INVITED REVIEW

# Mechanotransduction and auditory transduction in *Drosophila*

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**Abstract** Insects are utterly reliant on sensory mechanotransduction, the process of converting physical stimuli into neuronal receptor potentials. The senses of proprioception, touch, and hearing are involved in almost every aspect of an adult insect's complex behavioral repertoire and are mediated by a diverse array of specialized sensilla and sensory neurons. The physiology and morphology of several of these have been described in detail; genetic approaches in *Drosophila*, combining behavioral screens and sensory electrophysiology with forward and reverse genetic techniques, have now revealed specific proteins involved in their differentiation and operation. These include three different TRP superfamily ion channels that are required for transduction in tactile bristles, chordotonal stretch receptors, and polymodal nociceptors. Transduction also depends on the normal differentiation and mechanical integrity of the modified cilia that form the neuronal sensory endings, the accessory structures that transmit stimuli to them and, in bristles, a specialized receptor lymph and transepithelial potential. Flies hear near-field sounds with a vibration-sensitive, antennal chordotonal organ. Biomechanical analyses of wild-type antennae reveal non-linear, active mechanical properties that increase their sensitivity to weak stimuli. The effects of mechanosensory and ciliary mutations on antennal mechanics show that the sensory cilia are the active motor elements and indicate distinct roles for TRPN and TRPV channels in auditory transduction and amplification.

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## Introduction

As human commensals and laboratory companions, *Drosophila* share our living space, but they occupy a very different mechanosensory realm. At the scale of a fly, viscosity, drag, and stickiness far outweigh the forces of gravity, mass, and inertia in importance; sounds are felt as much as heard, and flight is akin to swimming through syrup. To navigate this world, the fly relies on thousands of diverse sense organs to detect, transduce, and integrate different mechanical stimuli (Fig. 1). Walking demands continual proprioceptive feedback to coordinate the action of six multijointed legs. Flight requires gyroscopes to detect changes in heading and attitude, and strain gauges to monitor each wing beat. Sensitive tactile bristles defend against parasites by triggering a cleaning reflex. Even reproduction begins with a male courtship song, heard by the antennal ear of a female fly—and by male rivals. In fact, mechanosensory feedback is the essential sense in *Drosophila*: In contrast to mutants that lack sight or smell, which can still thrive in laboratory cultures, flies with substantial mechanosensory defects are completely incapacitated by their lack of coordination.

The fly's mechanosensory world is somewhat simpler at the level of the individual sensory receptors because the many thousands of mechanosensory organs on an adult fly fall into a few basic classes (Fig. 1). Receptors in each class can transduce different stimuli, depending on their location, size, and attachment. This review will first survey the different types of mechanosensory receptor and the experimental approaches that have proven useful in their study,

**Fig. 1** The fly's mechanosensory world. *Arrows* link mechanosensory-dependent behaviors and mechanical stimuli that trigger or result from those behaviors to the four major classes of sense organs that are specialized to detect those stimuli
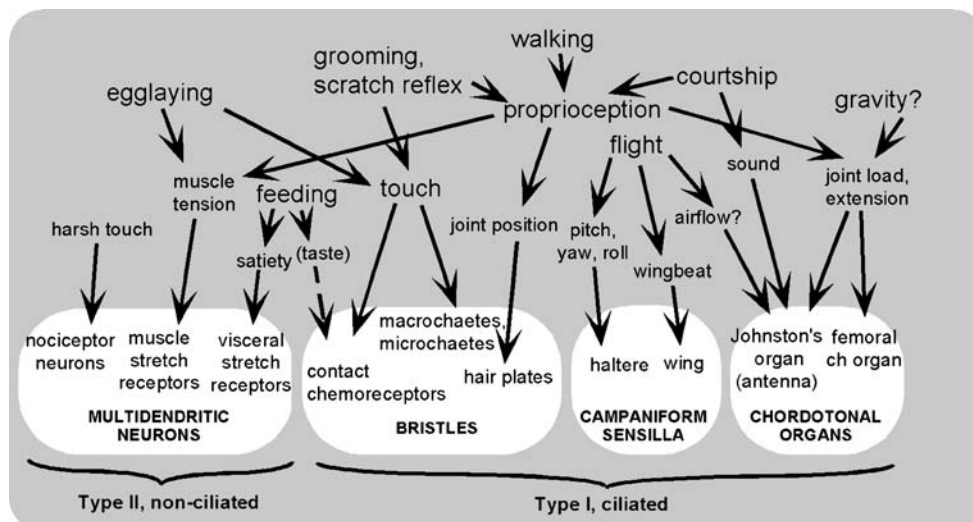


and then focus on the differentiation, structure, and operation of two mechanosensors: bristles and chordotonal organs, including the antennal chordotonal organ that is the sensory element of the fly's ear. I aim to integrate prior detailed descriptions of the early development and morphology of insect mechanoreceptors [43, 53, 106, 108], with new knowledge about the role of specific proteins in their differentiation and function.

Two themes will be apparent. One is the prevalence of TRP superfamily ion channels as the candidate transducers in mechanosensory cells: Members of three different TRP channel subclasses are implicated in transducing touch, sound, and mechanical pain. A second theme is the importance of cilia as the site of transduction in most of the fly's mechanoreceptors. The cilium forms a distinct cell compartment with its own specialized cytoskeleton and transport mechanisms, which are required to construct the specialized sensory endings and may also play a direct role in modulating and amplifying sensory responses.

## Mechanoreceptor types and sensory functions

Sense organs in *Drosophila* fall into two main types, both of which include mechanosensitive neurons. Type I organs, or sensilla, are multicellular organs, each comprising one to four sensory neurons and three or four individually specialized support cells (Fig. 2). Each type I neuron has a single dendrite or sensory process with a modified cilium or outer segment at its distal tip. Three surrounding support cells enclose the sensory process and construct accessory structures that distinguish the different sensilla. In contrast, type II organs consist simply of single, non-ciliated multidendritic (md) neurons with dendritic morphologies ranging from a simple T-shape to extensive arborizations.

