Mechanical systems biology of *C. elegans* touch sensation

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The sense of touch informs us of the physical properties of our surroundings and is a critical aspect of communication. Before touches are perceived, mechanical signals are transmitted quickly and reliably from the skin's surface to mechano-electrical transduction channels embedded within specialized sensory neurons. We are just beginning to understand how soft tissues participate in force transmission and how they are deformed. Here, we review empirical and theoretical studies of single molecules and molecular ensembles thought to be involved in mechanotransmission and apply the concepts emerging from this work to the sense of touch. We focus on the nematode *Caenorhabditis elegans* as a well-studied model for touch sensation in which mechanics can be studied on the molecular, cellular, and systems level. Finally, we conclude that force transmission is an emergent property of macromolecular cellular structures that mutually stabilize one another.

Keywords:
Caenorhabditis elegans; cytoskeleton; force transmission; mechanics; mechanosensation; tension; touch

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Abbreviations:
ECM, extracellular matrix; **MeT,** mechano-electrical transduction; **MS,** mechanosensitive; **MT,** microtubule; **TRN,** touch receptor neuron.

Introduction

The way we experience our environment and each other is deeply influenced by the body's ability to detect and respond to mechanical stimuli. The sense of touch provides immediate and intuitive access to physical properties of objects and our bodies such as density, texture, and shape [1, 2]. The perception of sound underlies language and allows us to enjoy many facets of music, from the deep grooves of house music to the high pitches of a soprano's aria. Less obviously, we also rely on mechanical cues for every beat of our heart [3, 4].

The flow of mechanical signals is influenced by the physical properties of the intervening materials. Just as the seismic waves of an earthquake travel faster on land than in water, mechanical energy travels faster through bone than through interstitial fluid. The process that links touching to feeling is no different: forces applied to the skin surface are transmitted through millimeters of tissue before reaching mechano-electrical transduction (MeT) channels that convert mechanical signals into electrical ones. Molecules [5], cells [6], tissues [7], and whole animals [8] all deform in response to externally applied forces. Importantly, any living structure that deforms under force could, in principle, be mechanosensitive (MS) [9]. The extent and dynamics of the deformation depends on constitutive material properties, such as elasticity and isometric tension. In general, stiff structures deform less than soft ones subjected to the same force, and tense structures propagate mechanical stimuli farther than relaxed ones.

Here, we review concepts of force propagation along cytoskeletal filaments and suggest a framework for understanding how mechanical loads applied to the skin might be transferred to MeT channels that decorate mechanoreceptor neurons. The approach we propose combines our understanding of the biophysical mechanisms of force transmission within and between living cells and our knowledge of the physics and physiology of touch sensation in the nematode *C. elegans*. Both arenas have been covered separately in several excellent reviews [9–13]. Here, we bring them together to develop an understanding of how the mechanical loads delivered in a touch result in neural responses.

Cytoskeleton mechanics affect mechanical signal transmission

The actin cytoskeleton experiences mechanical tension [14] generated by myosin contraction [6, 15], which is counterbalanced by structures that include microtubules (MTs) [16, 17], anchoring to the extracellular matrix (ECM) [11] or the osmotic pressure of the cytoplasm [18]. This preexisting mechanical tension has been proposed to help convey mechanical signals over long distances [13]. Examples include force transfer from the membrane to the nucleus [19], which elicits changes in gene expression and/or nucleolar organization [20, 21], and *src* kinase activation at cellular sites distant from the location of the applied force [22, 23], which initiates phosphorylation of kinase targets.

Mechanical pre-stress in the actin cytoskeleton plays a central role in transmitting force between physically distant parts of the cell [21, 22, 24–26]. Similar to the string in a tin can telephone, a cytoskeletal element under tension transmits mechanical deformation faster and further than a relaxed one [27]. Put differently, if the string is completely slack, then no mechanical energy can be transported along its length. In support of this idea, experimental manipulations that decrease actin tension or destroy actin stress fibers impair force propagation in cells [22, 25].

Theoretical modeling of cellular force propagation along cytoskeletal filaments has suggested that the bending rigidity, visco-elasticity, and pre-stress of the fiber as well as cytosolic viscous damping influence force transmission [12–14, 24–28]. For instance, when force is applied transversely (perpendicular to the direction of the fiber) [25, 27, 28], the fiber bends and is slightly stretched (Fig. 1A). The bending mode and resulting deformation depends on how the fiber ends are coupled to the boundaries (Fig. 1B). Due to the low flexural rigidity of both actin and MTs, bending has a minor contribution to the restoring force after deformation. As a result, the extent and rate of the propagation of this perturbation increases dramatically with pre-stress [27, 28]. In contrast, when a stimulus is applied longitudinally (along the fiber), the propagation speed is independent of the tension, but depends instead on the filament's elastic modulus [27, 28]. The distinction between these different modes of force application may have important biological consequences. For example, during touch sensation in *C. elegans*, forces are applied transversely to the long (anterior-posterior) axis of sensory neurites and such stimuli are known to activate MeT channels in vivo [29]. Consequently, pre-stress in the neurite might be necessary to facility fast long-range forces transmission to induce a behavioral response. It is conceivable, however, that biological materials do not experience purely transverse or axial deformations during mechanical stimulations, suggesting that both elasticity and tension can greatly enhance the spread of a mechanical signal through cells or tissues.

Whereas actin fibers and networks are the predominant cytoskeletal structures that provide shape and rigidity, other components, notably MTs, endow specific cell types with more specialized mechanical properties. MTs resist axial loads and experience pure compression up to a critical limit [16, 30]. This limit depends on MT length, L, and its flexural rigidity (or bending modulus), κ_{MT}. Beyond this limit, the MT will buckle into an arc with a characteristic wavelength $\lambda = 2L$, because the total deformation energy is smaller if the rod is bent. This behavior arises as a consequence of the high-compression and

