Feeling force: mechanical transduction by vertebrates and invertebrates

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Detection of mechanical stimuli requires conversion of the signal's inherent information into neuronal electrical signals. Studies of vertebrate hair cells suggest that this is accomplished by elastic links between stereocilia that control the opening of ion channels. Molecular genetics in *Caenorhabditis elegans* has identified candidate proteins that may be responsible for similar functions in this organism.

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Of what importance is mechanical transduction in the life of an organism? The information contained within mechanical stimuli, when transduced into neuronal electrical signals, powerfully illuminates important aspects of the external environment. Sound informs us of events at a distance, like the approach of a tiger, even if it is out of our line of sight; detection of gravity allows us to maintain body equilibrium, even when fleeing the racing tiger; and touch assures us that the tiger has yet to grasp us in its jaws.

When considering a particular sensory system, it is important to understand the nature of the initial stimulus. In the case of mechanical transduction, the stimulus is a force applied to a sensory cell's receptive structure. Sensory organs are designed so that force from only one type of stimulus is directed to the receptor.

Vertebrates and invertebrates alike seem to have settled on ion channels as the primary means of detection of mechanical force. Perhaps this common theme has arisen because ion channels directly alter the electrical properties of a sensory cell. In turn, the altered membrane potential of the sensory cell directly signals the central nervous system, via chemical synapses and electrical action potentials. Gating of ion channels can be extraordinarily fast; vertebrate transduction channels can be opened by mechanical stimuli on a microsecond time scale [1]. Thus, although mechanical receptors coupled to second-messenger systems or other types of transduction mechanisms can be imagined [1], ion channels appear to be predominant.

Given that forces are usually funneled to mechanically sensitive ion channels, what other common elements are mechanical transduction systems likely to possess? One may be some sort of elastic gating element, or gating spring, that controls the opening and closing of the transduction channel. External forces stretch this spring, which directly transmits the force to the gate of the channel.

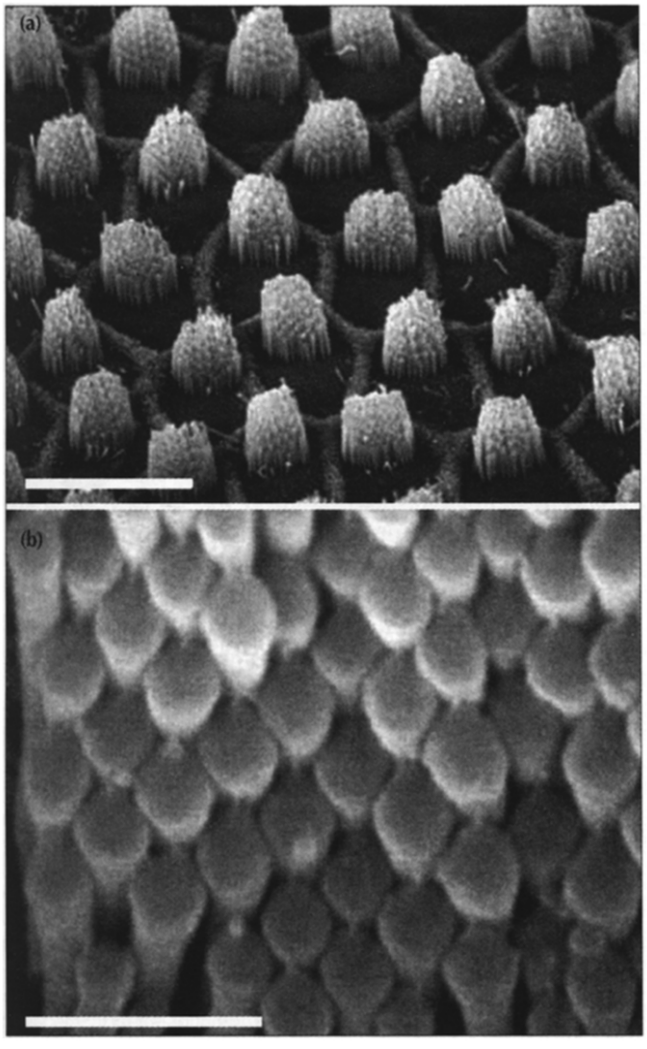
All mechanical transduction systems must also have optimized sensitivity. External forces presumably alter the energy of the closed or open states of a transduction channel. The distribution between states — and hence channel opening — must therefore depend on the Boltzmann relation [2], which describes the probability that a molecule will be in a particular state at a given temperature. The sigmoidal distribution of the number of channels that are open as force varies dictates that there will be an optimal range of sensitivity, which will not be at the point where all channels are closed. The sensory organ thus needs a gating-adjustment mechanism that keeps the channel poised at the position of maximal sensitivity. To be most sensitive, the sensory receptor must partially stretch its gating spring using this adjustment mechanism so that a tiny displacement will produce the maximum possible channel opening (or closing).

