel subunits required, and that other TRP channels could contribute to the transduction channel in mammals. Although several TRPV channels have been localized to hair cells in mammals, gene knockouts suggest that these channels do not play a role in transduction.

As both fast and slow adaptation mechanisms are regulated by Ca\(^{2+}\), the stereocilia mechanisms that control the free concentration of this ion also play central roles in transduction. Entering Ca\(^{2+}\) is thought to be buffered very rapidly by the mobile buffers parvalbumin 3, calbindin, and calretinin [22, 23]. Even before bound Ca\(^{2+}\) can diffuse out of stereocilia, it is pumped back out into the endolymph by isoform 2a of the plasma-membrane Ca\(^{2+}\)-ATPase (PMCA2) [24, 25] (see also Ca\(^{2+}\) transport in Ch. 5).

**DEAFNESS**

About 1 in 1,000 children are born deaf, and another 1 in 1,000 develop deafness by adolescence. Although some deafness can arise during development due to external sources, much deafness in children is due to mutations in specific genes. As many forms of deafness are nonsyndromic — there is no other associated abnormality — identification of the responsible genes can lead to characterization of proteins that are essential for the auditory system (and often the vestibular system, as well).

The ‘deafness gene’ approach has proven fruitful for finding genes important to the auditory system. The genes responsible for ~20 recessive and ~20 dominant nonsyndromic deafness syndromes have been identified in humans, and similar numbers have been identified in mice [26]. Because some genes are associated with both recessive and dominant syndromes, and because most genes identified in humans have also been identified in mice, this tally corresponds to about 30 different genes.

The identified genes code for a variety of proteins, including transcription factors that are important for inner-ear development, cytoskeleton proteins that are responsible for the specialized architecture of hair cells, extracellular matrix molecules that make up some of the specialized aacelullar structures of the inner ear, and still others whose function is not yet clear.

A variety of genes have been identified, including a set of genes that apparently interact with each other in the auditory and visual systems. An example of a gene important for transduction that was identified in this manner was PMCA2. The deaf mouse *deafwaddler* was shown to have a mutation in the PMCA2 gene, which is called Atppb2 [27]; knockout of this gene also leads to deafness [28]. These results demonstrate that the Ca\(^{2+}\)-pumping activity of stereocilia is essential for auditory (and vestibular function), and reinforce the central role played by this ion in hair cell function.

Usher syndrome is a deafness–blindness syndrome that is caused by mutations in a number of genes [29]. Usher syndrome is divided into three classes depending on the severity of the phenotype; Usher I produces profound deafness and vestibular dysfunction, as well as adolescent blindness; in Usher II, deafness is less profound. Usher III is less common; in these patients, hearing and vision loss develop progressively, and vestibular dysfunction is variable.

Of the seven mapped genes underlying Usher I, five have been identified. These included cadherin-23 and myosin-7a, both described above, harmonin and SANS, both scaffold proteins, and protocadherin-15, another member of the cadherin superfamily. Biochemical evidence and phenotypic similarity suggests that these proteins may assemble into a complex [29], although conclusive evidence for such a complex is lacking.

Usher II is caused by mutations in at least four genes; only one, usherin, has been identified. Usherin is an extracellular matrix protein of unknown function; it is found in basement membranes in the eye and ear (and in other tissues as well). Similarly, at least two genes underlie Usher III, but only one (clarin-1) has been cloned. The function of clarin-1 is unknown.

The surprise is that genes clearly involved in mechanotransduction have not yet been identified by the deafness-gene approach. Clearly disruption of hair-cell function at many levels can lead to deafness, so it is expected that deafness genes include those involved in inner-ear development, in ion balance in the endolymph, and in structural integrity of hair cells. Examination of deafness genes in zebrafish has been particularly thorough, however, and
investigators using this system have several useful assays that allow them to focus only on those mutants that clearly have consequences for transduction [30].

However, the deafness-gene approach has not been successful in identifying either known (e.g., myosin-1c) or unknown (e.g., the transduction channel) components of the transduction apparatus in fish, mouse, or human. This is surprising, as the genetic approach relies on few assumptions about the nature of the systems studied. Perhaps the lesson here is that some of the important molecules for transduction play essential roles elsewhere in the organism during development; given the widespread expression of Myo1c, this presumption seems reasonable.

**CONCLUSIONS**

Although only a relatively small number of hair-bundle proteins have been identified, many of these clearly play important roles for transduction and bundle structure. The increasing speed of identification of important proteins suggests that a thorough accounting of the major transduction proteins for hair cells should not be too far off. The next and more interesting phase of characterization of hair-cell transduction will then ensue, determining how these molecules interact and how the hair cell assembles them during development and following normal protein turnover, in order to make a remarkably sensitive transduction apparatus.

**REFERENCES**