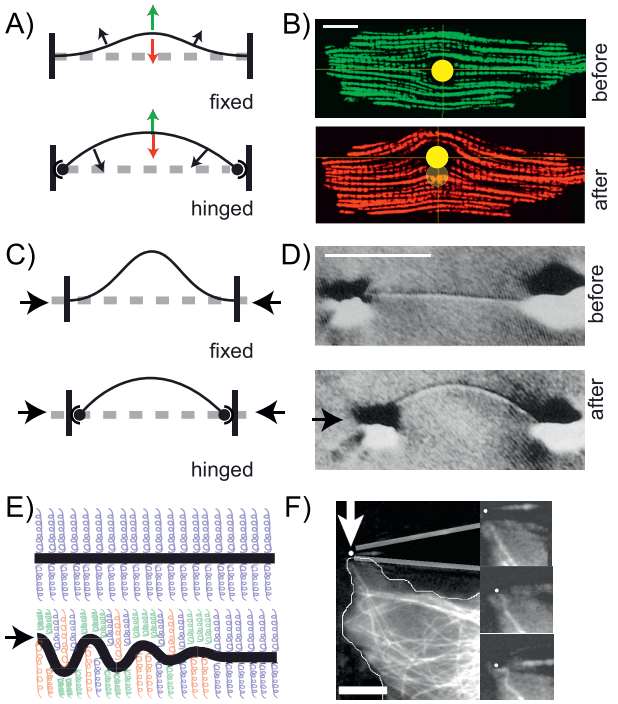

Figure 1. Force transmission along cellular filaments. **A:** A slender rod, e.g. a hypothetical cytoskeletal fiber, with fixed (upper schematic) and hinged ends that are free to pivot (lower schematic) is subjected to a transverse point load (green arrow). Red arrows indicate the restoring force due to pre-stress acting on the deformed fiber (direction of signal propagation). Black arrows indicate the restoring force due to bending elasticity. The two boundary conditions determine the relative contributions of pre-stress and bending modulus to the force transmission properties of the fiber. **B:** Cytoskeletal filaments are subjected to a transverse deformation in cultured cardiac myocytes. A thin glass pipette in the middle of the cell is moved up and down while imaging cytoskeleton-associated mitochondria. Reproduced from Dyachenko et al. [118]. **C:** Classical Euler buckling of an axially compressed rod. The ends are fixed in the upper image and free to pivot below. The energy of deformation is dominated by the bending rigidity of the rod. **D:** A single microtubule in a dumbbell configuration with hinged ends of an optical trap assay subjected to axial compression. The initially straight filament buckles into a single arc. Reproduced from Kurachi, et al. [119]. Scale bar: 10 μm. **E:** Attenuated short wavelength buckling of an axially compressed rod embedded in an elastic material. The deformation of the rod now depends on the mechanical interaction with its surroundings. An energetic competition between matrix deformation and bending of the rod drives local buckling behavior. Importantly, due to the low flexural rigidity of the rod, oscillatory buckling minimizes the energetic cost of matrix deformation. The extent of buckling, however, depends on the presence of the matrix and how the rod adheres to it. Blue: undeformed matrix; red: extension; green: compression deformation. **F:** A single microtubule within the cytoplasm of a Cos7 cell is subjected to axial forces by a micropipette. Local, attenuated short wavelength buckling is visible. Reproduced from Brangwynne et al. [16]. Scale bar: 10 μm.

low-bending modulus of the MT, which makes it more favorable to reduce end-to-end distance by buckling than by pure axial compression (Fig. 1C, D). For reasonable values of κ and L [31, 32], the critical force threshold is about 1 pN – less than the 4–6 pN force exerted by a single kinesin motor protein [33–35]. This begs the question—how are MTs protected from buckling in cells? They are embedded in a viscoelastic cytoplasm that includes high-molecular weight actin and intermediate filament (IF) cytoskeletons and constrains lateral movement. This is analogous to a composite material, like rebar embedded in concrete. Under these circumstances, MTs can withstand axial forces up to 100 pN before collapsing [16]. Moreover, the deformation mode changes and the buckling wavelength is reduced to $\lambda = 2\pi\left(\frac{\kappa}{G}\right)^{\frac{1}{4}}$ ([16, 30] and Fig. 1E, F), where κ is the MT bending modulus and G is the elasticity of the surrounding matrix, because the deformation of the matrix is energetically less favored than the bending of the MT. Under these conditions, the wavelength is independent of MT length and inversely proportional to G. As a result, measurements of buckling wavelength can be used to derive G (if κ is known or can be estimated) [16, 36–38]. In addition to the elasticity of the surrounding matrix, friction between the MT and the matrix also affects the buckling mode. Specifically, such frictional, viscous forces cause the buckling amplitude to decrease exponentially with distance from a site of force application known as attenuated short wavelength buckling (Fig. 1F) [16, 39, 40].

Cytoskeletal fibers rarely work alone: in specialized sensory and nerve cells, MTs frequently assemble into cross-linked bundles. Crosslinking individual MTs into a coherent bundle increases the resistance to bending deformation and persistence length of the bundle as compared to an isolated MT [41]. Thus, MT bundles might be an important aspect of mechanical signal transmission. In the context of an MT bundle, it is important to consider the material properties of individual MTs as well as those of the bundled superstructure. The flexural rigidity of the bundle (κ_B) increases with filament number N according to $\kappa_B = N \cdot \kappa_{MT}$ for a loosely crosslinked and $\kappa_B = N^2 \cdot \kappa_{MT}$ for a completely crosslinked bundle [42]. More complex models that incorporate crosslinker dynamics and compliance have been developed [43]. As with single MTs, if an MT bundle is embedded in an elastic matrix, the bundle can withstand higher forces before undergoing buckling instabilities. The shape and spatial dynamics of the buckling MT bundle is intimately coupled to the mechanical properties of the surrounding medium and the bundle itself. In principle, it should be possible to use MT shape as a local probe to read-out the forces acting in cells with high spatio-temporal resolution [36], similar to what has been achieved with carbon nanotubes on elastomeric substrates [38]. Taken together, MTs can in principle transmit compressive forces in living cells, and, due to their well-characterized mechanical properties, can be used as experimental probes to monitor cell mechanics and force propagation [16, 22, 39, 44].

IFs also contribute to mechanics, tissue integrity, and mechanical signal propagation [45–48]. IFs show high tensile strength and protect skin against mechanical damage [49]. However, compared to actin and MTs, IFs have received less attention regarding their function in cell mechanics. Stable assembly of neurofilaments (NFs) is critical to maintain neuron shape, and defects in NFs lead to morphological changes reminiscent of neurodegeneration [50]. Interestingly, all NFs have a domain that inhibits MT polymerization and helps to determine the number of MTs within an axon [51]. In theoretical work, it has been proposed that IFs stabilize MTs and protect them from undergoing buckling instability under axial compression [30]. A direct role of keratin IFs in mechanotransduction was revealed in a study in *Xenopus*; local tugging forces applied to isolated embryonic cells cause reorganization and reinforcement of the IF network towards the site of force application, and lead to a change in their migratory behavior [52]. Just recently, nuclear lamins have been identified as crucial determinants of cell mechanics and differentiation [48]. Finally, the IF protein IFB-1A both colocalizes with mechanosensory neurons in *C. elegans* and is required for mechanical integrity of the epidermis during muscle contraction [53], suggesting that it plays a critical role in bi-directional mechanical signal transmission between the cuticle and underlying cells.

Membrane mechanics and force transmission to ion channels

The mechanical properties of lipid bilayers are thought to be crucial for the activation of MS ion channels, including MeT channels that give rise to the senses of touch, proprioception, and hearing. The principles linking the physical properties of membranes to ion channel gating have been discussed in several other reviews [9, 54–58]. Some of these physical properties enable membranes to store mechanical energy: resistance to changing the angle between two lipid molecules (bending rigidity); preferred lateral spacing of lipid molecules (in-plane tension); and an adjustable, but well-defined thickness of the bilayer due to the length of the fatty acids chains [59]. When proteins such as ion channels are embedded in membranes, the lipid bilayer is deformed, creating a tension along this interface with a force proportional to the length of the interface between the protein and the bilayer. In principle, such a force could contribute to gating [60].

Among the best-studied examples is the *m*echanosensitive *c*hannel of *s*mall conductance (MscS) present in bacteria and plants [56], whose open conformation is stabilized by stresses in the lipid bilayer by a mechanism of hydrophobic mismatch [61, 62]. In this model, increased tension in the cell membrane leads to thinning of the bilayer and hence a mismatch between the hydrophobic core of the fatty acids and the hydrophobic region of the transmembrane helices of the channel. Because this is energetically unfavorable, the helices tilt with respect to the bilayer plane, thinning the channel into an open conformation that allows ions to pass through the channel. However, in an alternative model, tension applied locally to the plasma membrane leads to phase separation of different lipid moieties in the bilayer, which then stabilizes different conformations of an integral membrane protein [54].

Very little is known about how gating happens in eukaryotic MS channels, notably those that mediate the senses of touch and

hearing. We note that tension-dependent interactions between MS channels and the surrounding lipid membrane could operate in parallel with additional factors, like cytoskeletal tension [63, 64]. Moreover, the sufficiency of membrane tension does not exclude a role for protein tethers linking such channels to extracellular or intracellular structures. Indeed, it is possible that channels such as TREK and TRAAK, which are known to be activated by increased membrane tension in reduced and reconstituted systems [65], may also depend on protein tethers for mechanical signal transmission in vivo [66].