Type I organs are further subdivided into external sensory (es) organs, including bristle and campaniform sensilla, and chordotonal (ch) organs. *Sensory bristles* or *chaetae* are the most abundant and conspicuous sensilla on the adult fly: Each of its several thousand bristles is a separately innervated sense organ. Their external, cuticular parts include a curved bristle shaft, which is secreted by the trichogen cell and is seated in a socket, the product of the tormogen cell. Purely mechanosensory bristles have a closed bristle shafts, and each shaft is innervated at its base by a single type I neuron. Its ciliary outer segment extends to the base of the bristle and is entirely enclosed by the dendritic sheath, an extracellular matrix secreted by a sheath cell or thecogen, the innermost support cell. Single large macrochaetae are located at invariant positions on the head and thorax, along with patterned fields of smaller microchaetae. Both types are probably primarily tactile; smaller bristles on hair plates at leg joints provide proprioceptive feedback on limb position and locomotion. Indeed, es organs appear to provide most proprioceptive input in the adult fly; although chordotonal organs are sometimes referred to as the primary insect proprioceptors; the degree of uncoordination seen in mutants lacking bristle function is far more severe than in those with defective chordotonal organs.

Contact chemosensory bristles on the mouthparts, legs, and wings are innervated by a single mechanosensory neuron that contacts the bristle base, as well as multiple chemosensory neurons that extend long cilia into the porous bristle shaft. (Olfactory bristles on the third antennal segment and mouthparts are also innervated by multiple chemosensory neurons but lack a mechanosensory neuron.)

*Campaniform sensilla* are named for a cuticular dome that replaces the bristle shaft, but are otherwise structurally similar to bristles. They are activated by local deformations
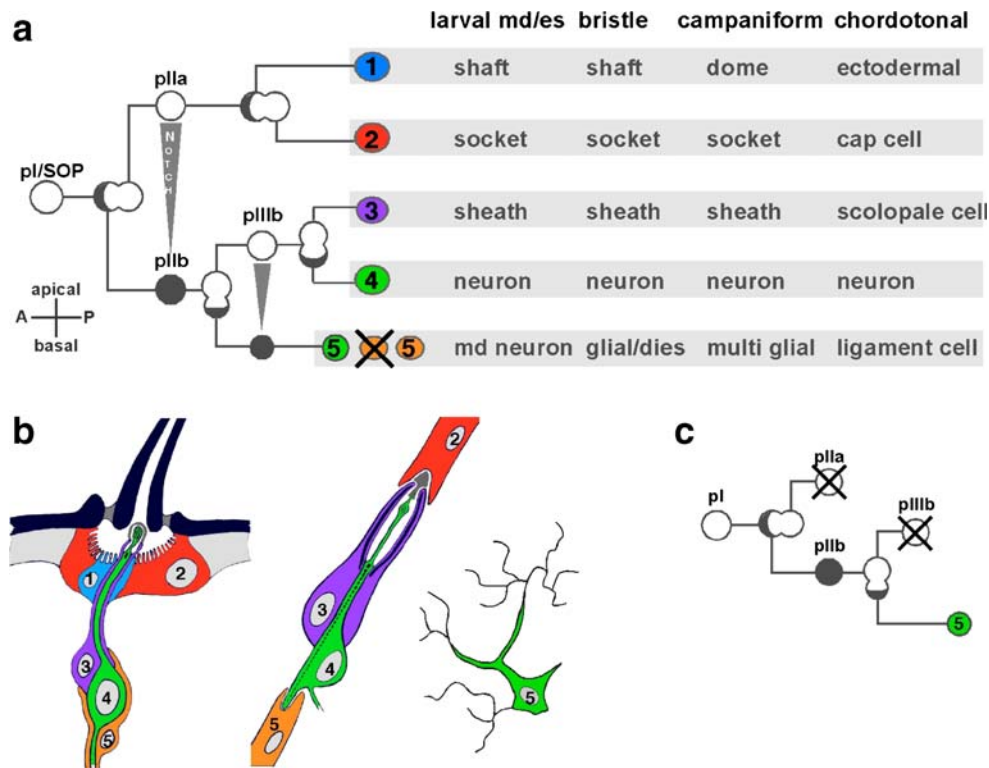
**Fig. 2** Cell lineage and fate in sense-organ development. **a** Starting from a single SOP cell, four asymmetric mitotic divisions give rise to the neuron and support cells that make up a type I sense organ and, in some cases, an additional type II neuron. Cell divisions are oriented as they occur, along an apical-basal axis or in the plane of the epithelium. The width of the *shaded triangles* indicates the relative strength of Notch signaling in the sister cells arising from each division; *shaded crescents* and *cells* indicate the asymmetric localization and inheritance of Numb, a determinant of neural fate and antagonist of Notch signaling. The fates of the five resulting cells in different organ subtypes are listed. Diagram based on figures and models by Lai and Orgogozo [59]. **b** Schematic of the different types of sense organs: two type I organs, a mechanosensory bristle, and a chordotonal organ, and a type II, multidendritic neuron. Cells are *colored* and *numbered* as in **a**. **c** Origin of "solo" md neurons by an extreme modification of the lineage in A, in which the IIa and IIIb cells undergo apoptosis, and the only remaining cell becomes an md neuron

of the cuticle, for instance, due to wing bending [20]. Campaniform sensilla are located near limb joints and along the wing blade, and are most numerous at the bases of the halteres, the club-shaped modified hindwings that evolved in the Diptera. The halteres beat in synchrony with the wings during flight, developing angular momentum so that a change in flight attitude or direction induces gyroscopic forces at their bases; these are detected by three arrays of campaniform sensilla, each oriented so as to sense the force resulting from rotation in a different plane [22].

*Chordotonal* (ch) organs, in contrast to es organs, lack visible external sensilla and are attached to the underside of the cuticle [108]. A chordotonal organ may include one to hundreds of individual sensory units or scolopidia. Each scolopidium contains a scolopale; a fluid-filled capsule encloses the ciliary sensory endings of one to three neurons. The cilia are attached at their tips to an extracellular dendritic cap: stimuli pull on the cap to stretch the cilia. Chordotonal organs, thus, essentially stretch receptors, but like bristles, may transduce different stimuli depending on their location and attachment. In the larva, groups of up to

five scolopidia attached to the body wall in each segment sense touch [54] and provide proprioceptive feedback on larval locomotion [12]. Adult chordotonal organs typically bridge the joint between two limb segments, so that flexion of the joint stretches the sense organ and stimulates the neurons. A femoral chordotonal organ in each leg contributes to proprioception, but a role in vibration detection or gravireception is also possible. The largest chordotonal organ in the fly is Johnston's organ, the adult fly's antennal ear. Located in the second antennal segment, it comprises hundreds of scolopidia that detect air-driven vibration of the distal segments due to near-field sound sources—for instance, the vibrating wing of a courting male fly [10].

