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THE MOLECULES OF MECHANOSENSATION

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ABSTRACT

Mechanosensation, the transduction of mechanical forces into a cellular electrochemical signal, enables living organisms to detect touch; vibrations, such as sound; accelerations, including gravity; body movements; and changes in cellular volume and shape. Ion channels directly activated by mechanical tension are thought to mediate mechanosensation in many systems. Only one channel has been cloned that is unequivocably mechanically gated: the MscL channel in bacteria. Genetic screens for touch-insensitive nematodes or flies promise to identify the proteins that constitute a mechanosensory apparatus in eukaryotes. In *Caenorhabditis elegans*, the *mec* genes thus identified encode molecules for a candidate structure, which includes a "degenerin" channel tethered to specialized extracellular and intracellular structural proteins. In hair cells of the inner ear, evidence suggests that an extracellular tip link pulls on a channel, which attached intracellularly to actin via a tension-regulating myosin 1β. The channel and the tip link have not been cloned. Because degenerins and MscL homologs have not been found outside of nematodes and prokaryotes, respectively, and because intracellular and extracellular accessory structures apparently differ among organs and species, it may be that mechanosensory channel complexes evolved multiple times.

INTRODUCTION

Channels that gate in response to mechanical forces are thought to form the basis for touch, hearing, balance, and osmosensation; they have been characterized biophysically in several mechanosensory cell types. In addition, channels that activate or inactivate when suction is applied through a recording patch pipette have been studied in many cell types and organisms; they may participate in the

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regulation of cell volume and shape. Despite the ubiquity of mechanotransduction and the variety of biophysical studies, little is known about the molecular composition of mechanosensors. A picture is emerging from the recent cloning of genes involved in prokaryote osmoregulation, invertebrate touch, and vertebrate hearing and balance.

OSMOREGULATION: A BACTERIAL MECHANOSENSORY CHANNEL

Escherichia coli contains at least three species of stretch-activated channels, MscL, MscS, and MscM (for mechanosensitive channel of large, small, and mini conductance, respectively) (Martinac et al 1987, 1990; Delcour et al 1989; Sukharev et al 1993a, 1994, Berrier et al 1996). These channels were identified by applying suction to patch pipettes on bacterial spheroplasts and were later shown to gate in response to osmotic changes (Cui et al 1995). Because the MscL protein could be extracted, fractionated, and reconstituted as a functional channel in artificial liposomes (Sukharev et al 1993a,b, Hase et al 1995), it could be purified biochemically and partially sequenced, and the *mscl* gene encoding it was cloned (Sukharev et al 1994a,b). Recombinant MscL constitutes a mechanosensitive channel in the absence of other proteins, indicating that tension is applied to the channel by surrounding lipids, not by accessory proteins. This is the first, and at present the only, cloned protein that is unquestionably a mechanically sensitive ion channel.

Functional reconstitution in the absence of other proteins also facilitates the analysis of the channel by in vitro mutagenesis. The channel is probably composed of six 17-kDa MscL subunits, each with two-membrane spanning α helices and a periplasmic loop that may form a P-domain like that proposed to line the pore in voltage-gated channels (Blount et al 1996a). Mutagenesis experiments implicate a lysine of the first transmembrane helix and a glutamine of the periplasmic loop in the kinetics and mechanosensitivity of channel gating (Blount et al 1996b). It is expected that an ongoing random mutagenesis experiment will reveal the structures that form the pore and the gate of the channel, and the mechanisms by which force opens the channel. The MscL type of mechanosensitive channel is widespread among prokaryotes: Homologues of *mscl* have been found in the genomes of gram-negative and gram-positive bacteria (Parra-López et al 1994, Fleischmann et al 1995, Matsushita et al 1995). No eukaryotic homologues have been detected so far.

TOUCH SENSITIVITY: GENETIC ANALYSIS IN INVERTEBRATES

In vertebrates, the understanding of touch lags behind that of all other major senses. This is probably because touch receptors are dispersed throughout the

body and intermixed with pain and thermal receptors, preventing the biochemical purification of their components, and because the sensory terminals are far from the cell bodies and embedded in other tissues, obstructing the access of patch pipettes for electrophysiological recordings. Inherited defects in touch in humans might reveal genes needed for cutaneous sensation (Dyck et al 1993). However, mapping and cloning of human disease genes is so cumbersome, especially if no candidate genes are available, that a genetic approach to human touch would be impractical. An alternative approach has been to identify touch-insensitive mutants in genetically tractable organisms, such as the ciliates *Paramecium tetraurelia* and *Paramecium caudatum* (Eckert & Brehm 1979, Saimi & Kung 1987), the nematode *Caenorhabditis elegans* (Sulston et al 1975, Chalfie & Sulston 1981, Kaplan & Horvitz 1993), or the fly *Drosophila melanogaster* (Kernan et al 1994).

Mechanical stimulation of the anterior part of *P. tetraurelia* and *P. caudatum* induces a depolarization which triggers an action potential that results in a reversal in the direction of movement. Stimulation of the posterior part results in a potassium-dependent hyperpolarization and an increase in the speed of forward movement. Although numerous mutations have been isolated that impair these responses, they all alter various aspects of the action potential but none affect the mechanoreceptor potential (Saimi & Kung 1987). Genes that participate in the generation of the mechanoreceptor potential may be essential for viability, or they may act in redundant pathways, impeding the isolation of specific mechanoreceptor-deficient mutants.

Genes Necessary for Gentle Touch Sensitivity in C. elegans

So far the most fruitful approach to a molecular understanding of touch has been with *C. elegans* gentle touch reception. Gently stimulating the body of the worm (but not the nose) with an eyebrow hair (a force of about 20 to 50 μN) (J García-Añoveros, unpublished data) causes worms to move away from the stimulus. Mutants were collected that failed to respond to this stimulus (the Mec phenotype), although they still responded to touch applied to the nose or to the harsher prod of a platinum wire (70 to 180 μN) applied anywhere along the body (Sulston et al 1975). Sensitivity to gentle touch is mediated by a set of six touch receptor neurons that have large-diameter, 15-protofilament microtubules (Chalfie & Thomson 1979, 1982). The processes of these microtubule cells run along the side of the animal and are embedded in the hypodermis (the "worm skin"), to which they appear to be glued by an extracellular material called the mantle (Chalfie & Sulston 1981) (Figure 1). Over 440 of these mutations in 15 genes have been identified (Sulston et al 1975, Chalfie & Sulston 1981, Chalfie & Au 1989, Savage et al 1989, Lundquist & Herman 1994, Shreffler et al 1995). These genes are termed *mec*, and their protein products MEC. Pleiotropic mutations with severe phenotypes such as uncoordination or