Mechanical systems govern force transmission during touch sensation in the worm

The process of touch sensation begins with mechanical signal transmission from the skin to MeT channels expressed in touch receptor neurons (TRNs), and culminates in electrical signals when external mechanical loads activate the MeT channel. To illustrate what is already understood as well as the key open questions, we focus on the roundworm *C. elegans* and its six TRNs as a model for mechanosensory signaling. Figure 2 shows the general anatomy of the TRNs and their position relative to other tissues. The epidermis is a single epithelial cell layer that secretes the collagenous cuticle, a protective, acellular layer important for the animal's shape, development, and physiology [67, 68], from its apical surface, and is bounded by a basement membrane along its basal surface. Body wall muscles assemble into quadrants attached to the dorsal and ventral aspects of the body. Also shown schematically are the MeT channels that localize to puncta with an average inter-punctum spacing of about 2 μm [69, 70].

C. elegans offers many experimental advantages for studies in basic biology, biophysics, and neurobiology, including a short generation time (2–3 days), a fully sequenced genome, and a fully mapped nervous system consisting of only 302 neurons. Aspects particularly important for our discussion include a transparent body that enables direct visualization of neuron shape and function in living animals, and the ability to deliver precise mechanical stimuli (force, indentation) to both immobilized and freely behaving animals [8, 71]. Because the neurons responsible for converting touch into simple behaviors, and the protein partners forming the MeT channel complex, are known, *C. elegans* enables us to build a unique perspective on mechanosensation that is integrated from molecules to behavior.

Whole body mechanics influence touch sensation and behavior

One of the first detailed experimental characterizations of *C. elegans* body mechanics was carried out using self-sensing, piezo-resistive cantilevers [72] to measure the deformation of the whole animal under a given pre-set force [73]. The resulting linear force-deformation relationship was modeled as a cortical shell, consisting of cuticle, muscles, and epidermal tissue under hydrostatic pressure. In these experiments, cuticle puncture and adaptation to hyperosmotic environments had only modest effects on body stiffness, especially when compared to the impact of mutations that altered the composition or collagen crosslinking of the cuticle [73]. These and other measurements support the idea that the cortical shell is a major determinant of *C. elegans* body stiffness [74, 75].

Body wall muscle tone also modulates body mechanics and touch sensitivity [8, 71]. Hyper-contraction of body wall muscles leads to stiffer worms and a reduced sensitivity of the worm to external forces. This observation is consistent with the idea that *C. elegans* body mechanics modulates the efficiency of force transfer to sensory neurons and therefore is critical to set optimal touch sensitivity [8, 71]. Taken together, these data strongly suggest that body mechanics influence touch sensitivity by modulating mechanical signal transmission in living animals.

Subsequent work linked changes in body stiffness to changes in touch sensitivity, showing that both softer and stiffer animals respond less efficiently than wild-type animals when exposed to the same force [71]. This result implies that worms are sensitive to indentation rather than force per se, and suggests that indentation-induced changes in neuronal strain

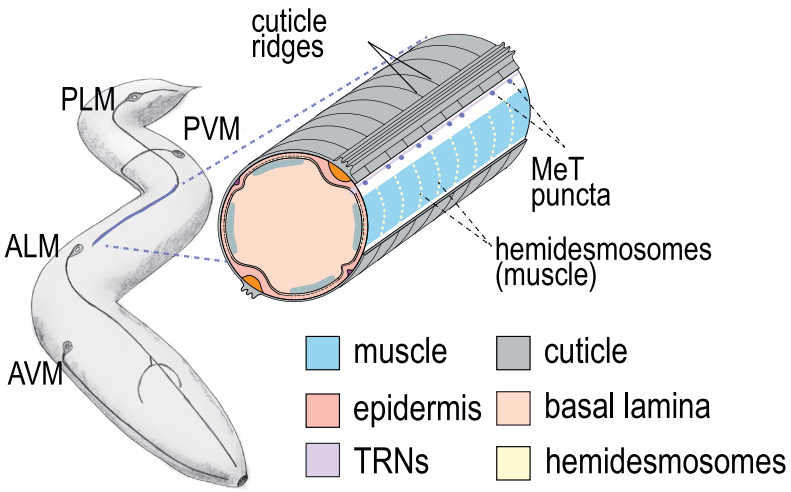

Figure 2. *C. elegans* schematic showing the TRNs, mechanoelectrical transduction (MeT) transduction channels and elements thought to contribute to mechanical signal transmission. Four of the six touch receptors are shown in the schematic drawing of a worm: AVM, ALM, PLM, and PVM. PLM and ALM exist as pairs on the left and right side of the body, of which only the left neuron is shown. A slab through the worm shows the cuticle ridges as circumferential rings and the alae, longitudinal projections on the ventral and dorsal sides. The cuticle is secreted by the epidermis below (pink), which attaches to the muscles (white) and the touch receptor neurons (purple). The hemidesomosomes, which attach the muscles and the TRNs to the hypodermis, are shown as white dots.

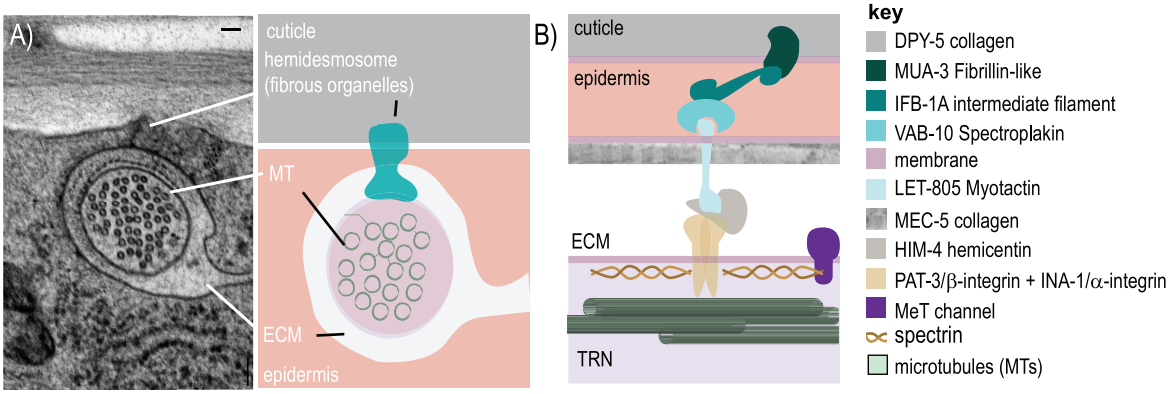

Figure 3. The molecular touch transmission machine. **A:** Electron micrograph and schematics of a TRN, embedded into the epidermis, underneath the cuticle. TRNs are densely filled with cross-linked MTs that are connected to the electron-dense cortical cytoskeleton by numerous tethers. The TRN and the epidermis are separated by a specialized extracellular matrix called the mantle, which is necessary for MeT channel trafficking and mechanosensation. Scale bar: 100 nm. **B:** Hypothetical assembly of mechanical components separating the stimulus from the touch response. Touch deforms the cuticle collagens and with it the hypodermal cells. Structural proteins in the epidermal cell are needed both to position the TRNs correctly and to provide mechanical coupling to the cuticle. A specialized ECM surrounding the neuron is required either for mechanical signal propagation and/or channel trafficking. Tension and bending rigidity of the membrane might influence ion channel gating and open probability. MTs and the spectrin cytoskeleton inside TRNs are critical for function on multiple levels. Both cytoskeletal elements likely contribute to TRN mechanical integrity (see text).

might account for activation of TRNs. However, it is not yet known whether sensitivity to indentation is a conserved feature of mechanoreceptor neurons across species and contexts: A recently published mechanical model of touch in mammals suggests that variations in skin thickness across individuals is more likely to favor behavioral control by force rather than indentation [76]. Additional experiments are needed to address whether worms sense stress or strain and whether or not this represents a conserved feature in metazoan animals.

How do mechanical signals propagate during touch?