*Type II* or *multidendritic* (md) neurons are not associated with any other specialized support cells or structures. Their sensory endings vary in complexity from bipolar dendrites (bp type) that innervate tracheae to extensive dendritic arborizations (da type) that extend across large areas of the body wall. The internal location and relatively unspecialized morphology of type II neurons have obscured their functions, but mutant phenotypes show that some sense

painful stimuli such as pinching or heat [97], while others may act as proprioceptors [2]. Sensory neurons that innervate muscles and internal visceral and reproductive organs also lack cilia and are classified as type II.

## Experimental approaches: genetic, behavioral, and electrophysiological

With experimentally accessible mechanosensory organs and a wealth of genetic resources, *Drosophila* is particularly well-suited to genetic studies of mechanotransduction. The early genesis and specification of its sense organs have long been studied as a model of neural development, and the morphology and physiological properties of similar sensilla in larger insects have also been described. But until recently, little was known about the molecular mechanisms by which ciliary mechanotransducers convert stimuli into receptor potentials. Many of the proteins needed for their differentiation and function have now been identified by genetic dissection. As applied to mechanotransduction, this involves random mutagenesis and screens to isolate mutants with defects in mechanosensory behavior; electrophysiological recording from sense organs identifies those mutants with sensory defects and positional cloning to locate the affected genes. The complementary approach, targeted mutagenesis of candidate genes, has also been informative; recently, comparative genomics of *Drosophila* and other model organisms has identified a well-conserved set of structural, transport, and other proteins common to sensory cilia, including those in type I mechanoreceptors [6, 16, 61]. The available *Drosophila* genetic reagents and resources need not be catalogued in this paper, but a few aspects particularly relevant to the genetics of mechanotransduction can be noted. A well-annotated genome and a large and expanding collection of transponson insertions have made positional cloning faster and enhanced our ability to generate targeted deletions of short intervals and selected genes. The ability to insert single-copy transgenes that express wild-type and modified proteins in their native context is especially important, given the complex architecture of type I mechanotransducers. Further, in these highly differentiated sensilla, the precise location of a novel protein is often key to understanding its function: Fluorescently tagged proteins expressed from transgenes have been highly informative in this regard.

*Drosophila* actually combines two experimental organisms in a single species, the larva and the adult fly. Each has a different set of sense organs and a different behavioral repertoire, and provides different experimental opportunities: For instance, larval behavior is easier to monitor directly, but the larger adult bristles are more accessible for recording. During metamorphosis, the larval peripheral nervous system is destroyed and the adult peripheral nervous system (PNS) develops anew. The larval and adult sense organs both include the same major classes of external and chordotonal sensilla, arise by similar developmental programs, and require similar sets of genes for their operation. Thus, most mechanosensory mutations isolated in larval behavioral screens also affect the adult sensilla, and vice versa. However, the peristaltic movements by which larvae crawl and eat are less dependent on sensory feedback [84], so even the most severely affected mutants do survive through larval and pupal development, and can be studied as both larvae and adults. Computer-assisted video analysis has been used to record and analyze changes in locomotor pattern due to mechanosensory defects [2, 12]. Gentle touch (i.e., stroking with an eyelash or fine probe) is sensed in the larva mainly by chordotonal organs and elicits a stereotypic withdrawal and turning. This response was used to screen for touch-insensitive mutants [54]. Harsher stimuli or noxious heat stimulate nociceptive md neurons to evoke a different and distinctive writhing behavior [97].

Direct observation of freely behaving adult flies usually requires video recording and analysis. However, decapitated flies live for a day or two, and although immobile unless provoked, they retain their normal stance, righting ability, and some motor skills. Scratch reflexes can be reliably elicited from a decapitated fly by touching bristles in defined receptive fields [98]. More complex behavioral sequences that depend on mechanosensory input include grooming, geotaxis, and courtship. To keep themselves clean and free of parasites and debris, flies spend much of their time grooming. This involves a stereotypic sequence in which each of a pair of legs are combed alternately over the thorax or abdomen, and then rubbed together. The behavioral sequence probably relies on both tactile and proprioceptive feedback, and a screen for inability to clean off colored powder has recovered mutants with PNS defects [73].

Flies show negative geotaxis: they tend to walk upward on vertical surfaces. It is not known how gravity is sensed, but the chordotonal organs in the antennae and legs are likely to detect changes in body orientation. In an early demonstration of genetic variation governing a complex behavior, Hirsch and coworkers used a choice maze in which flies progress through a series of vertical T junctions, in long-term selection experiments, to produce lines with extreme positive and negative geotaxis [44]; recently revisited in [95]. The same type of maze has been used to screen mutagenized lines for single-gene mutants affecting gravitaxis [4]. But the interaction of a freely-moving fly with even the simplest apparatus is a complex business: Even with direct observation, it is difficult to specify what aspects of sensory and/or motor function are being tested.

Courtship, the fly's most complex behavior pattern, involves the integration of auditory, tactile, visual, and

chemosensory stimuli. Courting males follow a strict sequence: first following and orienting toward a female, then "singing" to her by vibrating one extended wing in a sequence of pulses and hums before extending their proboscis to contact her genitalia and attempting copulation [40]. The courtship song conveys a species-specific signal—probably the interval between pulses—to a conspecific female. Other nearby males can also hear courtship song and respond by following the singing male; deaf mutants have been isolated by screening for the absence of this response [27].