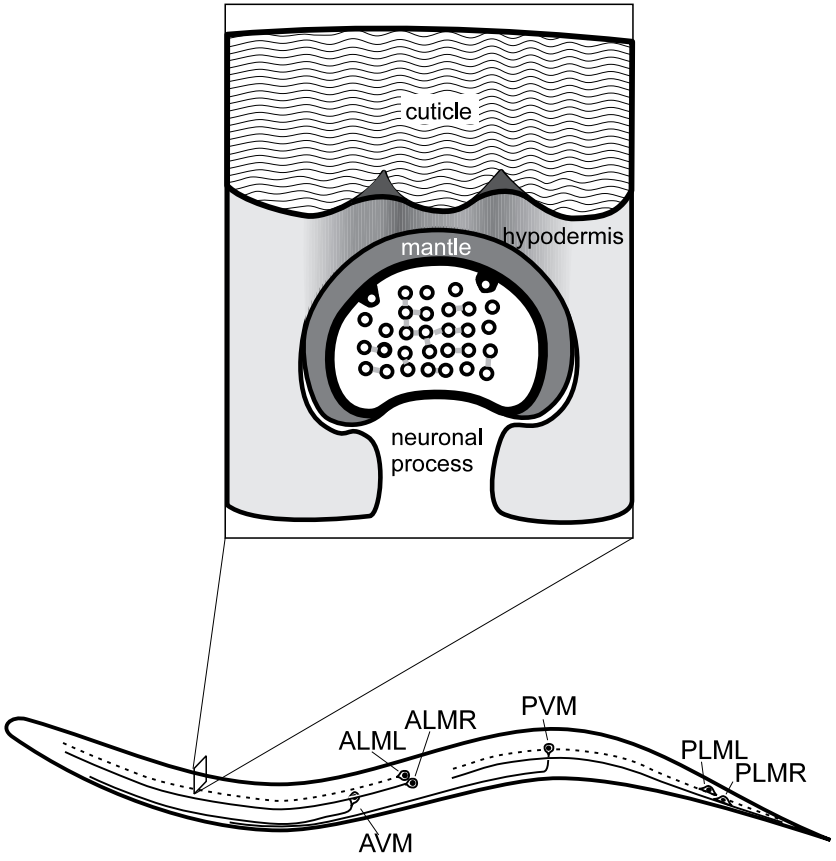


Figure 1 Anatomy of touch receptor neurons in *C. elegans*. (*Bottom*) A set of cells (ALML/R, AVM, PVM, PLML/R, all characterized by their 15-protofilament microtubules) mediates gentle touch through most of the body; because their receptive field corresponds to the extent of their processes, these microtubule cells are probably mechanosensitive throughout, and not just at their terminals. Other touch receptors not shown include certain types of neurons with ciliated endings (ASH, FLP, OLQ, IL1) that respond to touch at the nose, as well as the PVD and other yet unidentified cells that respond to harsher touch stimuli. (*Top*) Cross section through the process of a microtubule touch receptor, based on electron microscopy observations (Chalfie & Sulston 1981; Chalfie & Thomson 1979, 1982). The characteristic large-diameter microtubules appear interconnected with each other and terminate in close association with the membrane. The cuticle contains a darkly staining material that may connect to the extracellular structure known as the mantle through a thin portion of hypodermis, a syncitial layer serving an epidermal role. Thus a continuum exists between the cuticle surrounding the animal, to which touch is applied, and the intracellular microtubules extending along the touch receptor.

lethality were not usually tested for touch insensitivity, so genes needed for both mechanosensation and an additional process may have been missed. However, the screen was so saturated (33 to 63 mutations were identified in some *mec* genes) that a few of the *mec* mutations found may actually only impair the function in touch sensitivity of genes that also have other functions. Indeed, only one to a few mutations were identified in other *mec* genes, perhaps because most mutations in these genes produce additional defects.

Some *mec* genes encode transcriptional regulators needed for the generation and differentiation of the touch cells. MEC-3 is a homeobox-containing transcription factor necessary for the differentiation of the six touch cells and for the activation of at least several (and probably most) of the other *mec* genes. Other genes necessary for the generation of the microtubule cells, such as *unc-86* and *lin-32*, encode transcription factors also needed for the development of other cells (reviewed in Duggan & Chalfie 1995). The *mec-17* gene has not been cloned, but it seems necessary for the maintenance of expression of the *mec* genes activated by MEC-3 (Way & Chalfie 1988). *mec-8* encodes an RNA splicing factor functional in the six touch cells as well as in other sensory neurons and in muscles (Perkins et al 1986, Lundquist & Herman 1994, Lundquist et al 1996). The remaining 12 *mec* genes are not needed for the development of the touch cells but are needed for their function. Since gap junctions between microtubule cells and downstream interneurons are sufficient to mediate the touch response (Chalfie et al 1985), probably few, if any, *mec* genes are needed for events downstream of the mechanoreceptor potential, and the products of the other *mec* genes may constitute the building blocks of a macromolecular mechanosensory structure.

Some *mec* genes encode extracellular matrix components. MEC-5 is a collagen secreted not by the touch cells, but by the hypodermal cells along which the touch cell processes run (Du et al 1996). MEC-9 is secreted by the touch cells; it contains predicted epidermal growth factor (EGF) repeats and Kunitz-like serine-protease inhibitor domains (Du et al 1996). Missense mutations in *mec-9* occur in either type of domain, indicating their importance for MEC-9 function. MEC-9 is unique in that no other known protein contains both motifs. However, agrin, which clusters acetylcholine receptors, also consists of a combination of EGF and serine protease inhibitor repeats (albeit of the different, Kazal type) (Rupp et al 1991). In addition, dendrotoxin (Lucchesi & Moczydlowski 1991) and calcicludine (Schweitz et al 1994), two snake venom peptides that consist of a Kunitz domain, act as inhibitors of voltage-dependent K^+ channels and L-type Ca^{2+} channels, respectively. Therefore, MEC-9 may associate with ion channels, and perhaps even regulate them. *mec-1* has not been cloned, but mutations in this gene eliminate the mantle, which is the extracellular structure apposed to the touch cells (Figure 1); apparently as a result, the neuronal

processes of the touch cells detach from the hypodermis, and the animals are unable to respond to touch (Chalfie & Sulston 1981, Chalfie & Au 1989).

Some *mec* genes encode cytoskeletal elements. MEC-7 is a β-tubulin (Savage et al 1989) and MEC-12 is an α-tubulin (M Hamelin, M Chou & J Culotti, personal communication); both are necessary for the formation of the large-diameter microtubules that are unique to the touch cells (Chalfie & Thomson 1982). These microtubules form bundles that in electron microscopy cross sections appear to be cross-linked. Each microtubule in the bundle is much shorter (about 5%) than the neuronal process, and they seem to run obliquely to the axis of the process instead of parallel to it: Their proximal ends are generally toward the center of the microtubule bundle, and their distal ends are almost always at the periphery, in apparent association with the membrane through a diffusely staining material (Chalfie & Thomson 1979) (Figure 1).

Other *mec* genes encode membrane components. MEC-2 is homologous to the band 7.2b protein of red blood cells, an integral membrane protein also known as stomatin (Hiebl-Dirschmied et al 1991b, Stewart et al 1992). Biochemical experiments demonstrated that band 7.2b has a hydrophobic domain inserted into the membrane, but no portion of the molecule seems to be exposed to the extracellular environment, and both its amino and carboxy tails are cytoplasmic (Hiebl-Dirschmied et al 1991a, Stewart et al 1992). This protein is also associated with the red-blood-cell cytoskeleton, since when the membranes are dissolved with detergents, cytoskeleton extractions contain stomatin (Stewart et al 1992). Localization of a MEC-2–β-galactosidase fusion protein to neuronal processes is variably reduced by mutations in the tubulin coding genes *mec-7* and *mec-12*, suggesting that microtubules may interact, directly or indirectly, with MEC-2 (Huang et al 1995). But it is not clear whether this interaction would be needed for their function as components of a mechanosensory apparatus or merely for the axonal transport of MEC-2.

Stomatin, which is normally expressed in most cell types, is absent from the red blood cells of patients with stomatocitosis, a dominantly inherited disease characterized by abnormally shaped red blood cells with high intracellular sodium and low potassium. This has led to the suggestion that stomatin (and by analogy, MEC-2) regulates ion conductances, possibly through an interaction with ionic transporters or ion channels (Stewart et al 1992, 1993; Huang et al 1995). However, no mutations have been detected in the coding regions of the stomatin gene from patients with stomatocitosis (only exons have been sequenced), and the mRNA is present in their red-blood-cell precursors at normal levels (ruling out regulatory mutations in noncoding regions) (reviewed by Stewart et al 1993). Therefore, the primary defect in stomatocitosis is not the absence of stomatin, so there is little evidence that stomatin (or MEC-2) regulates an ion channel.