In order for a touch to the cuticle to be sensed by the TRNs, the mechanical deformation has to be transmitted through successive tissue layers (Figs. 3 and 4A). Though little is currently known about the molecules that comprise this mechanical signal transmission pathway, we can garner insight from investigations of the structures that attach muscles to the cuticle [77], which contain many of the same molecular components (Fig. 4). Among these proteins are LET-805 myotactin [78] (Fig. 4A), the IFB-1A IF [53] (Fig. 4B), and MUA-3 [79] (Fig. 4C), a protein that links the IF cytoskeleton to sites assumed to assist in the transfer of mechanical stress. Myotactin is a large transmembrane protein in the epidermis with 32 type-3 fibronectin repeats and at least four potential integrin-binding RGD/RLD motifs, that localizes to both muscles and TRNs ([78] and Fig. 4B). It has been suggested that myotactin binds to integrins in muscle cells and ensures their firm connection to the epidermis [78, 80]. *C. elegans* has two α-integrins encoded by the *ina-1* and *pat-2* genes, and one β-integrin encoded by *pat-3*. Intriguingly, reduced expression of the RGD-binding α-integrin PAT-2, but not the laminin-binding α-integrin INA-1, impairs touch sensitivity [81]. A myotactin-integrin interaction may therefore be a crucial part of the force transmission pathway in the TRNs as it is in muscle. Together, these observations suggest that hemidesmosomes transmit force from muscles to the cuticle [77], and, conversely, from the animal's exterior to TRNs (Fig. 4A). The exact pathway of force transmission to the MeT channel and how it is activated during touch is unknown in *C. elegans* or in other metazoans. Below, we consider structures that could support mechanical signal transmission in *C. elegans* TRNs and perhaps other mechanoreceptor neurons.

Is force transmitted through the membrane?

Whereas it is well established that forces due to osmotic swelling are transmitted through the membrane to MscS [56], the gating mechanism of eukaryotic MS channel during touch is less clear. Several reviews have discussed the potential effects of membrane mechanical properties on the gating mechanism of eukaryotic ion channels [9, 54, 57, 58, 82].

Piezo1 and Piezo2 belong to a family of evolutionary conserved ion channels involved in blood flow [4] and the sensation of touch (reviewed in [83]) in mammals, respectively. In addition, the sole Piezo ortholog in *Drosophila* has been shown to sense noxious stimuli and be activated by mechanical force [84]. *C. elegans* also has one Piezo ortholog, which has yet to be characterized [85]. Piezo ion channels have been proposed to rely on membrane tension for activation [55]. While Piezo1 is sufficient to induce MS curents in heterologous cells, mechanosensitivity has yet to be detected in Piezo channels reconstituted in pure lipid bilayers [86]. Thus, it remains uncertain whether or not the Piezo1 protein is sufficient to confer sensitivity to bilayer tension [82].

In *C. elegans*, mutations that prevent the synthesis of poly-unsaturated phospholipids increase the resistance of TRN membranes to deformation and also impair touch sensitivity [87]. These observations provide indirect evidence of a role for

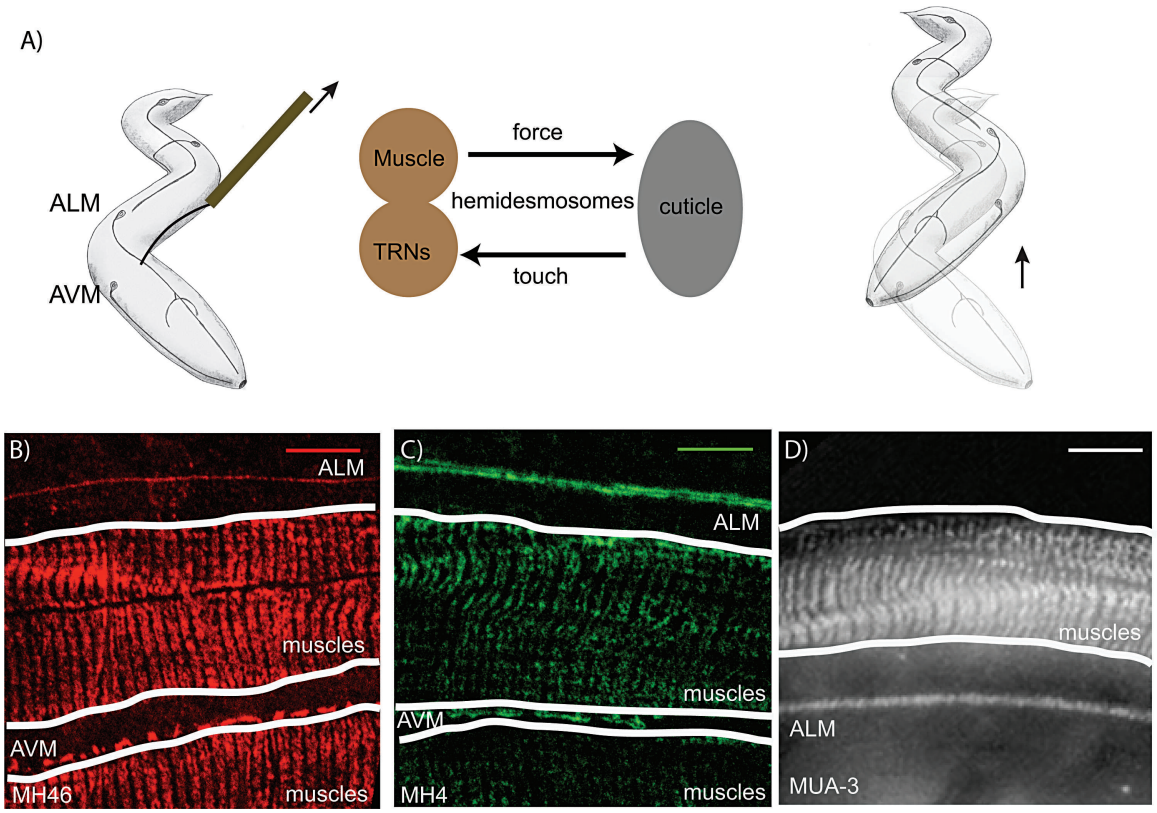

Figure 4. Bi-directional force signaling of fibrous organelles. **A:** Many molecular components, such as myotactin, intermediate filaments and integrins, are shared in structures that attach muscles to the basal lamina and TRNs to the mantle and epidermis, and ultimately to the cuticle. Muscle contraction produces a force that is transmitted outwards to the cuticle to drive body bending and translocation. Conversely, touch produces a stress in the cuticle, which can be transmitted along the fibrous organelles inwards to the TRNs. In the case of a touch to the anterior, a reversal response is elicited. **B:** Antibody stain (MH46) of myotactin, an epidermal cell adhesion molecule. **C:** Antibody stain (MH4) of intermediate filaments. Note that tracks are visible engulfing the TRNs. **D:** Antibody stain of MUA-3, a hypodermal protein necessary for hypodermis-cuticle attachment. Adapted from Bercher et al. (2001) [79]. Scale bars: 10 μm.

membrane mechanics in MeT channels activation. Additional evidence comes from work showing that mutations which reduce the ability of MEC-2 stomatin to bind lipids (palmitoylate and cholesterol) decrease touch sensation in vivo. Another stomatin homolog, UNC-24, co-localizes with MEC-2 and has a putative lipid-transfer domain. Thus, it has been proposed that these stomatin-related proteins regulate MeT channel function by modifying the lipid environment in the TRNs (reviewed in [10]). Whether or not the MeT channels in TRNs are directly activated by membrane stretch remains to be determined.

Is force transmitted through the extracellular matrix and basement membrane?

TRNs in *C. elegans* are surrounded by a specialized ECM, called the mantle. Several extracellular proteins have been identified [70, 88, 89] that might link the *C. elegans* MeT channel to its specialized ECM (reviewed in [10]). At least three proteins thought to localize to this ECM are crucial for touch sensation [70, 89]: MEC-1 and MEC-9 are EGF/Kunitz domain-containing proteins, and MEC-5 is a type IV collagen [70]. It is plausible that this matrix plays an important role in force transmission to the MeT channels, especially given that mutations in the MEC-1, MEC-5, and MEC-9 proteins all result in defects in touch sensitivity. However, all three proteins are also required for proper localization of the MeT channel in the neurite [70], complicating interpretation. Another collagen IV, LET-2, was proposed to be important for mechanical activation of a presumptive stretch-activated ion channel of the DEG/ENaC family expressed in muscles, UNC-105 [90]. However, uncertainty about this model remains [91].