A crucial step in identifying mechanosensory mutants is to establish if behavioral phenotypes are caused by a defect in sensory transduction, rather than in central processing or motor output. In *Drosophila*, this can be done directly by recording sensory receptor potentials and currents [18, 54, 99]. The methods used follow long-established techniques for recording from the sensilla of larger insects, including cockroach tactile spines [75] and sensory hairs in bees and blowflies [104], and take advantage of the hollow bristle shafts that give electrical access to extracellular space apical to the sensory ending (Fig. 3). Because this space is delimited by the hydrophobic cuticle and the high-resistance intercel-

lular junctions of the epithelium, electrical potentials and currents are localized within the sensillum and can be recorded by placing a saline-filled micropipet over the cut end of a bristle. With a reference electrode placed in basal extracellular space, an apical-positive transepithelial potential (TEP), due to electrogenic ion pumping by a support cell [87, 88], is recorded (Fig. 3). Displacements of the pipet and the bristle generated, for example, by a piezoelectric manipulator cause a rapid drop in the TEP. This reflects a depolarizing receptor potential in the sensory ending due to inward current flowing from the apical space; action potentials propagated back through the dendrite may appear as upward spikes superimposed on the receptor potential. Transepithelial and mechanoreceptor currents can be recorded in the same preparation by using a voltage-clamp amplifier to control the TEP [99]. It is important to note that only the TEP, not the neuronal membrane potential, is controlled in these preparations, as is evident from the current spikes due to neuronal action potentials in response to stimulation (Fig. 3).

Campaniform sensilla lack the bristle shaft that provides access to the neuron, but an elegant method for testing their responses is to record action potentials from the cut nerve endings of an excised wing while stimulating the isolated campaniform sensilla on the wing with a sharp probe [21]. Receptor potentials have not been recorded from individual chordotonal organs. However, stimulation of the large auditory chordotonal organ in the antenna generates a substantial compound potential in the antennal nerve, which can be recorded with extracellular electrodes. These sound-evoked potentials are absent in *atonal* mutants, confirming their chordotonal origin [26].

Each of these preparations is quick, robust, and well-suited to screening relatively large numbers of behavioral mutants for sensory defects. However, the opaque cuticle and the support cells and sheaths that enclose the sensory endings hinder more direct access to the membranes where transduction occurs. Ion concentrations in the receptor lymph cannot easily be controlled by perfusion because the tight epithelial junctions and apical septa limit diffusion into the apical extracellular cavity. A semi-intact preparation that conserved the important structural features of the sensilla but gave more direct access to the sensory neuron would be a useful advance.

Measurements of the mechanical correlates of transduction, although less often achieved, are of crucial importance in fully understanding how it works. One revealing instance is the use of laser vibrometry to record sound-evoked and spontaneous movements of the fly's auditory antennae [37]. This has revealed their adaptive, non-linear mechanical behavior and shown that active mechanics are integral to auditory transduction. But while the mechanical stimulus delivered to a bristle or antenna can be precisely controlled,
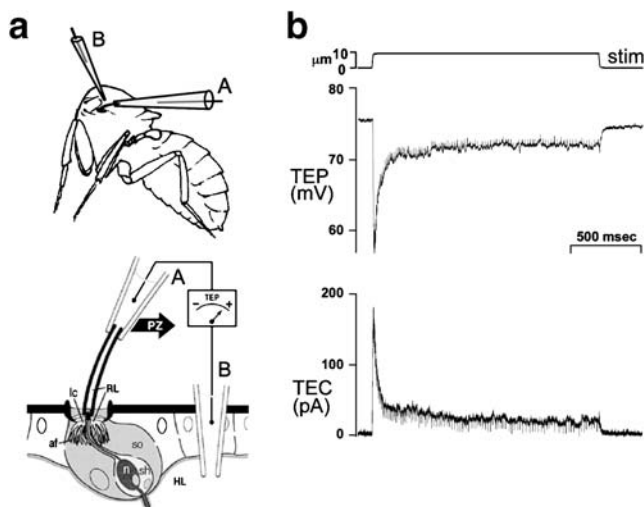


**Fig. 3** Receptor potentials and currents in mechanosensory bristles. **a** Bristle recording preparation. In a decapitated but otherwise intact fly, an apical recording electrode (**a**) placed over the cut end of a macrochaete bristle contacts the receptor lymph, and a reference electrode (**b**) contacts basal extracellular space. Transepithelial potentials are recorded as $(V_a–V_b)$. The bristle is stimulated by displacing the recording electrode with a piezoelectric manipulator (*PZ*). *af* Apical folds of socket cell, *hl* hemolymph, *n* neuron, *rl* receptor lymph, *so* socket cell, and *sh* sheath cell. *Upper panel* from [4]; *lower* from [59]. **b** Mechanoreceptor potential (*middle trace*) and current (*lower trace*) recorded from the same wild-type bristle during a half-second ramp-and-hold displacement (*stim, top trace*). Receptor potentials and currents adapt rapidly during a sustained stimulus. Neuronal action potentials are also visible on each trace. Data from E. Shishido and M. Kernan

we can, in most cases, still only guess at the nature and magnitude of the forces that impinge on the transducing apparatus at a molecular level.

## Early development of sense organs

*Drosophila* sensory neurons arise in peripheral epithelia and, from there, extend axons that project to specific regions within the central nervous system. The adult bristles in particular are conspicuously patterned, and the large macrochaetes are invariant and individually identified. Mutations affecting their formation and distribution have been the basis for a long-running and comprehensive research program that has made the fly bristle one of the best-studied systems in developmental biology. Only the briefest outline is sketched in this paper (Fig. 2); sense-organ development has been reviewed many times; this outline is based on a recent comprehensive review [59].

Development of a sense organ begins with the expression of one of several "proneural" helix–loop–helix transcription factors in a small group of epithelial cells under the influence of local patterning signals. Notch-mediated signaling among the group of cells then selects a single sense organ precursor (SOP) cell from the group. Different proneural transcription factors are required for different sensilla; expression of Achete–scute complex (AS-C) proteins initiates bristle formation, while Atonal is required in chordotonal organ precursors [47]. The later expression of subtype selector genes also contributes to sense organ identity; for instance, the Cut homoeodomain protein promotes an external sensory over a chordotonal fate [11], while the paired-homeodomain Pox-neuro protein specifies poly-innervated, chemosensory bristles [7].

The SOP undergoes a sequence of precisely choreographed, asymmetric cell divisions, ultimately producing up to five terminally differentiated cells (Fig. 2) [59]. Asymmetry arises from the polarity of the dividing cells relative to the apicobasal or anterior-posterior axes and results in the unequal distribution of determinants such as Numb, an inhibitor of Notch signaling, to one of the two daughter cells. In the development of a microchaete bristle, the first division produces a secondary precursor, pIIa, that undergoes one further division to produce the two cells that will construct the bristle shaft and socket. The other secondary precursor, pIIb, first divides to give rise to pIIIb and one other small cell. pIII divides to produce the sensory neuron and the innermost support cell, the thecogen.