A sensitive mechanical transduction apparatus should thus have at least three key elements: a mechanically sensitive channel, a gating spring, and a gating-adjustment mechanism. To summarize the mechanical-transduction systems that have been most thoroughly studied, I will examine transduction by vertebrate hair cells, worm touch receptors, and insect sensory cells. For more detail and other examples, the reader is referred to comprehensive reviews of transduction by hair cells [2–4], other vertebrate cells [5], and invertebrates [6].

Mechanical transduction by vertebrate hair cells

Vertebrate hair cells provide the best-understood example of mechanical transduction [2]. Hair cells transduce mechanical stimuli using a specialized organelle, the hair bundle (Fig. 1). Hair bundles consist of 30–500 stereocilia, each of which is a plasma membrane encased protrusion of the cell body containing hundreds of crosslinked actin filaments. Each stereocilium pivots at the point where filaments, reduced in this region to only a few dozen, enter the cell body. The rigid stereociliary shafts, flexible basal insertions, and tight coupling of the stereocilia within the hair bundle by an assortment of extracellular linkages, allow the bundle to move as a unit, with adjacent stereocilia sliding along each other during bundle movement (Fig. 2). Bundles are asymmetric with respect to the length of stereocilia, with a gradation from short to long along an axis of mirror symmetry within the

Figure 1



Vertebrate hair bundles. **(a)** A view of several dozen chicken hair cells. Each hair cell is surrounded by narrow extensions of the microvilli-rich supporting cells, which form a hexagonal grid around the hair cells. Hair bundles protrude from each hair cell; ~100 stereocilia are found in the typical bundle. Scale bar, 10 μm. **(b)** At high magnification, the asymmetric organization of the stereocilia is apparent. Tip links, connecting short stereocilia with their tallest neighbor, can be seen for most stereociliary pairs along the axis of mirror symmetry. Scale bar, 1 μm.

bundle. Channel gating is coupled to bundle movement in a polarized fashion; movement of the bundle towards the tallest stereocilia opens channels, and movement towards the short stereocilia closes channels.

The gating-springs hypothesis [1,2] states that displacement of the bundle stretches an elastic gating spring, which raises the energy level of the channel's closed state. The distribution between open and closed states shifts to favor the open state, and cations enter the cell through the open channel (Fig. 2). What is the gating spring? Electron-microscopic studies have indicated that a likely candidate is the tip link [7]. Tip links run along the bundle's axis of mechanical sensitivity from a short stereocilium to its tallest neighbor (Fig. 1b). Eradication of tip links with Ca^{2+}-chelators simultaneously disrupts gating springs, suggesting that they are one and the same [8]. The tiny amounts of tip-link protein, around an attomole in a typical hearing organ, have so far prevented its molecular identification.

Transduction channels are equally scarce, again on the order of an attomole per organ, and few distinguishing pharmacological characteristics have been ascribed to them. Circumstantial evidence has suggested that transduction channels might belong to the amiloride-sensitive epithelial Na^+-channel family [9], but data supporting this claim are not conclusive [4].

In hair cells, the adjustment mechanism responsible for maintaining optimal gating-spring tension is called the adaptation motor. Indirect evidence suggests that the adaptation motor comprises several dozen molecules of myosin, yoked together to generate substantial force [3]. Three myosin isozymes have been identified in hair bundles: myosin Iβ [10], myosin VI [11], and myosin VIIA [12]. Mutations in myosins VI and VIIA can produce deafness in mice; indeed, myosin VIIA mutations underlie a major class of human deafness, Usher 1B [13]. The effects of myosin Iβ mutations remain unknown.