Collagen IV is not the only component of the ECM in *C. elegans*. Amongst other members of the laminin superfamily, α-laminin EPI-1 is also needed for axon outgrowth and recruitment of fibulin-1C FBN-1 during the assembly of hemidesmosomes [92]. Although FBN-1 is dispensable for the sense of touch in *C. elegans* [93], it is worth mentioning in light of recent data showing that a tether of yet unknown identity connects murine dorsal root ganglion neurons to a laminin-111 matrix and is important for mechanosensation [94, 95]. Importantly, removal of these tethers by protease treatment abolishes the response to mechanical stimuli [94], suggesting that anchoring of the neurons to the surrounding ECM is required for touch sensitivity [96]. These tantalizing hints suggest that mechanical connections between stretch-sensitive channels and the ECM are likely important for physiological function, at least in some cases. How this

connection is established at the molecular level, to our knowledge, remains unknown.

The MT cytoskeleton has a significant role during C. elegans touch

The *C. elegans* TRN neurite contains a cross-linked bundle of as many as 50 MTs [69, 97, 98] (Fig. 3A). These distinctive MTs, which contain 15 protofilaments, depend on expression of MEC-12 α-tubulin, MEC-7 β-tubulin, and MEC-17 α-tubulin acetyltransferase [98, 99]. Mutations that result in a loss of these MTs cause impaired touch sensation and altered sensitivity of MeT channels to external forces [29, 100]. The integrity and stability of the cross-linked bundle depends on the *C. elegans* doublecortin protein ZYG-8 [101] and the MT minus-end binding protein PTRN-1 [102]. As discussed above, doublecortin and other MT-associated proteins can increase the bending rigidity of the MTs [32], suggesting that the partial defects in touch sensitivity seen in *zyg-8* and *ptrn-1* mutants could result from a change in the mechanics of the MT bundle and the TRN. Consistent with this idea, MT-associated proteins PTL-1 and ELP-1, which are known to increase MT bending rigidity in vitro [32], are also necessary for full touch sensitivity [103, 104]. Experiments have yet to reveal whether or not MTs participate directly in relaying mechanical signals along TRN neurites.

The presence of a specialized MT cytoskeleton in TRNs suggests that it is directly or indirectly involved in mechanosensation, e.g. provides a rigid mechanical support. Many filaments connect the MTs to the cortex of the TRNs [69, 97]. It is not known whether these tethers connect to the membrane directly or the membrane-bound actin-spectrin network. As suggested by Bounoutas et al. [100], unbinding of these tethers could release tension and help regulate MeT channel closure following activation. Such a mechanism is analogous to the tension release model [105] of hair cell adaptation. It will be interesting to discover whether such tethers contribute to membrane tension, MeT channel closure, or both.

Does force propagate along the actin-spectrin cytoskeleton?

A membrane subjacent actin-spectrin network characterizes many cell types, including neurons [106]. Spectrin assembles into tetramers containing two molecules each of α- and β-spectrin [107]. Such tetramers form through interactions between the C-terminus of β-spectrin and the N-terminus of α-spectrin; mutations in this tetramerization domain interfere with network formation and elasticity [108–111]. Whereas actin has been implicated in mechanical activation of MeT channels by direct force transfer [112], the role played by spectrin in mechanotransduction is less well studied.

Leveraging mutations in UNC-70 β-spectrin, we recently showed that TRNs are held under spectrin-dependent mechanical pre-stress, and interact mechanically with the engulfing epidermis [111]. In parallel, we also showed that defects in UNC-70 β-spectrin alter touch sensitivity. Both the severity of mechanical defects (buckling under compressive stress) and touch defects were correlated with the severity of the molecular defect in UNC-70. Taken together, these findings imply that the mechanical properties of TRNs are tuned to provide optimal sensitivity to external forces, similar to the overall body mechanics.

A mechanical systems perspective

A central question remains: How do these components interact to enable mechanical signal transmission and touch sensation? In *C. elegans*, external mechanical loads activate 20–30 MeT channels in one millisecond or less [29]. As the channels localize to puncta separated by 2–4 μm [29, 69], these experimental findings imply that mechanical signals travel over distances of 60–100 μm in, at most, one millisecond. As discussed above, theoretical considerations indicate that when mechanical deformation occurs orthogonal to the axis of a fiber, it propagates much farther along a pre-stressed fiber than along a slack one [27, 28, 113]. As touches delivered to the *C. elegans* cuticle and transverse to the TRNs are sufficient to elicit robust behavioral responses, we speculate that pre-stress may facilitate force transmission by increasing the distance of mechanical signal propagation along TRN neurites.

The contribution of a pre-stressed actin-spectrin cytoskeleton to mechanical signal transmission could exist on other levels. For instance, a pre-stressed actin-spectrin network could have a role in stabilizing MTs inside TRNs. As suggested from theoretical studies, embedding MTs in an active matrix such as a pre-stressed actin-spectrin network would increase their effective stiffness [114]. Thus, mechanical interactions between sub-structures within the TRN cytoskeleton could result in a mutual stabilization. But, the converse could also be true. Tension in the spectrin network could depend on the state of MTs inside axons [115]. Future experiments directed towards deciphering whether or not such a tight mechanical interaction between MTs and the neuronal spectrin network exists will be illuminating.

Pre-stressed actin-spectrin networks would also help keep the plasma membrane under tension and could, therefore, contribute to MeT gating according to the force-from-lipid model [58]. Alternatively, the actin-spectrin network might bind directly to the MeT channel, and thus transmit tension directly to the channel itself. In either case, tension in the network could shift the MeT channel to a point of maximal responsiveness, analogous to the gating spring in hair cells [116]. Tension release within the actin-spectrin network or between the membrane and its associated proteins could account for closure of MeT channels following their activation [29, 53, 100, 117]. However, whether mechanical pre-stress in the spectrin-actin network in fact tunes MeT activity remains to be established directly.

Conclusions and outlook

Here, we combined biophysical considerations about mechanical signal transmission within living cells with knowledge

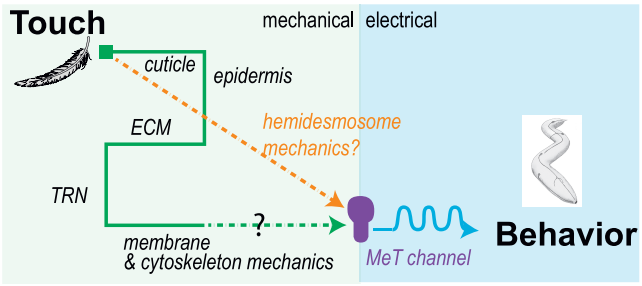

Figure 5. Hypothetical force transmission pathway during touch. A mechanical stimulation at the cuticle has to be transferred to the MeT channel through stratified tissues such as the cuticle, epidermis, and ECM surrounding the TRNs. Lipid bilayer mechanics and the cytoskeleton likely influence MeT channel function. Alternatively, a direct force transmission pathway via fibrous organelles (red dotted arrow) might be a fast track to MeT channel activation and behavior.

about the structures and proteins needed for touch sensation in *C. elegans*. The picture emerging from this synthesis is that a mechanical signal transmission pathway links the physical stimulus of touch to neuron activation, and hence to perception and behavior (Fig. 5). In the case of *C. elegans*, we envision a transverse pathway that transmits cuticle indentation through the epidermis and ECM, culminating in a local deformation of the TRN itself. It will be exciting to uncover the influence of the mechanics of fibrous organelles on touch sensation of freely moving animals, and whether or not they directly participate in mechanical signal transmission. Further, we speculate that spectrin-dependent tension within the TRNs contributes to lateral transmission and activation of the MeT channels, which in turn convert the mechanical signal into an electrical one. Thus, touch sensation depends on multiple cellular machines that act in concert to direct the mechanical signal from its source on the surface of the skin to MeT channels within the sensory neuron. It follows from this scenario that any genetic defect that alters the mechanical properties of the intervening links has the potential to impair touch sensation. Within this context, challenges for the future are to develop a holistic understanding of how proteins interact genetically, biochemically, and mechanically, and to take physical principles into account when developing mechanisms and models of sensory mechanotransduction. Therefore, quantitative and predictive models that link molecular properties to physiological function will be indispensable to understand how our body communicates with the physical world using the sense of touch.