Although this schema describes the formation of a thoracic bristle, other type I sensilla and even type II neurons arise from variations on this lineage (Fig. 2) [59]. For instance, the smaller pIII daughter cell adopts different fates in different sensilla, becoming a glial cell or undergoing apoptosis in adult microchaetes [31, 32]. In some larval "md–es" lineages, it becomes a neural cell that migrates away from the differentiating type I sensillum to form a separate, type II sensory neuron [71]. Indeed, even isolated "solo–md" neurons arise by an extreme variation of the canonical lineage in which the pIIa and pIIIb precursors both undergo apoptosis, leaving an md neuron as the sole product (Fig. 2c).

The chordotonal cell lineage also may fit this schema (Fig. 2) [59], with the scolopale cell as the lineal and functional equivalent of the thecogen, the pIIa-derived cells differentiating into apical cap and attachment cells, and the smaller pIII derivative forming the basal ligament cell. Although outwardly dissimilar, chordotonal features such as the scolopale, dendritic cap, and ciliary rootlet are enlarged versions of structures also constructed by their es counterparts [64], and specific molecular markers expressed in the corresponding support and glial cells further support their equivalence [17, 59].

## es Organs: structure and differentiation

The morphogenesis of four cells—neuron, thecogen, trichogen, and tormogen—into a differentiated bristle has been well described [42, 43, 53]. The trichogen and tormogen cells both become enlarged and polytene, each undergoing two rounds of DNA endoreplication [5] before constructing the massive (on a cellular scale), cuticular bristle and socket. The bristle shaft is held within the socket by an elastic supporting collar.

Bristle shaft assembly is controlled at a high level by the Pax-2 transcription factor, the expression of which is reduced in the trichogen in *shaven* (*sv*) mutants, and which can promote shaft formation when expressed ectopically [52]. Shaft assembly has been studied in detail [90] as a model for the assembly of actin-framed structures. The bristle shaft is secreted over a framework of short actin fascicles that are assembled and joined end-to-end within an apical extension of the trichogen. Cross-linking proteins such as fascin stabilize the bundle structure; mutations in *singed*, the fascin gene, or in *forked*, which encodes another actin-cross-linking protein, cause short or crooked bristles [91]. These mutations do not drastically impair mechanosensation: even misshapen bristle shafts still effectively transmit stimuli to the neuronal sensory endings.

Adult mechanosensory bristles are visibly asymmetric: In thoracic bristles, the socket is thicker on its anterior rim, and the shaft is tilted and curved posteriorly. Asymmetry is apparent from the earliest stages of elongation: An extension of the socket cell overlies the anterior side of the emerging trichogen process [89]. A

thin process that stains for the protein cofilin and was identified as the neuronal dendrite [89] also always lies along the upper surface of the trichogen extension and, thus, may also determine its initial orientation. However, mutants that cannot form neuronal outer segments [8, 25, 41] still have normal bristle orientation and morphology, so either this process is not required to orient the bristle, or it extends from a non-neuronal cell—possibly the thecogen.

After secreting the bristle cuticle, the trichogen extension withdraws and the trichogen degenerates to leave a hollow bristle shaft opening into an apical extracellular cavity. The tormogen, in contrast, persists after secreting the socket cuticle. Its apical membrane expands and becomes highly infolded to sustain the ion transport mechanism that will generate the receptor lymph and TEP. A subcuticular socket septum, which is supported by the socket and forms an annulus around the sensory ending, is probably also a tormogen product, but its makeup is unknown. Septate and adherens junctions connect the remaining cells to each other, maintaining the integrity of the epithelium and forming a diffusion barrier and high-resistance electrical seal necessary to maintain the TEP [42, 53].

Socket cell differentiation requires a persistent activity of the transcription factor Suppressor of Hairless (Su(H)/CBF1). A Notch signaling effector Su(H) is required as a Notch signalling effector throughout bristle development, but it is also subsequently upregulated specifically in the tormogen and independently of Notch [9, 15]. Upregulation requires autoregulatory enhancer elements downstream of the *Su(H)* gene: Deleting or mutating all nine of these elements results in bristles that are outwardly normal, but have reduced TEPs and mechanoreceptor currents. The tormogen cell is present, but its apical membrane folds are reduced or absent [9].

A similar support cell defect is seen in *nompH* mutants. The apical membrane folds of the tormogen are reduced, the trichogen extension persists instead of degenerating, and the neuronal outer segment is missing [103]. *nompH* encodes Cyp303a1, one of about 80 cytochrome P450 enzymes in *Drosophila*. These are membrane-associated, heme-binding oxygenases that metabolize a wide variety of substrates. The *nompH* mutations alter conserved residues in the substrate-binding domain or close to the heme-binding site, so the catalytic activity of Cyp303a1 is probably required for its function. *nompH* is expressed only in the socket cell—the protein is located in its apical membrane folds [103]—so its effects on the trichogen and neuronal outer segment are non-autonomous, suggesting that it is needed to generate a secreted or transcellular signal. Alternatively, normal secretory and electrogenic activity of the socket cell could be required for the late differentiation of the other cells.

Thecogen and dendritic sheath; *nompA*

The tormogen and trichogen also wrap concentrically around an apical extension of the thecogen. The neuronal sensory process penetrates to the apical surface through this extension as the cell bodies of the neuron and thecogen withdraw basally so that the theogen forms a continuous sleeve around the sensory process [43, 53]. The ciliary outer segment extends from the distal tip of the sensory process, but is tightly enclosed by an extracellular, electron-dense dendritic sheath (Fig. 4). The sheath matrix is composed in
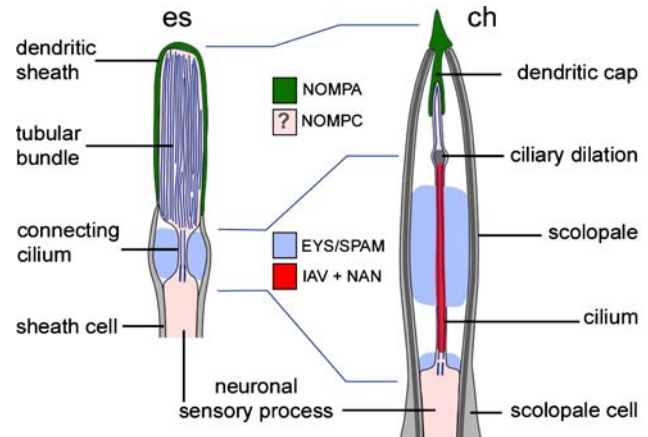