MEC-4 and MEC-10 are putative ion channel subunits that have been called degenerins because certain mutations in them cause cells to swell and die. Although there is no proof that degenerins actually form channels, there ought to be little doubt about this. First, they share extensive sequence similarity and overall structure with a superfamily of proteins that includes the three subunits of the mammalian epithelial sodium channel (α-, β-, and γ-ENaC) (Canessa et al 1993, 1994a; Lingueglia et al 1993; Voilley et al 1994) and a FRMFamide peptide–gated sodium channel from the snail *Helix aspersa* (Lingueglia et al 1995). These and other proteins constitute the DEG/ENaC superfamily of ion channels (Corey & García-Añoveros 1996) (Figure 2). The predicted topology of all DEG/ENaC proteins includes two transmembrane domains, intracellular amino and carboxy termini, and a large extracellular loop with two or three cysteine-rich domains. This topology has been demonstrated by protease, glycosidase, and antibody-based studies for the rat α-ENaC (Canessa et al 1994a, Renard et al 1994, Snyder et al 1994) and for MEC-4 (Lai et al 1996). The second transmembrane domain in all these proteins contains a highly conserved region with an P-loop–like structure predicted to line the pore of the channel (Renard et al 1994, García-Añoveros et al 1995).

A second reason to think that these proteins form channels comes from experiments in which the predicted pore-forming domain of α-ENaC was replaced by that of MEC-4. When the chimerical subunit was reconstituted in *Xenopus laevis* oocytes with ENaC β and γ subunits, a channel was formed with pore properties that differed from that of the normal ENaC (Waldmann et al 1995). Similarly Hong & Driscoll (1994) found that a chimera made by replacing MEC-4 with a portion of the predicted pore-forming domain of α-ENaC was functional in touch cells, since animals with null mutations in *mec-4* but expressing the chimera under a *mec-4* promoter were touch sensitive. Thus far there have been no reports of a channel reconstituted with MEC-4 or MEC-10 subunits, probably because an essential third subunit of the channel is missing. By analogy, β- and γ-ENaC do not form functional channels unless they are coexpressed with α-ENaC (Canessa et al 1994b). The third essential subunit of the touch cell channel is likely to be the product of the *mec-6* gene (see below), which has not yet been cloned.

In spite of the inability to study the degenerin channels electrophysiologically, knowledge of the structure and regulation of these proteins has been gained by means of genetic analysis. Rare gain-of-function Deg mutations in *mec-4* (Driscoll & Chalfie 1991) and a similar gene of unknown function, *deg-1* (Chalfie & Wolinsky 1990, García-Añoveros et al 1995), lead to the swelling and eventual death of some or all of the neurons in which they are expressed. These include the six touch receptor neurons in the case of *mec-4* (Herman 1987, Mitani et al 1993) and a different set of cells for *deg-1* (García-Añoveros

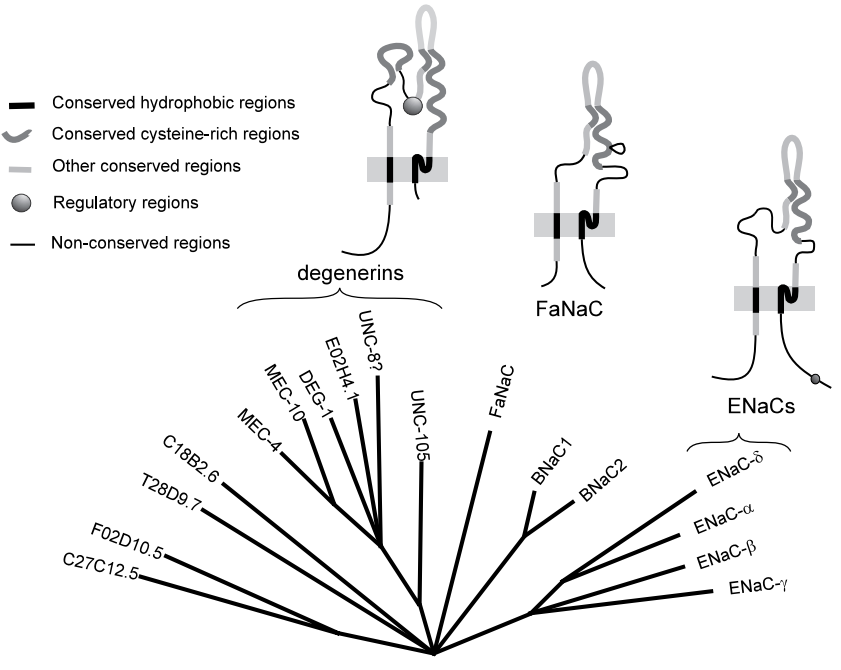


Figure 2 Phylogenetic and structural comparison of members of the DEG/ENaC superfamily of sodium channel subunits. The degenerins, characterized by an extracellular inhibitory region and other sequences (García-Añoveros et al 1995), have only been cloned from nematodes and are equally divergent to all other members of the DEG/ENaC superfamily. MEC-4 and MEC-10 have suspected mechanosensory roles in touch receptor neurons, and UNC-105 may play a similar role in muscle (Liu et al 1996); all other degenerins (DEG-1, E02H4.1, R13A1.4, and C27C12.5) are of unknown function. The amiloride-sensitive epithelial sodium channel subunits (ENaC) have been found in various vertebrate species; their intracellular regulatory site participates in channel retrieval from the membrane (Snyder et al 1995). FaNaC is an amiloride-sensitive FMRFamide-peptide–activated sodium channel found in a mollusk (Lingueglia et al 1995). The mammalian BNaC1 and BNaC2 and the nematode C18B2.6 constitute two new families of the DEG/ENaC superfamily. C27C12.5, R13A1.4, E02H4.1, and C18B2.6 are generic names assigned by the *C. elegans* genome sequencing consortium (Wilson et al 1994). R13A1.4 maps roughly to the same chromosomal region as the gene *unc-8*, which is indeed suspected to encode a degenerin (Shreffler et al 1995). Sequences were aligned with the CLUSTALV program (Higgins et al 1992), and the phylogenetic tree was deduced by comparing the hydrophobic regions (63 amino acids) of each of these proteins with the PAUP program (Swofford 1993). The accession numbers of these sequences are Z69883 (C47C12.5), Z48045 (UNC-105), U40798 (R13A1.4, possibly UNC-8), Z68003 (E02H4.1), L34414 (DEG-1), L25321 (MEC-10), P24612 (MEC-4), P37089 (α-ENaC), U38254 (δ-ENaC), P37090 (β-ENaC), P37091 (γ-ENaC), X92113 (FANaC), U40413 (C18B2.6), (U57352) BNaC1 and BNaC2 (not submitted).

1995). Loss-of-function mutations in either gene do not produce this phenotype. Similar degenerations are produced by gain-of-function mutations that disrupt a pore-lining residue of a *C. elegans* acetylcholine-receptor channel, and these degenerations may be prevented by cholinergic antagonists (Treinin & Chalfie 1995). Therefore, the gain-of-function Deg mutations most likely result in abnormally permeable channels by disrupting gate or pore regions of these proteins (García-Añoveros et al 1995). In fact, many Deg mutations in *deg-1* (García-Añoveros et al 1995, Shrefler et al 1995), *mec-4* (Driscoll & Chalfie 1991), and *mec-10* (Huang & Chalfie 1994) alter an equivalent alanine residue of the predicted pore-forming domain.