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References

1. **Mattens F**. 2009. Perception, body, and the sense of touch: phenomenology and philosophy of mind. *Husserl Studies* **25**: 97–120.
2. **O'Shaughnessy B**. 1989. The sense of touch. *Aust J Phil* **67**: 37–58.
3. **Drummond HA, Price MP, Welsh MJ, Abboud FM**. 1998. A molecular component of the arterial baroreceptor mechanotransducer. *Neuron* **21**: 1435–41.
4. **Li J, Hou B, Tumova S, Muraki K**, et al. 2014. Piezo1 integration of vascular architecture with physiological force. *Nature* (in press), doi: 10.1038/nature13701
5. **del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P**, et al. 2009. Stretching single talin rod molecules activates vinculin binding. *Science* **323**: 638–41.
6. **Krieg M, Arboleda-Estudillo Y, Puech PH, Kafer J**, et al. 2008. Tensile forces govern germ-layer organization in zebrafish. *Nat Cell Biol* **10**: 429–36.
7. **Mgharbel A, Delanoe-Ayari H, Rieu JP**. 2009. Measuring accurately liquid and tissue surface tension with a compression plate tensiometer. *HFSP J* **3**: 213–21.
8. **Petzold BC, Park SJ, Ponce P, Roozeboom C**, et al. 2011. *Caenorhabditis elegans* body mechanics are regulated by body wall muscle tone. *Biophys J* **100**: 1977–85.
9. **Anishkin A, Loukin SH, Teng J, Kung C**. 2014. Feeling the hidden mechanical forces in lipid bilayer is an original sense. *Proc Natl Acad Sci USA* **111**: 7898–905.
10. **Chalfie M**. 2009. Neurosensory mechanotransduction. *Nat Rev Mol Cell Biol* **10**: 44–52.
11. **DuFort CC, Paszek MJ, Weaver VM**. 2011. Balancing forces: architectural control of mechanotransduction. *Nat Rev Mol Cell Biol* **12**: 308–19.
12. **Ingber DE, Wang N, Stamenovic D**. 2014. Tensegrity, cellular biophysics, and the mechanics of living systems. *Rep Prog Phys* **77**: 046603.
13. **Wang N, Tytell JD, Ingber DE**. 2009. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nat Rev Mol Cell Biol* **10**: 75–82.
14. **Kumar S, Maxwell IZ, Heisterkamp A, Polte TR**, et al. 2006. Viscoelastic retraction of single living stress fibers and its impact on cell shape, cytoskeletal organization, and extracellular matrix mechanics. *Biophys J* **90**: 3762–73.
15. **Pellegrin S, Mellor H**. 2007. Actin stress fibres. *J Cell Sci* **120**: 3491–9.
16. **Brangwynne CP, MacKintosh FC, Kumar S, Geisse NA**, et al. 2006. Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement. *J Cell Biol* **173**: 733–41.
17. **Stamenovic D, Mijailovich SM, Tolic-Norrelykke IM, Chen J**, et al. 2002. Cell prestress. II. Contribution of microtubules. *Am J Physiol Cell Physiol* **282**: C617–24.
18. **Stewart MP, Helenius J, Toyoda Y, Ramanathan SP**, et al. 2011. Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding. *Nature* **469**: 226–30.
19. **Hu S, Chen J, Butler JP, Wang N**. 2005. Prestress mediates force propagation into the nucleus. *Biochem Biophys Res Commun* **329**: 423–8.
20. **Desprat N, Supatto W, Pouille PA, Beaurepaire E**, et al. 2008. Tissue deformation modulates twist expression to determine anterior midgut differentiation in Drosophila embryos. *Dev Cell* **15**: 470–7.
21. **Poh YC, Shevtsov SP, Chowdhury F, Wu DC**, et al. 2012. Dynamic force-induced direct dissociation of protein complexes in a nuclear body in living cells. *Nat Commun* **3**: 866.
22. **Na S, Collin O, Chowdhury F, Tay B**, et al. 2008. Rapid signal transduction in living cells is a unique feature of mechanotransduction. *Proc Natl Acad Sci USA* **105**: 6626–31.
23. **Wang Y, Botvinick EL, Zhao Y, Berns MW**, et al. 2005. Visualizing the mechanical activation of Src. *Nature* **434**: 1040–5.
24. **Canadas P, Laurent VM, Oddou C, Isabey D**, et al. 2002. A cellular tensegrity model to analyse the structural viscoelasticity of the cytoskeleton. *J Theor Biol* **218**: 155–73.
25. **Wang N, Suo Z**. 2005. Long-distance propagation of forces in a cell. *Biochem Biophys Res Commun* **328**: 1133–8.