| Extracellular | | |
|---|---|---|
| Dendritic cap, sheath | *nompA* | PAN + ZP domain |
| Sheath/scolopale space | *eys/spam* | EGF + LamG repeats |
| **Cilium, connecting cilium** | | |
| Basal body | *unc* | coiled-coil |
| Anterograde IFT | *nompB* | IFT88: TPR |
| | *Klp64D, KAP* | kinesin-2 |
| Retrograde IFT | *oseg1* | IFT122: WD + TPR |
| | *btv* | cytoplasmic dynein |
| **Ion channels** | | |
| TRPN channel | *nompC* | TRP + 29 ANK repeats |
| TRPV channel | *iav, nan* | TRP + 5 ANK repeats |

**Fig. 4** Identified molecular components and their locations in sense organs. **a** Sensory endings in an external mechanosensory bristle (es) and chordotonal organ (ch). The distal end of the neuronal sensory process and the ciliary outer segments are shaded in *pink* and the distal parts of the sheath and scolopale cells in *gray*; microtubules are indicated as *blue lines*. Cilia in both es and ch organs can be divided into proximal and distal sections based on the non-overlapping locations of the dendritic cap protein NOMPA [17] and EYS/SPAM, the MAb21A6 antigen [46]. The location of the *nompC* TRPN channel is unknown, but may be in the outer segment of es organs, and by analogy, the distal part of ch cilia. TRPV channels occur only in ch organs and are restricted to the zone proximal to the ciliary dilation. **b** Genetically identified components of sensory endings, listing mutant loci and modules or domains in the encoded proteins. *ANK* Ankyrin, *EGF* Epidermal growth factor-like repeat, *IFT* intraflagellar transport, *Lam G* laminin globular domain-like, *PAN* plasminogen N-terminal module, *TPR* tetratricopeptide repeat, *TRP* transient receptor channel superfamily member, *WD* B-propeller motif, *ZP* zona pellucida domain

part of a zona pellucida (ZP) domain protein. When this is disrupted in *nompA* mutants, transduction is eliminated, and the neuronal sensory processes are sometimes visibly detached from the overlying sensilla [17]. ZP-domain proteins are found in a wide variety of extracellular matrices and egg coats, frequently forming structured apical matrices; a relevant example is the tectorial "membrane" that overlies and stimulates hair cells in the mammalian inner ear. They are typically synthesized and brought to the site of matrix deposition as transmembrane preproteins, then released by protease cleavage at an extracellular site located between the ZP and transmembrane domains [49, 50]. NOMPA shares these features; a GFP-tagged NOMPA protein is synthesized in the thecogen cell body, transported along the apical extension, and deposited in the dendritic sheath [17]. Modified forms of the protein, expressed in transgenic flies, suggest that the ZP domain is necessary and sufficient for the incorporation of NOMPA into the sheath matrix and that the tetrabasic motif, transmembrane, and cytoplasmic domains are involved in its directed transport and deposition (Y.D. Chung and M. Kernan, unpublished data).

Distal to the ZP domain, NOMPA includes five plasminogen N-terminal (PAN) modules—short domains with a well-defined fold stabilized by three disulfide bonds [96]. PAN modules are also found as single modules in some vertebrate-secreted proteins, but proteins combining ZP and PAN domains are known only in invertebrates: The fly and nematode genomes each contain a diverse set of ZP–PAN proteins. No function or interaction partner has been defined for any PAN module, but the NOMPA modules are tightly conserved, with up to 97% amino acid identity between the corresponding modules in the fly and mosquito homologs, indicating strong functional constraints. The results of deleting or exchanging individual NOMPA modules (Y.D. Chung & M. Kernan, unpublished data) also suggest that individual PAN modules have specific functions. They could interact with specific neuronal outer segment proteins to make the mechanical link necessary for transduction, but interactions with other sheath components are also possible. Dendritic sheaths and caps are disorganized but still present in *nompA* mutants, so they must include other components.

A "dot" of staining by the monoclonal antibody 21A6 at the neuronal endings of es organs was originally thought to correspond to the dendritic sheath, but is proximal to the sheath as marked by GFP–NOMPA and instead appears to surround the short connecting cilium that joins the outer and inner dendritic segments [17] (Fig. 4). Mutants lacking this antigen have recently been identified. The affected gene, *spacemaker* [111] /*eyes shut* [46], encodes an agrin-related proteoglycan that is also expressed in the eye, where it fills the extracellular, intraommatidial space, optically isolating individual photoreceptors. However, *spam/eys* mutants do not show the severe uncoordination typical of mechanosensory bristle mutants, so it does not appear to be essential for bristle function.

Neuron and sensory process: ciliogenesis and intraflagellar transport

Differentiation of the mechanosensory neuron involves extension of the single sensory process, and the ciliary outer segment at its tip, as well as the projection of an axon to form synapses in the CNS. In es organs, the cilium is highly modified. Distal to a short connecting cilium, it expands to contain a tubular bundle, consisting of many tightly packed microtubules embedded in an electron-dense matrix (Fig. 4). In contrast to mammals, in which cilia are ubiquitous and ciliary signaling is essential for early development, *Drosophila* construct cilia or flagella only in type I sensory neurons and in sperm. Thus, mutants lacking cilia are frequently recovered in mechanosensory behavioral screens, with severe proprioception defects being their salient phenotype. For example, *uncoordinated* [54] encodes a basal body coiled-coil protein required for ciliary extension in sensory neurons, and flagellar integrity in sperm [8].

Many other such mutations affect an intraflagellar transport (IFT) complex, which is conserved throughout the eukaryotes and required for the assembly of almost all cilia and flagella [77, 80] (*Drosophila* sperm tails being a notable exception [41, 79]). IFT moves discrete protein particles along axonemal microtubules. Anterograde transport, towards the tip of the cilium, is driven by kinesin-2, and retrograde IFT depends on a cytoplasmic dynein. The 12–16 proteins that make up each IFT particle fall into A and B subcomplexes; in general, mutations affecting the B subcomplex proteins affect anterograde transport, and A subcomplex mutations disrupt retrograde transport. Many genes encoding IFT and other ciliary proteins are regulated by a winged-helix transcription factor, Rfx/DAF-19 [85]; the presence of an Rfx recognition site (X-box) in or near the first exon of a gene is a reliable indicator of its ciliary expression and function in nematodes [28] and flies [6].