All these degenerations require a functional *mec-6* (Chalfie & Wolinsky 1990, Huang & Chalfie 1994, García-Añoveros 1995). The degenerations induced by the gain-of-function mutation in *mec-10* also require a functional *mec-4*, but the reverse is not true (Huang & Chalfie 1994). Lai & Driscoll (C Lai & M Driscoll, personal communication) were able to co-immunoprecipitate MEC-4 and MEC-10 after in vitro translation in the presence of pancreatic microsomes; they demonstrated that indeed these two proteins can interact inserted into a membrane. An interpretation of these results is that although an imperfect channel can be assembled from MEC-6 wild-type and MEC-4 mutant subunits to cause degeneration, a channel must contain MEC-4, MEC-10, and MEC-6 wild-type subunits for the cells to function as touch receptors. The suppression of dominant degeneration-causing mutations in one copy of *deg-1* by a suppressor mutation in the other copy may be explained if the products of both *deg-1* alleles interact (García-Añoveros et al 1995, Shrefler et al 1995). Similar transdominant suppressors have been reported for *mec-4* (Hong & Driscoll 1994) and *mec-10* (Huang & Chalfie 1994). Therefore, the degenerin channel of the microtubule touch cells probably contains at least two subunits of MEC-4 and MEC-10 and one or more subunits of MEC-6.

Since most *mec* genes have been cloned by now, and only *mec-4*, *mec-10*, and possibly *mec-6* seem to encode subunits of a channel, the degenerin channel is very likely the mechanotransducing channel. However, some of the uncloned *mec* genes (such as *mec-14*, *mec-15*, and *mec-18*) may encode yet another channel or additional components of the degenerin channel (such as the non-pore-forming subunits of voltage-gated sodium and potassium channels) (Isom et al 1992, Rettig et al 1994, Scott et al 1994).

A MODEL STRUCTURE FOR MECHANOSENSORY TRANSDUCTION For either *mec-4* or *deg-1*, degenerations can be caused not only by substitution of the alanine in or near the predicted pore, but also by deletion of a region of 9 extracellular residues, or by substitution of one of those residues (an alanine) by larger side-chain amino acids. This suggests that those amino acids form or are part of a sterically constrained inhibitory domain situated in the extracellular

portion of the channel. Since these residues are in a region of 22 amino acids that is conserved among the *C. elegans* degenerins, yet absent from the ENaCs and the FMRFamide-gated channel, they are thought to form a regulatory domain specific for the presumed mechanosensory role of the degenerins (at least in the case of MEC-4 and MEC-10) (García-Añoveros et al 1995). MEC-5, MEC-9, and probably MEC-1 are extracellular components, which fits well with the idea that they may interact, directly or indirectly, with the extracellular portion of the degenerin channel and convey mechanical forces to pull it open.

The *unc-105* gene encodes another member of the degenerin family (Liu et al 1996) (Figure 2). It is expressed in muscle, and it has been proposed to a mechanosensitive channel that regulates contraction. Dominant mutations in this gene are suppressed by rare mutations in *sup-20* (also known as *let-2*), which encodes a type IV basement collagen expressed in muscle (Park and Horvitz 1986, Sibley et al 1993, Liu et al 1996). This suggests that regulation of degenerins by collagens (SUP-20 or MEC-5) may be common.

By analogy to models proposed for vertebrate hair-cell transduction (Pickles et al 1984, Pickles & Corey 1992, Hudspeth & Gillespie 1994), we might expect channels to be attached to both intracellular and extracellular linking proteins that convey force from relatively rigid structures. Using their homology to other proteins as a guide, we can speculate about the functions of the different MEC proteins in such a system (Figure 3). Intracellularly, the bundle of cross-linked microtubules (most likely made up of MEC-12 α-tubulins and MEC-7 β-tubulins) probably forms a rigid structure within the touch cell processes. The stomatin-like MEC-2 protein might link microtubules to channels, which are most likely a heteromultimer of MEC-4, MEC-6, and MEC-10. Genetic interaction experiments suggest that an ion channel includes at least two MEC-4 subunits and at least two MEC-10 subunits. The worm's cuticule and mantle would constitute the opposing rigid structure; they might be connected to an extracellular domain of the channel by proteins associated with the mantle, possibly MEC-1, MEC-5, or MEC-9. There is no evidence to indicate which of these might link directly to the channel. Gentle touch would cause the touch cell microtubule bundles to move relative to the cuticule, increasing tension in the linking proteins and thus tension between different domains of the channel proteins. Similarly, compression of membrane-attached microtubule bundles induces a receptor potential in insect cuticular mechanoreceptors, and Thurm (1983) has proposed that this is the mechanism of sensory stimulation. Because the 15-protofilament microtubules appear to be linked to each other with cross bridges, they are probably compressed not individually but as a bundle. If every microtubule is associated at one end with a channel, several channels can be gated at once, generating a sizable receptor potential. In other mechanosensitive

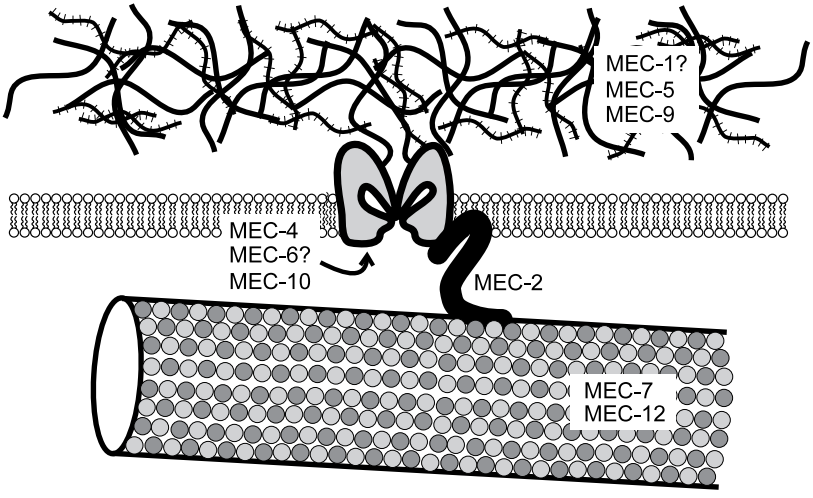


Figure 3 Putative arrangement of MEC proteins as part of a mechanosensory apparatus. Touch-induced displacement of the microtubules (made up of MEC-7 and MEC-12 tubulins) or the extracellular mantle (perhaps composed of MEC-1, MEC-5, and/or MEC-9) with respect to each other would convey mechanical forces to and gate the channel.

cells, a few tens of channels in a cell are sufficient to cause a receptor potential (Howard & Hudspeth 1988, Crawford et al 1991, Denk et al 1995), and a few tens of picoNewtons force is sufficient to open each channel. We estimate the force of a gentle touch stimulus to be 20 μN, so very little of that force needs to reach the channels to produce a response.

CAVEATS This is a tentative model, however, and it is worth considering some limitations in the evidence for it.

With the exception of interactions between MEC-4 and MEC-10, there is little evidence for interactions among the MEC proteins. The dominant enhancements of temperature-sensitive mutations in *mec-4*, *mec-5*, *mec-6*, and *mec-12* by normally recessive mutations in *mec-2*, *mec-4*, *mec-5*, *mec-6*, *mec-7*, *mec-9*, *mec-10*, *mec-12*, *mec-14*, or *mec-15* (Huang et al 1995, Gu et al 1996) are expected for genes that when mutated, produce the same phenotype and that are necessary for the function of the same cells, as all these *mec* genes are. But these interactions are not allele specific, and are not evidence that the products of these genes interact. True allele-specific suppressions rarely occur and have not been reported among *mec* alleles. But perhaps the most conclusive evidence would be obtained by biochemical means, such as the two-hybrid system or co-immunoprecipitation after in vitro translation in the presence of

microsomes [as Lai & Driscoll (C Lai & M Driscoll, personal communication) have done to prove an interaction between MEC-4 and MEC-10].