26. **Wang N, Tolic-Norrelykke IM, Chen J, Mijailovich SM**, et al. 2002. Cell prestress. I. Stiffness and prestress are closely associated in adherent contractile cells. *Am J Physiol Cell Physiol* **282**: C606–16.
27. **Hwang Y, Barakat AI**. 2012. Dynamics of mechanical signal transmission through prestressed stress fibers. *PLoS ONE* **7**: e35343.
28. **Hwang Y, Gouget CL, Barakat AI**. 2012. Mechanisms of cytoskeleton-mediated mechanical signal transmission in cells. *Commun Integr Biol* **5**: 538–42.
29. **O'Hagan R, Chalfie M, Goodman MB**. 2005. The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals. *Nat Neurosci* **8**: 43–50.
30. **Brodland GW, Gordon R**. 1990. Intermediate filaments may prevent buckling of compressively loaded microtubules. *J Biomech Eng* **112**: 319–21.
31. **Gittes F, Mickey B, Nettleton J, Howard J**. 1993. Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *J Cell Biol* **120**: 923–34.
32. **Mickey B, Howard J**. 1995. Rigidity of microtubules is increased by stabilizing agents. *J Cell Biol* **130**: 909–17.
33. **Hunt AJ, Gittes F, Howard J**. 1994. The force exerted by a single kinesin molecule against a viscous load. *Biophys J* **67**: 766–81.
34. **Svoboda K, Block SM**. 1994. Force and velocity measured for single kinesin molecules. *Cell* **77**: 773–84.
35. **Howard J**. 1996. The movement of kinesin along microtubules. *Annu Rev Physiol* **58**: 703–29.
36. **Brangwynne CP, Koenderink GH, Mackintosh FC, Weitz DA**. 2008. Nonequilibrium microtubule fluctuations in a model cytoskeleton. *Phys Rev Lett* **100**: 118104.
37. **Jiang HQ, Zhang JP**. 2008. Mechanics of microtubule buckling supported by cytoplasm. *J Appl Mech-T Asme* **75**: 061019.
38. **Xiao J, Jiang H, Khang DY, Wu J**, et al. 2008. Mechanics of buckled carbon nanotubes on elastomeric substrates. *J Appl Phys* **104**: 033543.
39. **Das M, Levine AJ, MacKintosh FC**. 2008. Buckling and force propagation along intracellular microtubules. *Eur Phys Lett* 84.
40. **Shan WL, Chen Z, Broedersz CP, Gumaste AA**, et al. 2013. Attenuated short wavelength buckling and force propagation in a biopolymer-reinforced rod. *Soft Matter* **9**: 194.
41. **Tolomeo JA, Holley MC**. 1997. Mechanics of microtubule bundles in pillar cells from the inner ear. *Biophys J* **73**: 2241–7.
42. **Guo Y, Liu Y, Tang JX, Valles JM, Jr**. 2007. Polymerization force driven buckling of microtubule bundles determines the wavelength of patterns formed in tubulin solutions. *Phys Rev Lett* **98**: 198103.
43. **Bathe M, Heussinger C, Claessens MM, Bausch AR**, et al. 2008. Cytoskeletal bundle mechanics. *Biophys J* **94**: 2955–64.
44. **Wu J, Misra G, Russell RJ, Ladd AJ**, et al. 2011. Effects of dynein on microtubule mechanics and centrosome positioning. *Mol Biol Cell* **22**: 4834–41.
45. **Buehler MJ**. 2013. Mechanical players-The role of intermediate filaments in cell mechanics and organization. *Biophys J* **105**: 1733–34.
46. **Fletcher DA, Mullins RD**. 2010. Cell mechanics and the cytoskeleton. *Nature* **463**: 485–92.
47. **Herrmann H, Bar H, Kreplak L, Strelkov SV**, et al. 2007. Intermediate filaments: from cell architecture to nanomechanics. *Nat Rev Mol Cell Biol* **8**: 562–73.
48. **Swift J, Ivanovska IL, Buxboim A, Harada T**, et al. 2013. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* **341**: 1240104.
49. **Pan X, Hobbs RP, Coulombe PA**. 2013. The expanding significance of keratin intermediate filaments in normal and diseased epithelia. *Curr Opin Cell Biol* **25**: 47–56.
50. **Nguyen MD, Shu T, Sanada K, Lariviere RC**, et al. 2004. A NUDEL-dependent mechanism of neurofilament assembly regulates the integrity of CNS neurons. *Nat Cell Biol* **6**: 595–608.
51. **Bocquet A, Berges R, Frank R, Robert P**, et al. 2009. Neurofilaments bind tubulin and modulate its polymerization. *J Neurosci* **29**: 11043–54.
52. **Weber GF, Bjerke MA, DeSimone DW**. 2012. A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Dev Cell* **22**: 104–15.
53. **Woo WM, Goncharov A, Jin Y, Chisholm AD**. 2004. Intermediate filaments are required for *C. elegans* epidermal elongation. *Dev Biol* **267**: 216–29.
54. **Anishkin A, Kung C**. 2013. Stiffened lipid platforms at molecular force foci. *Proc Natl Acad Sci USA* **110**: 4886–92.
55. **Delmas P, Coste B**. 2013. Mechano-gated ion channels in sensory systems. *Cell* **155**: 278–84.
56. **Haswell ES, Phillips R, Rees DC**. 2011. Mechanosensitive channels: what can they do and how do they do it. *Structure* **19**: 1356–69.
57. **Sukharev S, Sachs F**. 2012. Molecular force transduction by ion channels: diversity and unifying principles. *J Cell Sci* **125**: 3075–83.
58. **Teng J, Loukin S, Anishkin A, Kung C**. 2014. The force-from-lipid (FFL) principle of mechanosensitivity, at large and in elements. *Pflugers Arch* (in press), doi: 10.1007/s00424–014-1530–2 .
59. **Rawicz W, Olbrich KC, McIntosh T, Needham D**, et al. 2000. Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophys J* **79**: 328–39.
60. **Wiggins P, Phillips R**. 2004. Analytic models for mechanotransduction: gating a mechanosensitive channel. *Proc Natl Acad Sci USA* **101**: 4071–6.
61. **Christensen AP, Corey DP**. 2007. TRP channels in mechanosensation: direct or indirect activation. *Nat Rev Neurosci* **8**: 510–21.
62. **Killian JA**. 1998. Hydrophobic mismatch between proteins and lipids in membranes. *Bba-Rev Biomembranes* **1376**: 401–16.
63. **Heureaux J, Chen D, Murray VL, Deng CX**, et al. 2014. Activation of a Bacterial Mechanosensitive Channel in Mammalian Cells by Cytoskeletal Stress. *Cell Mol Bioeng* **7**: 307–19.
64. **Liang X, Madrid J, Gartner R, Verbavatz JM**, et al. 2013. A NOMPC-dependent membrane-microtubule connector is a candidate for the gating spring in fly mechanoreceptors. *Curr Biol* **23**: 755–63.
65. **Brohawn SG, Su Z, MacKinnon R**. 2014. Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K+ channels. *Proc Natl Acad Sci USA* **111**: 3614–9.
66. **Lauritzen I, Chemin J, Honore E, Jodar M**, et al. 2005. Cross-talk between the mechano-gated K2P channel TREK-1 and the actin cytoskeleton. *EMBO Rep* **6**: 642–8.
67. **Chisholm AD, Xu S**. 2012. The *Caenorhabditis elegans* epidermis as a model skin. II: differentiation and physiological roles. *Wiley Interdiscip Rev Dev Biol* **1**: 879–902.
68. **Page AP, Johnstone IL**. 2007. The cuticle. *WormBook* 1–15.
69. **Cueva JG, Mulholland A, Goodman MB**. 2007. Nanoscale organization of the MEC-4 DEG/ENaC sensory mechanotransduction channel in *Caenorhabditis elegans* touch receptor neurons. *J Neurosci* **27**: 14089–98.
70. **Emtage L, Gu G, Hartwieg E, Chalfie M**. 2004. Extracellular proteins organize the mechanosensory channel complex in *C. elegans* touch receptor neurons. *Neuron* **44**: 795–807.
71. **Petzold BC, Park SJ, Mazzochette EA, Goodman MB**, et al. 2013. MEMS-based force-clamp analysis of the role of body stiffness in *C. elegans* touch sensation. *Integr Biol (Camb)* **5**: 853–64.
72. **Park SJ, Petzold BC, Goodman MB, Pruitt BL**. 2011. Piezoresistive cantilever force-clamp system. *Rev Sci Instrum* **82**: 043703.
73. **Park SJ, Goodman MB, Pruitt BL**. 2007. Analysis of nematode mechanics by piezoresistive displacement clamp. *Proc Natl Acad Sci USA* **104**: 17376–81.
74. **Backholm M, Ryu WS, Dalnoki-Veress K**. 2013. Viscoelastic properties of the nematode *Caenorhabditis elegans*, a self-similar, shear-thinning worm. *Proc Natl Acad Sci USA* **110**: 4528–33.
75. **Fang-Yen C, Wyart M, Xie J, Kawai R**, et al. 2010. Biomechanical analysis of gait adaptation in the nematode *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **107**: 20323–8.
76. **Yuxiang W, Marshall KL, Baba Y, Lumpkin EA**, et al. 2013. World Haptics Conference Natural variation in skin thickness argues for mechanical stimulus control by force instead of displacement. p. 645–650.
77. **Francis R, Waterston RH**. 1991. Muscle cell attachment in *Caenorhabditis elegans*. *J Cell Biol* **114**: 465–79.
78. **Hresko MC, Schriefer LA, Shrimankar P, Waterston RH**. 1999. Myotactin, a novel hypodermal protein involved in muscle-cell adhesion in *Caenorhabditis elegans*. *J Cell Biol* **146**: 659–72.
79. **Bercher M, Wahl J, Vogel BE**, et al. 2001. Mua-3, a gene required for mechanical tissue integrity in *Caenorhabditis elegans*, encodes a novel transmembrane protein of epithelial attachment complexes. *Lu C* **154**: 415–26.
80. **Bosher JM, Hahn BS, Legouis R, Sookhareea S**, et al. 2003. The *Caenorhabditis elegans* vab-10 spectraplakin isoforms protect the epidermis against internal and external forces. *J Cell Biol* **161**: 757–68.
81. **Calixto A, Chelur D, Topalidou I, Chen X**, et al. 2010. Enhanced neuronal RNAi in *C. elegans* using SID-1. *Nat Methods* **7**: 554–9.
82. **Martinac B**. 2014. The ion channels to cytoskeleton connection as potential mechanism of mechanosensitivity. *Biochim Biophys Acta* **1838**: 682–91.
83. **Vasquez V, Scherrer G, Goodman MB**. 2014. Sensory biology: it takes Piezo2 to tango. *Curr Biol* **24**: R566–9.
84. **Kim SE, Coste B, Chadha A, Cook B**, et al. 2012. The role of Drosophila Piezo in mechanical nociception. *Nature* **483**: 209–12.