*Drosophila* mutants lacking *Rfx* [25], the IFT-B protein IFT88 (*nompB*; [41]), or subunits of the heterotrimeric kinesin-2 (*Klp64D*, *KAP*; [79]) lack sensory cilia and mechanoreceptor responses. Mutants affecting the IFT-A retrograde subcomplex protein Oseg1/IFT122 have reduced outer segments [6]. Interestingly, the *Drosophila* homolog of the retrograde IFT dynein is encoded by the *beethoven* (*btv*) gene, which seems to be required for transduction in chordotonal organs but not bristles [26], suggesting that IFT-A proteins, but perhaps not retrograde transport per se, may be required to construct the highly modified outer

segment and tubular bundle. IFT is intrinsic to the ciliated neuron, but the effects of *nompH* and *Su(H)* mutations on the neuronal outer segment suggest that socket cell-derived signals, or emergent properties of the whole sensillum, are also needed for assembly or maintenance of the modified cilium. How such extrinsic influences interact with the IFT machinery to regulate ciliogenesis or ciliary maintenance is unknown, but very intriguing.

## Ion transport and transduction in es organs

The apical receptor lymph that is the ion source for the receptor current is unusual in having K+ and Na+ concentrations more like intracellular fluid. K+ in the haltere receptor lymph of the blowfly *Calliphora* was measured at 121 mM [39], in contrast to 13–25 mM in hemolymph. Potassium transport appears to be electrogenic. It correlates with the apical-positive TEP. A TEP was first observed in insect bristles by Küppers, Thurm, and Wessel [58, 88] who found that it depends acutely on oxidative metabolism and can be quickly reduced by oxygen deprivation or by inhibiting ATP synthesis. Similar TEPs and K+ concentrations were recorded in mechanoreceptor and contact chemoreceptor bristles in several insect orders (but not in the otherwise similar slit sensilla of spiders) [39, 58, 88]. As the receptor current is primarily carried by potassium, the driving force for the current is almost entirely electrical, being the sum of the TEP and the neuronal transmembrane potential relative to basal extracellular space.

The potassium-rich receptor lymph is strikingly similar to the endolymph that bathes the apical surfaces of the sensory hair cells in the mammalian cochlea. There, $K^+$ is transported into the endolymph, by the combined action of a $Na^+/K^-$ATPase and a $Na^+/K^+/2Cl^-$ cotransporter in the basolateral membrane of the supporting marginal cells, via a KCNQ1/KCNE1 channel in their apical membranes (reviewed in [100]). Does a homologous mechanism operate in fly bristles? Probably not. In the tormogen, it is the apical membrane that is highly infolded, and the more likely site of the ion pump. Further, the TEP is insensitive to the $Na^+/K^+$ ATPase inhibitor ouabain [87], and mutants lacking the only *Drosophila* KCNQ channel show no evidence of mechanosensory defects [101]. $K^+$ transport by the tormogen more likely involves the coupled activity of a proton-exporting V-ATPase and a $H^+/K^+$ exchanger (as yet unidentified in *Drosophila* [33]), which energizes the apical surfaces of insect gut, excretory, and salivary gland cells [102]. V-ATPase subunits are highly expressed in sensory tissues and are located on the apical membranes of sensory support cells, probably the tormogen or its equivalent, in moth sensilla [57]. *Drosophila* mutant for

V-ATPase subunits die as early larvae [19, 24], so mutants affecting this mechanism would not have been recovered in behavioral screens. Thus, mammalian cochlea and the insect bristle seem to have converged on an electrically driven $K^+$ influx as an energetically efficient receptor current, but have probably achieved this end by different physiological means. However, mutations in some human V-ATPase subunits can lead to sensorineural deafness [51, 83]; the disease etiology is unclear but again raises the possibility of some functional homology between the two systems.

Transduction, receptor potentials, and currents: *nompC*

Mechanical stimuli are transmitted to the neuronal outer segment by the bristle shaft, socket septum, and dendritic sheath. Bristles act as first-order levers, the shaft pivoting in the socket so that its base impinges on the dendritic sheath, compressing it against the outer segment membrane [86]; the minimum detectable displacement at the bristle base may be as small as 1–2 nm [99]. The functional morphology is clearest in the haltere campaniform sensilla: The outer segment in each sensillum is flattened at its distal tip, where it and the enclosing dendritic sheath are pinched between the jaws of the thickened socket septum; indentation of the dome forces the two halves of the septum together [53]. The tubular bundle that fills the outer segments is required for mechanotransduction. Disassembly of the tubular bundle in cricket sensory hairs during extended vinblastine treatment correlated with loss of mechanosensitivity [30]. High-resolution electron micrographs show "membrane-integrated cones" extending from the outermost layer of the microtubules to the ciliary membrane, specifically on the tubular bundle face that is compressed. Thus, compression of the ciliary membrane between the dendritic sheath and the tubular bundle is the proximate stimulus in es organs. However, gating arrangements that convert compressive forces on the membrane into shear or even tension on individual channels cannot be excluded.

An electrode in contact with apical space, via the hollow bristle shaft typically records an apical-positive TEP of 20 to 80 mV [54]. Displacement of the bristle tip by about 10 μm—a deflection of about 10°—causes a rapid drop in the TEP, reflecting a depolarizing receptor potential (Fig. 3). Clamping the TEP close to its resting level reveals a corresponding increase in the transepithelial current, reflecting an inward, mainly K+ current from the apical extracellular space into the neuron [99]. Consistent with the lever action of the bristle, the response is asymmetric. Deflections from a resting position downward in the plane of curvature of the bristle shaft show the greatest response, while upward or orthogonal movements cause little or no

change in TEP [99]; the absence of any hyperpolarizing potentials implies that little or no mechanogated current flows at rest. The response is detectable 200 μs after the stimulus onset. This latency, which includes any mechanical delay in relaying the stimulus to the neuronal membrane, is much too fast for the generation and diffusion of even the fastest second-messenger mediated pathways, so channel gating must be directly linked to the deformation of the overlying dendritic sheath.