The MEC proteins may play alternative, less direct roles in mechanotransduction. The microtubules may not attach to the channel at all, but instead may provide resistance to the stressed neuronal process. The channel composed of MEC-4 and MEC-10 subunits may have a nonsensory role in touch cell function. Alternatively, as Kernan & Zuker (1995) suggested, it may contribute to a unique extracellular ionic composition such as that necessary for mechanosensory transduction by hair cells (Kuijpers & Bontig 1970) and insect bristles (Grünert & Gnatzy 1987). However, some of the microtubule touch cell processes run in the ventral cord adjacent to many other neurons, and therefore, they do not appear to require a specialized extracellular environment. In general, any of the *mec* genes may not be needed for the generation of the mechanoreceptor potential, but they may be needed, for example, for downstream events such as the transmission of this signal to interneurons. Since whole-cell patch-clamp recordings of *C. elegans* neurons have recently been obtained (Lockery 1994, Avery et al 1995), some of these questions may be resolved in the near future.

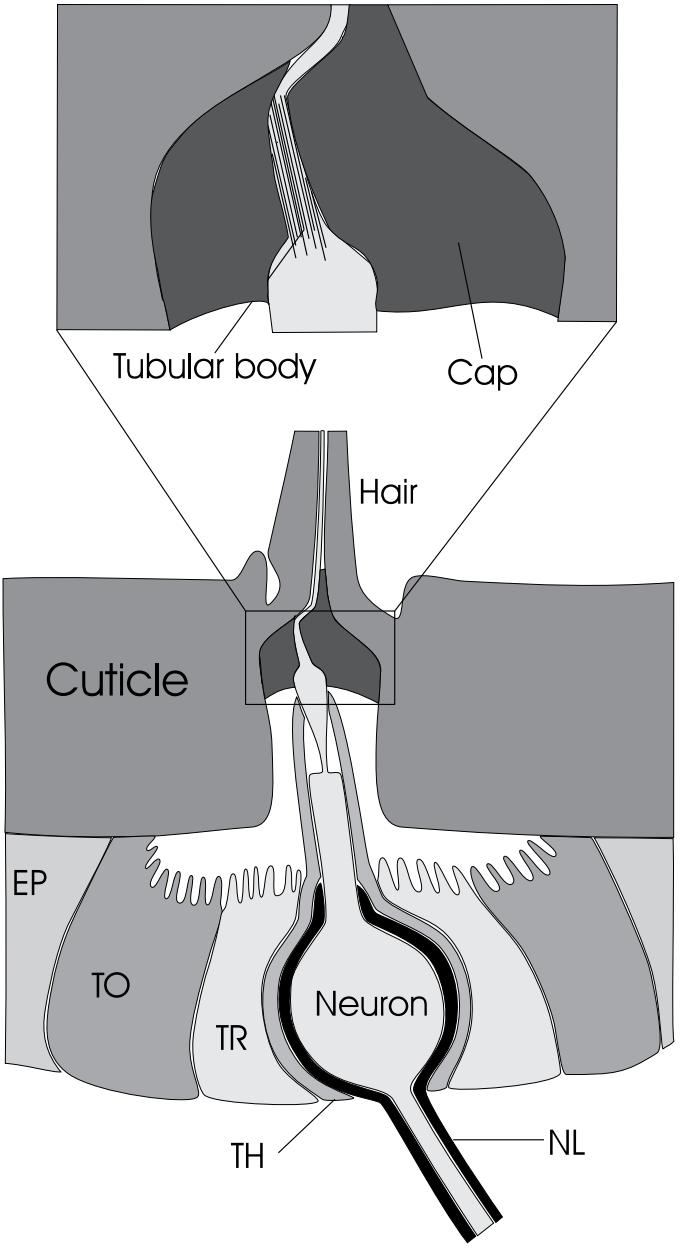
Finally, any model may be altered once the sequences of the uncloned *mec* genes (notably *mec-6*, *mec-14*, *mec-15*, and *mec-18*) are reported. Even then not all the elements of the mechanosensory apparatus may be known because the behavioral screens for Mec mutants (Chalfie & Sulston 1981, Chalfie & Au 1986) avoided animals with additional phenotypes, so genes necessary for both mechanotransduction and another process may have been missed. Although the proposed function of the MEC proteins in mechanotransduction remains tentative at best, the *C. elegans* system has been remarkably productive in identifying likely components of a mechanosensory complex.

Other Genetic Approaches to the Study of Touch

CILIATED MECHANOSENSORY NEURONS OF *C. ELEGANS* Some of the difficulties in interpreting the function of the *mec* genes have been addressed in more recently designed screens for touch-insensitive animals. A group of four types of sensory neurons with ciliated endings (ASH, FLP, OLQ, and IL1) mediate the response to touch applied to the most anterior part of *C. elegans*, the nose. Nose-touch mutants are nevertheless sensitive to touch throughout the rest of the animal; conversely, Mec mutants are nose-touch sensitive but otherwise touch insensitive. Two of these cells, the ASH pair of neurons, are polymodal receptors required not only for nose-touch sensitivity but also for withdrawal in response to osmotic shock, garlic, and volatile repellents (Bargman et al 1990, Kaplan & Horvitz 1993). Hart & Kaplan (J Kaplan, personal communication; Hart et al 1995) have identified mutations that render the animals nose-touch insensitive (the Not phenotype), yet animals with mutations in several *not* genes still respond to osmotic stimuli. Conversely, mutations in several of the *osm*

genes eliminate osmotic avoidance but spare nose-touch sensitivity. Therefore, these *osm* and *not* genes are specifically needed for one or the other form of sensitivity (osmotic shock may or may not be a mechanical sense), but not for the normal physiology of the ciliated ASH cell. Despite the logical clarity of the approach, Hart et al (1995) and Maricq et al (1995) found that *not-3* (now renamed *glr-1*) encodes a postsynaptic glutamate receptor that apparently receives glutaminergic transmission from the touch cell but does not participate in touch transduction. Other *not* and *osm* genes may very well encode components of mechanosensory apparatuses. Perhaps the main drawback in the genetic dissection of nose touch sensitivity is that wild-type animals do not always respond to nose touch, and mutant animals sometimes do. Therefore, animals have to be tested multiple times to come up with a significant phenotypic assessment, making the genetic analysis tedious (Kaplan & Horvitz 1993; J Kaplan, personal communication). Although *mec*-3 (Way & Chalfie 1989), *mec-7* (Hamelin et al 1992), and *mec-10* (Huang & Chalfie 1994) are expressed in the FLPs, none of the remaining ciliated mechanosensory neurons express any of the *mec* genes whose pattern of expression has been determined, and no mutations have been reported that impair both nose- and body-touch sensitivity. Therefore, the molecular components of each type of receptor are different, underscoring the importance of identifying genes needed for mechanotransduction in several cell types and in more than one organism.