85. **Hobert O**. 2013. The neuronal genome of *Caenorhabditis elegans*. *WormBook* 1–106.
86. **Coste B, Xiao B, Santos JS, Syeda R**, et al. 2012. Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature* **483**: 176–81.
87. **Vasquez V, Krieg M, Lockhead D, Goodman MB**. 2014. Phospholipids that contain polyunsaturated fatty acids enhance neuronal cell mechanics and touch sensation. *Cell Rep* **6**: 70–80.
88. **Huang M, Chalfie M**. 1994. Gene interactions affecting mechanosensory transduction in *Caenorhabditis elegans*. *Nature* **367**: 467–70.
89. **Du H, Gu G, William CM, Chalfie M**. 1996. Extracellular proteins needed for *C. elegans* mechanosensation. *Neuron* **16**: 183–94.
90. **Liu JD, Schrank B, Waterston RH**. 1996. Interaction between a putative mechanosensory membrane channel and a collagen. *Science* **273**: 361–4.
91. **Jospin M, Mariol MC, Segalat L, Allard B**. 2004. Patch clamp study of the UNC-105 degenerin and its interaction with the LET-2 collagen in *Caenorhabditis elegans* muscle. *J Physiol* **557**: 379–88.
92. **Muriel JM, Xu X, Kramer JM, Vogel BE**. 2006. Selective assembly of fibulin-1 splice variants reveals distinct extracellular matrix networks and novel functions for perlecan/UNC-52 splice variants. *Dev Dyn* **235**: 2632–40.
93. **Muriel JM, Dong C, Hutter H, Vogel BE**. 2005. Fibulin-1C and Fibulin-1D splice variants have distinct functions and assemble in a hemicentin-dependent manner. *Development* **132**: 4223–34.
94. **Hu J, Chiang LY, Koch M, Lewin GR**. 2010. Evidence for a protein tether involved in somatic touch. *EMBO J* **29**: 855–67.
95. **Chiang LY, Poole K, Oliveira BE, Duarte N**, et al. 2011. Laminin-332 coordinates mechanotransduction and growth cone bifurcation in sensory neurons. *Nat Neurosci* **14**: 993–1000.
96. **Poole K, Moroni M, Lewin GR**. 2014. Sensory mechanotransduction at membrane-matrix interfaces. *Pflugers Arch* 1–12.
97. **Chalfie M, Thomson JN**. 1979. Organization of neuronal microtubules in the nematode Caenorhabditis elegans. *J Cell Biol* **82**: 278–89.
98. **Cueva JG, Hsin J, Huang KC, Goodman MB**. 2012. Posttranslational acetylation of alpha-tubulin constrains protofilament number in native microtubules. *Curr Biol* **22**: 1066–74.
99. **Savage C, Hamelin M, Culotti JG, Coulson A**, et al. 1989. Mec-7 is a beta-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans*. *Genes Dev* **3**: 870–81.
100. **Bounoutas A, O'Hagan R, Chalfie M**. 2009. The multipurpose 15-protofilament microtubules in *C. elegans* have specific roles in mechanosensation. *Curr Biol* **19**: 1362–7.
101. **Bellanger JM, Cueva JG, Baran R, Tang G**, et al. 2012. The doublecortin-related gene zyg-8 is a microtubule organizer in *Caenorhabditis elegans* neurons. *J Cell Sci* **125**: 5417–27.
102. **Richardson CE, Spilker KA, Cueva JG, Perrino J**, et al. 2014. PTRN-1, a microtubule minus end-binding CAMSAP homolog, promotes microtubule function in *Caenorhabditis elegans* neurons. *eLife3:* e01498.
103. **Gordon P, Hingula L, Krasny ML, Swienckowski JL**, et al. 2008. The invertebrate microtubule-associated protein PTL-1 functions in mechanosensation and development in *Caenorhabditis elegans*. *Dev Genes Evol* **218**: 541–51.
104. **Hueston JL, Herren GP, Cueva JG, Buechner M**, et al. 2008. The *C. elegans* EMAP-like protein, ELP-1 is required for touch sensation and associates with microtubules and adhesion complexes. *BMC Dev Biol* **8**: 110.
105. **Stauffer EA, Scarborough JD, Hirono M, Miller ED**. 2005. Fast adaptation in vestibular hair cells requires myosin-1c activity. *Neuron* **47**: 541–53.
106. **Xu K, Zhong G, Zhuang X**. 2013. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. *Science* **339**: 452–6.
107. **Bennett V, Baines AJ**. 2001. Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. *Physiol Rev* **81**: 1353–92.
108. **Sleep J, Wilson D, Simmons R, Gratzer W**. 1999. Elasticity of the red cell membrane and its relation to hemolytic disorders: an optical tweezers study. *Biophys J* **77**: 3085–95.
109. **Ipsaro JJ, Harper SL, Messick TE, Marmorstein R**, et al. 2010. Crystal structure and functional interpretation of the erythrocyte spectrin tetramerization domain complex. *Blood* **115**: 4843–52.
110. **Li D, Harper SL, Tang HY, Maksimova Y**, et al. 2010. A comprehensive model of the spectrin divalent tetramer binding region deduced using homology modeling and chemical cross-linking of a mini-spectrin. *J Biol Chem* **285**: 29535–45.
111. **Krieg M, Dunn AR, Goodman MB**. 2014. Mechanical control of the sense of touch by beta-spectrin. *Nat Cell Biol* **16**: 224–33.
112. **Hayakawa K, Tatsumi H, Sokabe M**. 2008. Actin stress fibers transmit and focus force to activate mechanosensitive channels. *J Cell Sci* **121**: 496–503.
113. **Rowghanian P, Grosberg AY**. 2012. Propagation of tension along a polymer chain. *Phys Rev E Stat Nonlin Soft Matter Phys* **86**: 011803.
114. **Kikuchi N, Ehrlicher A, Koch D, Kas JA**, et al. 2009. Buckling, stiffening, and negative dissipation in the dynamics of a biopolymer in an active medium. *Proc Natl Acad Sci USA* **106**: 19776–9.
115. **Dennerll TJ, Joshi HC, Steel VL, Buxbaum RE**, et al. 1988. Tension and compression in the cytoskeleton of PC-12 neurites. II: quantitative measurements. *J Cell Biol* **107**: 665–74.
116. **Bechstedt S, Howard J**. 2007. Models of hair cell mechanotransduction. *Curr Top Membr* **59**: 399–424.
117. **Arnadottir J, O'Hagan R, Chen Y, Goodman MB**, et al. 2011. The DEG/ENaC protein MEC-10 regulates the transduction channel complex in touch receptor neurons. *J Neurosci* **31**: 12695–704.
118. **Dyachenko V, Christ A, Gubanov R, Isenberg G**. 2008. Bending of z-lines by mechanical stimuli: an input signal for integrin dependent modulation of ion channels. *Prog Biophys Mol Biol* **97**: 196–16.
119. **Kurachi M, Hoshi M, Tashiro H**. 1995. Buckling of a single microtubule by optical trapping forces: direct measurement of microtubule rigidity. *Cell Motil Cytoskeleton* **30**: 221–8.