Because of its compact size and localization at stereociliary tips, myosin Iβ remains the most attractive candidate for the mediator of the adaptation mechanism. Myosin VI seems more likely to have a structural role [11], and the larger size and greater bundle distribution of myosin VIIA suggest that it is unlikely to reside at the tip link's upper insertion, the probable location of the adaptation motor.

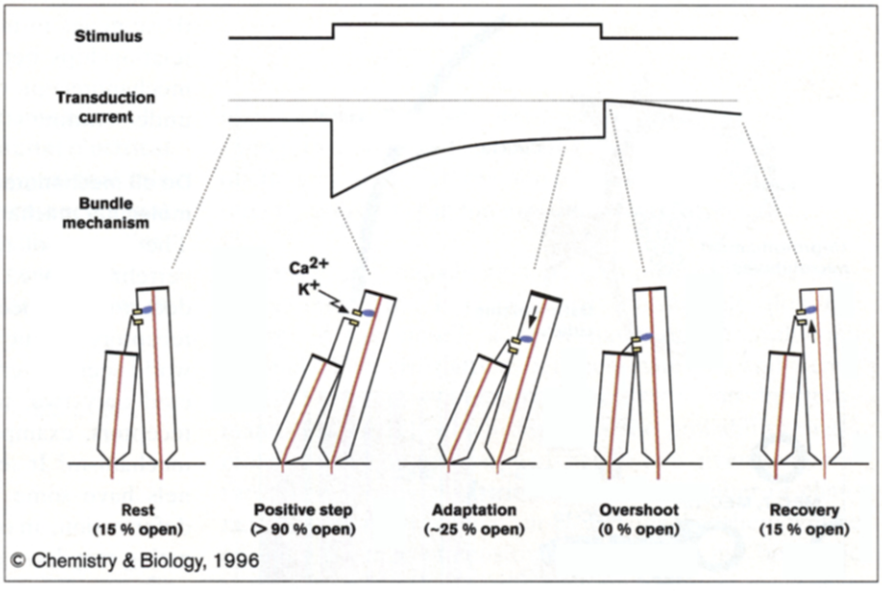
Touch transduction by *Caenorhabditis elegans*

By virtue of its well mapped cell lineage, rapid generation time, genetic accessibility, and repertoire of behaviors, *Caenorhabditis elegans* has proven to be an exceptionally useful model organism. This is no less true for mechanical transduction, where most of the genes responsible for touch-receptor function have been cloned and sequenced and a comprehensive model can be constructed.

Reception of light touch (experimentally applied using a hair) is mediated in *C. elegans* by the touch cells, six cells located along the body wall of the nematode. Each cell sends a long process running longitudinally along the body wall; it is this process which apparently is responsible for transduction. The processes are endowed with large-diameter microtubules consisting of 15 protofilaments; specialized α and β tubulin genes have been identified (*mec-12* and *mec-7*), which are essential for touch-receptor function. Exactly how force is directed to

Figure 2

Mechanical transduction by vertebrate hair cells. Tip links are stretched by excitatory mechanical stimuli, and the heightened tension increases the fraction of channels that are open. Transduction channels permit entry of a wide variety of small cations; with the K^+-rich endolymph bathing the hair bundle, K^+ (and, to a much smaller extent, Ca^{2+}) is the major current-carrying ion. Increased tension and entry of Ca^{2+} trigger adaptation. The adaptation motor slips down the actin cytoskeleton, decreasing tip-link tension and permitting channels to re-close. At the end of a stimulus (or after an inhibitory stimulus), tip-link tension plummets; motors then ascend the cytoskeleton and reopen channels.



mechanosensitive elements remains unclear, although the mantle surrounding the touch-receptor process or the overlying cuticle may provide a firm substrate for attaching a gating spring to a mechanically sensitive channel that can move relative to the extracellular substrate.