INSECT MECHANOSENSORY BRISTLES Insects also contain cuticular mechanoreceptors that have a cilium at their sensory dendrite. The ciliary tips, known as the tubular bodies, are embedded in the cuticle and contain bundles of microtubules that are connected to integral membrane structures, probably proteins. Thus there appears to be a mechanical connection from the microtubules to the cuticle. Different cuticular accessory structures such as hairs or bristles occur in different sensory organs. Each of these external bristles is innervated by a single sensory neuron whose nerve ending is enclosed by the socket- and sheath-supporting cells (McIver 1985) (Figure 4). The bristle and socket cells secrete a potassium-rich receptor lymph (Grünert & Gnatzy 1987) that results in a transepithelial potential difference, measurable with extracellular electrodes situated in the apical and basal sides of the organ (Thurm & Kuppers 1980). Upon deflection of the bristle in one direction, the tubular body at the tip of the cilium is compressed. This results in a decrease of the transepithelial potential, which most probably corresponds to an increase in current entering the receptor cell. Deflection of the bristle in the opposite orientation apparently results in a decrease in current (Thurm 1983). The receptor potential can be detected less than 100 μs after the deflection (Thurm 1983); as has been argued for vertebrate hair cells (Corey & Hudspeth 1983), transduction probably does not involve a slow second messenger pathway but most likely results from the


Tubular body
Cap
Hair
Cuticle
EP
TO
TR
Neuron
TH
NL

direct mechanical gating of channels. Some of these findings, originally obtained in the cricket *Acheta domesticus* and in other insects, have been recently reproduced in the fly *Drosophila melanogaster* (Kernan et al 1994; R Walker & C Zuker, personal communication).

Deflection of sensory hairs or bristles in larvae and adult flies elicits a withdrawal response (in the larvae) or a scratch reflex (in the fly). Kernan et al (1994) found that mutations in the *unc* (*uncoordinated*) and *uncl* (*uncoordinated like*) genes result in bristle dysfunction: While *unc* and *uncl* larvae are touch insensitive but viable, adults are severely uncoordinated and often die, probably because some of the bristles in the joints of the legs and in the neck act as external proprioceptors. Thus they (Kernan et al 1994) screened for adult lethals that resulted from severe uncoordination (a more efficient screen than the one for touch-insensitive larvae they previously performed). In order to avoid regulatory genes needed for the generation of the bristle structures, such as those in the *achaete-scute* complex (Villares & Cabrera 1987), mutant animals with no bristles were discarded. As the screen nears saturation (only the II chromosome is not saturated, although mutations in it have been characterized), about half of the more than 40 mutants display abnormal mechanosensory behavior with wild-type mechanoreceptor potential (*ump*, for *uncoordinated with mechanoreceptor potential*); they probably disrupt genes needed for downstream events such as synapse formation or function. Two mutations eliminated the transepithelial potential and are likely to disrupt the supporting cells that generate the potassium-rich receptor lymph. However, over 20 mutations in 14 genes produced animals with bristles that appear wild type and with normal transepithelial potentials but no mechanoreceptor potentials (*nomp* mutations). These 14 genes probably encode the building blocks of a mechanosensory structure (Kernan et al 1994; C Zuker, personal communication).

HEARING AND BALANCE: MOLECULES INVOLVED IN VERTEBRATE HAIR CELL FUNCTION

Mechanotransduction by Inner-Ear Hair Cells

The most detailed morphological and physiological understanding of a mechanosensory system has come from vertebrate hair cells. These are the receptor

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Figure 4 Anatomy of an insect mechanosensory bristle (after Thurm 1964, Thurm & Küppers 1980). Deflection of the hair compresses the cap, a specialized portion of the cuticle, which in turn compresses the tubular body (the nerve ending rich in small-diameter microtubules) and elicits a mechanoreceptor potential. The tormogen (TO), trichogen (TR), and thecogen (TH) are supporting cells derived from an otherwise unspecialized epidermal layer (EP). The neurilemma (NL) is a glial sheath cell that surrounds the axon of the mechanoreceptor neuron.

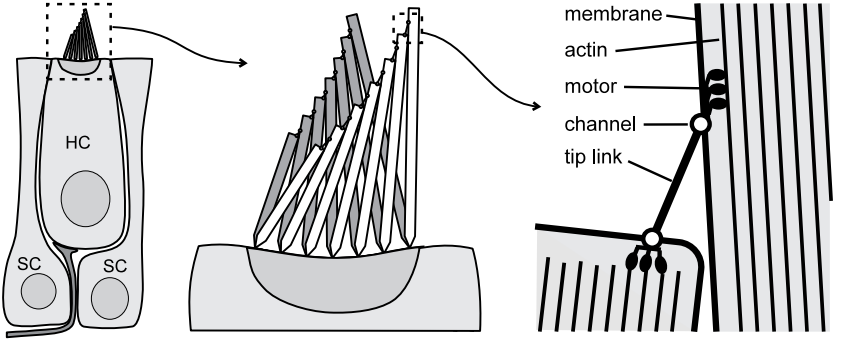

Figure 5 Hair cell structure. (*Left*) The sensory epithelium is composed of hair cells (HC) and supporting cells (SC). Nerve fibers contact hair cells on their basolateral surfaces. (*Center*) Stereocilia of the mechanosensory hair bundle are rigid rods that pivot at their bases when the bundle is deflected but still touch at their tips. A positive deflection is towards the right; it would stretch tip links. (*Right*) Hypothesized elements of the mechanotransduction complex. The membrane, actin filaments, and tip link are clearly visible in electron micrographs; other elements are inferred from the physiology.

cells of both the auditory and vestibular systems and occur in specialized sensory epithelia of the inner ear (Figure 5, *left*). These epithelia separate fluids of markedly different ionic composition (a high-K^+ endolymph and normal extracellular perilymph). Hair cells do not have axons, but they release neurotransmitter onto fibers of the eighth cranial nerve. The mechanosensory organelle of the hair cell is a bundle of cilia protruding from the apical (lumenal) surface of the cell (Figure 5, *center*). In nearly all hair-cell organs, this hair bundle is attached to a secreted extracellular structure (the tectorial membrane of the cochlea or basilar papilla, the otolithic membrane of saccule and utricle, or the cupula of the semicircular canal). In different organs, different physical stimuli cause movements of these accessory structures, but in each case they result in deflection of the hair bundle.

The bundle is composed of two types of cilia: 30–300 stereocilia, which have the actin core characteristic of microvilli, and a single kinocilium, which has the $9 + 2$ arrangement of microtubules characteristic of motile cilia. (The kinocilium is lost in mature hair cells of the mammalian cochlea.) Hair bundles range in height from about 2 μm to about 60 μm, but are typically 4–10 μm. Stereocilia are usually 300–400 nm in diameter, and taper to $\sim$100 nm at their bases. Where the bases insert into the apical surface of the cell, they are anchored in the cuticular plate, a dense meshwork of filaments. In all organs, the stereocilia are graded in height along the axis of symmetry, such that each row of stereocilia is 0.5–1.0 μm taller than the previous row, and the kinocilium is

adjacent to the tallest row. Stereocilia are joined by three or more extracellular filamentous structures: *Ankle links* run between all adjacent stereocilia at the level of the tapers (Csukas et al 1987, Richardson et al 1990, Jacobs & Hudspeth 1990). *Shaft links* (that may be the same material) run between stereocilia along their whole length in hair cells of some organs (Richardson et al 1990). *Upper cross links* run laterally between the tips of adjacent stereocilia, apparently holding them in close apposition. In contrast to these links, which laterally connect all adjacent stereocilia, there is a set of *tip links*, which run only parallel to the bundle axis (Figure 5, *right*). Tip links extend upwards from the tip of each stereocilium to the side of the next taller adjacent stereocilium; they are about 150 nm long and 5 nm thick, and in some cases appear to branch at their upper insertions (Pickles et al 1984). At the upper end of the tip link, a dense insertional plaque of 30–50 nm diameter lies between the membrane and the core of the stereocilium (Furness & Hackney 1985, Jacobs & Hudspeth 1990).

The physiological response to bundle deflection has been extensively studied. Deflection of the bundle towards the tallest stereocilia (defined as positive) opens ion channels, increasing inward current and depolarizing the hair cell (Hudspeth & Corey 1977). These transduction channels have a resting open probability of 10–20%, so some current flows into the cell at rest; a negative deflection closes all channels and hyperpolarizes the cell. Deflections perpendicular to the bundle axis have no effect (Shotwell et al 1981). The transduction channels are nonselective cation channels, passing most cations up to $\sim$0.7 nm diameter (predominantly K^+ in vivo but including Ca^{2+}) (Corey & Hudspeth 1979, Ohmori 1985). The single-channel conductance is apparently 90–100 pS (Crawford et al 1991, Denk et al 1995). A number of studies have indicated that transduction channels are located at or near the tips of the stereocilia (Hudspeth 1982, Jaramillo & Hudspeth 1991, Denk et al 1995, Lumpkin & Hudspeth 1995). Channels open within microseconds following positive bundle deflection (Corey & Hudspeth 1983, Crawford et al 1989), suggesting that the mechanical stimulus does not act via a second messenger but is communicated directly to an ion channel that is itself mechanically sensitive. These features of the hair cell response suggested a gating spring model for transduction, in which positive bundle deflection causes some elastic link to be stretched, increasing tension on the channel protein, which in turn promotes the conformational change of channel opening (Corey & Hudspeth 1983). Strong support for this model came from direct observation of changes in bundle stiffness associated with channel opening, and movement of the bundle associated with channel blockade (Howard & Hudspeth 1988, Denk et al 1992). Stiffness measurements, adjusted by a recent study on the number of channels per gating spring, suggested that the channel moves by $\sim$2 nm in opening (Howard & Hudspeth 1988, Denk et al 1995).

The discovery of the tip links and the localization of the transduction channels provided a specific correlation between physiologically and morphologically defined elements (Pickles et al 1984). This tip-links hypothesis proposed that the tip links are the gating springs and that they are connected directly to the transduction channels. A positive deflection would cause the tip links to be stretched, pulling on the channels to open them, whereas a negative deflection would relax the tip links and allow channels to close (Pickles et al 1984). This model is consistent with nearly all structural and physiological observations and is further supported by the finding that treatment with low Ca^{2+} simultaneously abolishes tip links and mechanical sensitivity (Assad et al 1991). A recent study, which used a calcium indicator dye to assess the presence of channels in individual stereocilia, concluded that channels are most likely positioned at both the upper and lower ends of tip links (Denk et al 1995).

Hair cells also have an adaptation mechanism, which apparently acts by continuously adjusting tension in the tip links. Channel opening caused by a maintained positive deflection is followed by channel closure over a period of 20–100 ms, and closure caused by maintained negative deflection is followed by reopening (Eatock et al 1987). Details of the physiology are consistent with an adaptation mechanism that works by moving the attachment point of the gating spring without changing its stiffness (Howard & Hudspeth 1987, 1988; Hacohen et al 1989). Adaptation occurs more rapidly when the Ca^{2+} concentration inside the tips of the stereocilia is elevated (Assad et al 1989, Crawford et al 1989), both localizing the mechanism and suggesting a Ca^{2+} regulatory element. In addition, manipulations of intracellular Ca^{2+} that change both the rate of adaptation and the open probability of channels also cause movement of unrestrained hair bundles, in further support of a mechanical basis of adaptation (Assad & Corey 1992).

Howard & Hudspeth (1987) proposed a specific structural model for adaptation, in which the membrane channel at the upper end of each tip link is attached to an intracellular molecular motor that can move along the side of the stereocilium. The motor is oriented on its substrate such that it always tends to climb upwards (dragging along the channel and tensioning the tip link). Positive deflections increase tip-link tension and open channels but cause the motor to slip down and reduce tension; negative deflections reduce tension and allow channels to close but also allow the motor to climb to restore tension. At rest, a balance between the intrinsic climbing and the tension-dependent slipping sets a resting tension that keeps channels open $\sim$10% of the time (Howard & Hudspeth 1987). Physiological details of the adaptation indicate that the motor may also be attached to the core by an additional extent spring of about one quarter the stiffness of the tip link, and that a stop limits the climbing to about 60 nm from rest (Shepherd & Corey 1994). Morphological support for this

adaptation model has been difficult to obtain, but preliminary observations are at least consistent with it (Shepherd et al 1991).

Molecular Identification of Transduction Elements

A structural picture of the transduction apparatus has evolved from these experiments that is persuasive although certainly not definitive (Figure 5, *right*): In this model, the bundle of crosslinked filaments constituting the stereocilium core provides a rigid structure for transmission of force. Bound to the core at the upper end of the tip link is a cluster of motor molecules, perhaps a few dozen in number, that constitutes the insertional plaque (Hudspeth & Gillespie 1994, Gillespie 1995). Each motor is associated with Ca^{2+}-dependent regulatory molecules. Also bound to the filamentous core is the extent spring linked to the motor complex, and the stop element. It seems unlikely that a few dozen motor molecules could all be attached to one channel protein, so there may be an intervening motor-channel linker protein. The transduction channel spans the membrane, and attaches to the tip link at an extracellular domain. At the lower end of the tip link, another channel spans the membrane at the top of the next lower stereocilium and attaches to another intracellular linker. For symmetry, we suggest that the channel linker at the lower end is bound to the same motor molecules, but we expect that these do not move because they are already at the end of the core filaments.

What is the molecular identity of these elements? Despite the development of ingenious methods for isolation of stereocilia (Shepherd et al 1989, Gillespie & Hudspeth 1991), the paucity of material has made biochemical identification extremely difficult. Use of antibodies directed against candidate proteins has provided additional information, so some progress has been made.

The core filaments have been identified as actin by a variety of methods, including labeling with the S1 fragment of myosin, phalloidin, and anti-actin antibodies (Tilney et al 1980, Flock et al 1981, Sobin & Flock 1983, Shepherd et al 1989). Amplification by polymerase chain reaction (PCR) of cDNAs for different actin isoforms detected β actin mRNA in hair cells (Pickles 1993). Actin filaments in stereocilia are also crosslinked at regular intervals (Tilney et al 1980, 1983). These cross bridges are likely to be fimbrin, since immunocytochemistry and immunoblots have demonstrated the presence of fimbrin in stereocilia (Sobin & Flock 1983, Tilney et al 1989, Shepherd et al 1989).

Myosin has long been an attractive candidate for the adaptation motor (Howard & Hudspeth 1987, Hacohen et al 1989). Myosins are the only proteins known to move on actin. Actin in stereocilia is polarized such that myosin climbs upwards during the power stroke (Tilney et al 1980), in the direction required for the adaptation model. When stereocilia are isolated from bullfrog hair cells and demembranated with detergent to expose the actin cores, beads coated with

chicken muscle myosin move upwards along them, at a speed appropriate for adaptation (1–2 μm/s) (Shepherd et al 1990). Adaptation was blocked by the introduction into the hair-cell cytoplasm of ADPβS, suggesting the involvement of myosin or a similar ATPase (Gillespie & Hudspeth 1993). Phosphate analogs that are well-characterized inhibitors of myosins, such as vanadate, beryllium fluoride, and aluminum fluoride, also inhibit adaption (Yamoah & Gillespie 1996).