Screens for touch-receptor mutants have identified thirteen genes essential for touch detection, most of which may be directly involved in transduction (for further references to *C. elegans* touch-receptor genes, see [14]). Perhaps the most interesting are *mec-4*, *mec-10*, and *deg-1*, dominant mutations of which lead to degeneration of touch-receptor cells. Strikingly, these genes are homologous to the epithelial Na^+ channel [15]; indeed, by virtue of inhibitor blockage and cellular localization, the hair cell's transduction channel had previously been proposed to belong to this family [9]. Indirect evidence strongly suggests that the *C. elegans* proteins are channels [16,17].

The original dominant mutations of *mec-4*, *mec-10*, and *deg-1* all lie within putative pore regions. But a missense mutation or a deletion of a short region found in *mec-4* and *deg-1* but not the epithelial Na^+ channel genes also produced the dominant phenotype. Because this region is predicted to be extracellular, and because mutations in the putative pore regions that substitute bulkier amino acids for small ones suppress the dominant phenotype, mutations of the extracellular region may lead to a channel that remains open at all times. Implicit in this description is the suggestion that mutations in *mec-4*, *mec-10*, and *deg-1* that produce constitutively open channels are dominant and lead to the degeneration phenotype, whereas mutations that plug the channel's pore would be recessive.

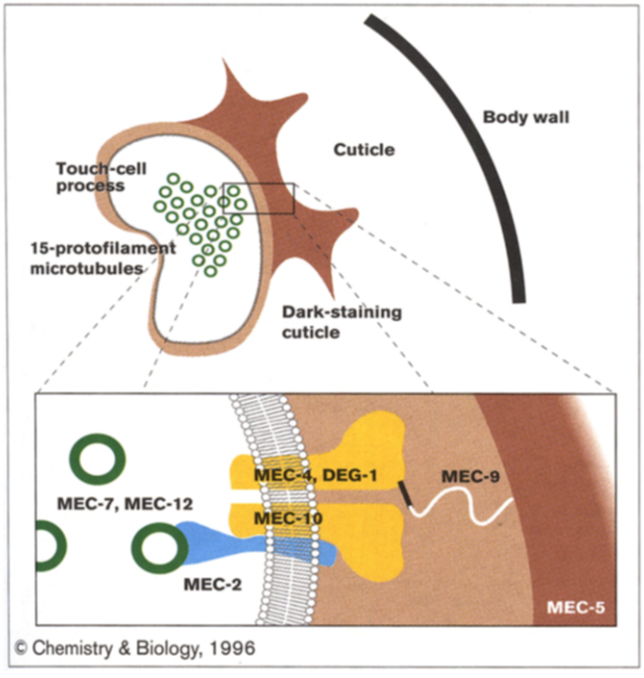
Other genes responsible for touch-receptor function that have been cloned and sequenced include *mec-2*, which is expressed intracellularly and may be responsible for anchoring the putative transduction channels to the cytoskeleton, *mec-5*, which encodes a protein that has similarity to collagens, and *mec-9*, which encodes an extracellular protein with putative Ca^{2+}-binding sites and domains found in cell-adhesion molecules. Despite the absence of direct evidence, it is nonetheless attractive to propose that *mec-9* encodes an extracellular gating spring that interacts with the putative transduction channel [14]. A highly speculative but coherent model based on the available data is shown in Figure 3. Similarities to the vertebrate hair-cell model should be obvious.

Although genetic tools have advanced the study of touch transduction in *C. elegans* to an impressive extent, the touch assay — a crude prod — is insufficient to dissect the biophysics of touch transduction. For example, it remains unclear how forces applied to the animal's surface are transmitted to the putative transduction apparatus. The sensitivity of touch receptors remains equally unclear. Vertebrate hair cells respond to mere piconewtons of force; do *C. elegans* receptors require more? A gating-adjustment mechanism has yet to be identified; indeed, it is unclear whether such a mechanism exists in this case. Development of a physiological assay measuring the effects of defined forces upon touch cells should greatly assist the field in answering these and related questions.