The myosin superfamily comprises at least 14 branches, with most branches including multiple members (Cheney et al 1993). Which of these might be the adaptation motor? Solc et al (1994) used PCR to amplify cDNAs representing all or most of the myosins expressed by bullfrog hair cells and supporting cells. They found ten different myosins, some occurring in multiple splice forms, that represent six different branches. The difficulty with this approach is that narrowing the group to a single candidate requires antibody probes specific to each isoform. Gillespie et al (1993) labeled putative myosins in stereocilia by vanadate trapping of radiolabeled uridine triphosphate (UTP); they identified proteins of 120, 160, and 230 kDa. Although this narrows the choices, it does not provide molecular identification of the bands. Fortunately, Gillespie et al (1993) found that antibodies raised against bovine brain myosin Iβ labeled the 120-kDa myosin candidate and also labeled the tips of stereocilia in bullfrog. The amount of myosin Iβ in each stereocilium was estimated to be 130–150 molecules (Gillespie et al 1993, Walker & Hudspeth 1996). The full-length myosin Iβ from bullfrog saccule has now been cloned (Solc et al 1994, Metcalf et al 1994). Antibodies raised against the tail domain of bullfrog myosin Iβ also label the tips of stereocilia (Corey et al 1996). Although intriguing, these results do not prove that myosin Iβ is the motor. Indeed, two other myosins identified in the PCR screen also occur in stereocilia: Myosin VI, first identified in pig kidney cells, is the gene defective in a deaf mouse mutant, *Snell's waltzer* (Avraham et al 1995). Antibodies to myosin VI label stereocilia (although without localization to the tips) and recognize the 160-kDa band in isolated frog stereocilia (Corey et al 1996). Myosin VIIa, first identified in pig kidney and human testis (Bement et al 1994, Hasson et al 1995), is the gene defective in both a deaf mouse mutant, *shaker-1*, and in the human inherited deafness, Usher syndrome 1B (Gibson et al 1995, Weil et al 1995). Antibodies to myosin VIIa label stereocilia and recognize the 230-kDa band in isolated stereocilia (Corey et al 1996). Although myosin Iβ remains the best candidate, definitive identification of the motor will require localization with electron microscopic resolution and demonstration of physiological effects on adaptation by using probes specific for this myosin isoform.

Myosin Iβ from both frog and cow contains three IQ domains, which occur in all myosins and are thought to bind regulatory light chains, in many cases calmodulin (Cheney and Mooseker 1992). Calmodulin occurs in stereocilia,

especially at the tips at a concentration in excess of 100 μM (Shepherd et al 1989, Walker et al 1993). Myosin Iβ in stereocilia has been shown by a gel overlay method to bind calmodulin in a Ca^{2+}-dependent manner (Walker et al 1993, Walker & Hudspeth 1996), and recombinant cow myosin Iβ binds up to three calmodulin molecules (Zhu & Ikebe 1996). Importantly, calmodulin inhibitors block the adaptation process (Walker & Hudspeth 1996). Whichever myosin is the motor, it seems likely that calmodulin mediates its Ca^{2+} regulation.

There are presently few hints as to the identities of the motor-channel linker, the extent spring, or the adaptation stop. Some proteins have been identified that bind intracellular domains of ion channels; these include PSD95 (certain K^+ channels and NMDA receptors) (Kim et al 1995), gephyrin (glycine receptors) (Prior et al 1992), and rapsyn (acetylcholine receptors) (Frail et al 1988). The diversity of such proteins makes it difficult to guess about a transduction channel linker.

The tip link is most likely a glycoprotein; it is unaffected by detergent treatment (Assad et al 1991). Based on its dimensions, it is probably in excess of 1000 kDa if it is a single protein. Physiological experiments suggest that it can stretch to three times its rest length without breaking (Shepherd & Corey 1994). Proteins with similar size and elasticity have been described, such as titin in muscle (see Keller 1995, for review); otherwise there is little to suggest the nature of the tip link.

The transduction channel, perhaps the most interesting of these proteins, may be the most obscure. Its conductance and ionic selectivity are known, but these features offer few clues, since they are not generally conserved among members of an ion channel family. At present, there are no high-affinity ligands, no antibodies, and no sequence information for the channel. Hackney & Furness (1992) have suggested that this channel may be related to the amiloride-sensitive sodium channel of epithelia (ENaC, mentioned above), since amiloride blocks the hair-cell channel, and an antibody to ENaCs binds stereocilia near the tips. This seems unlikely: First, the order of potency of amiloride analogs differs between ENaCs and hair cells, and amiloride is not a particularly specific blocker of transduction channels (Rusch et al 1994). Second, the antibody labeled the point of contact between stereocilia rather than either end of the tip link, which implies a model that is inconsistent with the directional sensitivity of transduction. In the case of the transduction channel, a candidate protein approach is not promising. The channel may eventually be identified as a binding protein for a motor or tip-link protein or as the product of a deafness gene.

CONCLUDING REMARKS

The large number of genes needed for the function of the *C. elegans* microtubule cells (up to 12 *mec* genes, and probably some other genes) and the

fly bristles (more than 14 *nomp* genes), and the morphological and physiological complexity of hair-cell transduction, are consistent with the idea that mechanosensory structures are composed of a complex assembly of proteins. The bacterial channel, which apparently functions alone, thus seems fundamentally different from channels involved in eukaryotic mechanosensation. Future cloning and sequencing of *not, unc, norp,* and the remaining *mec* genes will reveal whether the various nematode and fly touch receptor neurons use similar genes, and whether homologues of any of these genes are used by the mechanoreceptor cells of other organisms, notably vertebrates. These homologues would be good candidate genes for certain inherited peripheral sensory neuropathies (Dyck et al 1993).

The evidence thus far, however, does not point toward a single conserved apparatus for mechanosensation. For example, large-diameter microtubules occur in certain *C. elegans* touch cell processes, small-diameter microtubules fill the insect ciliary endings (Thurm 1964), and actin fills the hair-cell stereocilia and the vertebrate Pacinian corpuscle sensory endings (Flock et al 1982, Iggo & Andres 1982). Homologues of the mechanosensitive channel in bacteria, *mscL*, have not been found in eukaryotes, and close homologues of the putative channel subunits in worms, MEC-4 and MEC-10, have not been found in vertebrates (Figure 2). Perhaps mechanosensation has evolved multiple times. It is easy to imagine why.

The detection of touch, osmotic gradients, cell swelling, position of body parts, movements, accelerations, gravity, sound, and other vibrations all involve the transduction of mechanical forces into a cellular signal. Mechanotransduction is at once so varied and so important that its acquisition has posed strong selective pressures in multiple evolutionary scenarios. One can easily envision how a mechanosensory channel might evolve from another kind of channel. Attachment of a cytoskeletal or extracellular linkage to a voltage sensor domain or a ligand-binding site could turn a voltage-gated, ligand-gated, or second-messenger-gated channel into a mechanically gated channel. Most channels already have attachments to cytoskeletal or extracellular matrix components and could be adapted to a mechanotransducing role. Indeed, the response of the NMDA receptor to agonist is modulated by pressure (Paoletti & Ascher 1994), and a hyperpolarization-activated channel from smooth muscle can also be activated by stretch (Hisada et al 1991). Degenerins belong to a protein superfamily of which other members are not thought to be mechanically sensitive: It includes constitutively active channels with attachments to the cytoskeleton (Smith et al 1991, Rotin et al 1994) and a ligand-gated channel (Lingueglia et al 1995). The acquisition of the extracellular regulatory domain that characterizes degenerins may have conferred upon them the ability to be mechanically gated (García-Añoveros et al 1995). It is therefore important to identify independently

the molecular components of mechanosensors acting in different cell types and organisms. Despite molecular heterogeneity, different mechanosensory systems may employ common structural and biophysical principles, and these can guide a search for specific molecules.

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