Other invertebrate mechanoreceptors

Several invertebrate preparations, such as the wolf spider's slit sense organ [18], permit exquisite biophysical recordings,

Figure 3



Organization of gene products responsible for touch transduction by *C. elegans*. Based on sequence similarities to proteins of known function, many of the *mec* gene products can be assembled into a coherent but untested model. MEC-12 and MEC-7 are specialized α and β tubulin subunits required for the touch-receptor microtubules; MEC-2 may connect ion channels to these microtubules. The transduction channel may consist of MEC-4, DEG-1, MEC-10 (and perhaps MEC-6), with MEC-4 (and perhaps DEG-1) contributing to the extracellular gate. The extracellular protein MEC-9 may be the gating spring, interacting both with the putative transduction channel and the firm extracellular substrate. MEC-5 may be a part of this substrate. Mechanical stimulation (not shown) must lead to displacement of the touch-process microtubules relative to the extracellular substrate, stretching the extracellular gating spring and opening channels.

yet study of these systems is ultimately limited by the lack of defined molecular techniques. The combination of genetics and electrophysiology, as well as biochemical methods (if at all possible), seems crucial for teasing out the molecular mechanism of mechanical transduction.

A highly promising start towards reaching this goal has been made by Maurice Kernan, Charles Zuker, and their colleagues [19]. These authors carried out a large-scale screen of the *Drosophila* genome and identified several dozen genes that are necessary for mechanical transduction. Although sequences for these genes have yet to be reported, the powerful molecular genetic techniques available to researchers working on *Drosophila* should allow rapid identification and characterization of these genes and their gene products. Furthermore, the combination of impressive molecular genetic tools and sophisticated electrophysiological assays for *Drosophila* mechanoreceptors should allow those working on fly receptors to understand the functions of the products of these genes in mechanical transduction, and to tease out relationships between them. Those studying *Drosophila* mechanoreceptors seem best poised for a comprehensive understanding of a mechanical transduction system.

Do all mechanoreceptors use the same molecular mechanism?

The variety of mechanoreceptors is enormous. A single vertebrate species might use a dozen different types of detectors of mechanical forces, from hair cells to baroreceptors to touch receptors in the skin. The invertebrate world seems even more diverse; although we have considered only two, worm touch receptors and fly mechanoreceptors, examples abound. Do they all use a common mechanism? It seems unlikely; many unrelated ion channels have some sensitivity to membrane stretch or cell deformation, an observation that indicates that mechanical gating may have arisen independently many times during evolution. Furthermore, the underlying cytoskeletal structures of mechanoreceptors seem strikingly different, from a hair cell's elaborate actin-based hair bundle to the extended microtubule array in a worm touch receptor to the intricate structure of a fly's mechanoreceptor.

Still, it seems premature to suggest that all mechanoreceptors arose independently. Indeed, many (if not most) mechanoreceptors derive from ciliated cells, and the hair cell is no exception. All hair bundles include one microtubule-based process, the kinocilium, although it degenerates in hair cells of some hearing organs later in development. Not responsible for transduction in mature bundles, the kinocilium may nevertheless be a vestige of an early mechanoreceptor. In addition, fly mechanoreceptors and vertebrate hair cells both expose their mechanoreceptive organelles to a K^+-rich, Na^+-poor extracellular fluid. This unusual strategy probably reduces metabolic load; the net inward driving force promotes apical K^+ entry (and hence transduction current) through transduction channels, but K^+ subsequently escapes passively across the basolateral surface where the extracellular K^+ concentration is much lower. That both types of mechanoreceptors use this mechanism implies either that they share a common origin or that it is such a useful (and unusual) strategy that it has evolved independently on two occasions. Finally, although not conclusive, several lines of evidence do implicate members of the epithelial Na^+-channel family both in hair-cell transduction [9] and in *C. elegans* touch reception [15].

Even if the varied mechanoreceptors use different mechanisms, gaining a comprehensive picture of one mechanical transduction system from a genetic approach to invertebrate transduction should prove thoroughly illuminating for the study of transduction in all systems. I

expect that the next several years will be exciting. If a physiological assay is developed for the worm touch receptor, and if the *Drosophila* mechanoreceptor genes are identified, comparison of the genes necessary in these two organisms and their functions in mechanical transduction should provide a molecular and mechanistic framework for determining how transduction is carried out by other cell types, including the hair cell.

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