The channel that generates bristle receptor potentials is, thus, most likely located in the ciliary outer segment and gated directly by mechanical action. It must be permeable to potassium, the charge carrier in vivo, but is most likely cation selective because receptor potentials are unaffected when with either $K^+$ or $Na^+$-containing saline fills the recording pipet. *nompC* mutations, which eliminate the major, adapting component of the bristle mechanoreceptor current, identified the locus that encodes the only known subunit of this channel [99]. NOMPC is a 1,619-amino-acid protein that, with its nematode and vertebrate homologs, constitutes the TRPN branch of the TRP channel superfamily. TRP channels vary greatly in their activation modes and physiological functions, but many form non-specific cation channels with high calcium permeability [66, 67, 69, 72]. Like other TRP channel subunits, NOMPC has cytoplasmic N and C termini, and six transmembrane alpha-helices with a pore loop located between the fifth and sixth transmembrane regions. The strongest evidence for a direct role of NOMPC in producing the receptor current is a missense mutation in the third extracellular loop that alters mechanoreceptor current kinetics, increasing the adaptation rate and reducing the total charge flow [99]. The subcellular location of NOMPC has not been confirmed in *Drosophila*, but its nematode ortholog, TRP-4, is expressed in the *Caenorhabditis* dopamine neurons CEP and ADE and is localized to their ciliary outer segments, which have tubular bundles like those in fly es organs [62, 99]. TRP-4 is also expressed in the nematode DVA and DVC interneurons where it is distributed along sensory axons and required for a proprioceptive response to body bending [62]. A TRPN homolog is found in zebrafish where it is required for normal transduction by lateral line hair cells [82]. In amphibian hair cells, it is concentrated on the kinocilium, the "true cilium" (as opposed to the actin-based stereocilia or stereovilli) of hair cells [81]. However, no TRPN homologs are encoded in the genomes of pufferfish, or of any mammal or bird—perhaps, it was discarded by terrestrial vertebrates as their auditory stimuli shifted into a higher, airborne range. Its retention or loss may reflect the extent to which the kinocilium and ciliary mechanisms participate in transduction in each species.

The N-terminal cytoplasmic regions of TRPV, TRPA, and TRPN proteins include different numbers of ankyrin repeats: NOMPC and its nematode and vertebrate orthologs have 29, the largest number in any TRP channel. Ankyrin repeats, each comprising a pair of short alpha-helices followed by a loop or hairpin, and tandem repeats can stack in a helical array [65]. The 29 repeats in NOMPC would form a full helical turn that could be an intracellular gating spring [45]; atomic force microscope measurements show that tandem ankyrin repeats can indeed behave as a spring [60]. The four N-terminal repeat domains in a tetrameric channel may together correspond to the "membrane-integrated cones" that link the ciliary membrane to the tubular body cytoskeleton [53]. A high degree of conservation of individual repeats between the NOMPC orthologs suggests that the N-terminal region may also have functions requiring more specific molecular interactions.

Most current models for sensory transduction also posit a direct connection between the channel and extracellular elements that are displaced or deformed by stimulation. However, the predicted topology of NOMPC shows no large extracellular domains: If transduction requires a mechanically stable connection between the channel and the dendritic sheath, it must be supplied by a different, as yet unidentified subunit. The persistence of a small, non-adapting receptor current in *nompC* null mutants also suggests the existence of a secondary or partially functional channel.

Bristle receptor potentials and currents both adapt within tens of milliseconds to give a peak followed by a shallow plateau that persists for seconds-long stimuli and returns to baseline at its offset (Fig. 3). Bristle adaptation reflects a shift in the displacement–response relationship rather than a reduction in sensitivity: a second, superimposed step stimulus gives a second response peak, while a positive response is seen at the offset of a sustained negative deflection [99]. This behavior and the time-scale of adaptation are reminiscent of the slow, myosin-driven adaptation seen in vertebrate hair cells, suggesting that bristle adaptation is also likely to be due to a motor-driven adjustment in the channel gating force. However, the adaptation mechanism is unknown. Given the microtubular cytoskeleton of the outer segment, as opposed to the actin filaments in the hair cell sterovilli, myosins are unlikely to be involved. Candidate microtubule-based adaptation motors include the kinesins and cytoplasmic dyneins associated with IFT, or the axonemal dyneins.

## Chordotonal organ structure and function

The distinctive scolopidial unit structure of chordotonal organs is conserved across insects and crustaceans [108]. The scolopale capsule, its central feature, encloses the ciliary outer segments of one to three neurons (Fig. 4);

support cells connect the scolopale and neurons to apical and basal attachment sites on the body wall. Scolopidia in different organs and insect species vary in the number of innervating neurons, their ciliary morphology, and the nature of their attachment to the body wall [108]. Scolopidia in *Drosophila* larvae each have a single neuron with a cilium attached to an elongated cap cell with a short dendritic cap; in most adult chordotonal organs, the scolopale encloses a pair of similarly attached cilia.

Each chordotonal cilium extends from two axially aligned basal bodies at the end of the neuronal sensory process. Unlike the highly modified outer segments of es organs, a ch cilium retains the axonemal structure of nine microtubule doublets for most of its length; its apical tip, where the cilium is enclosed by the dendritic cap, the doublets reduce to single microtubules. The axoneme is also modified at about two thirds of its length, where the doublets bulge out to form a ciliary dilation that encloses an electron-dense inclusion with a tubular or paracrystalline substructure; the function of this structure is unknown. As in es organs, assembly of chordotonal cilia depends on IFT. Cilia are absent in mutants lacking the transcription factor RFX, IFT-B proteins [41], or the IFT-associated kinesin-2 [79] and truncated in IFT-A subcomplex mutants (our unpublished data). The ciliary dilation is disrupted in *btv* mutants [26].

Chordotonal cilia are probably capable of active movement, and this may be essential for transduction. Grasshopper femoral chordotonal organs, when fixed in a stimulated position, were observed to be flexed [68]. Because each cilium is held at both tip and base, an active stroke will cause a helical writhe that shortens the overall ciliary length in opposition to the stimulus force, increasing the tension on any elements connected in series and making a sharp bend at the base [68, 94]. The dynein arms that drive axonemal bending in motile flagella and cilia are not prominent in sectioned chordotonal cilia, but axonemal-class dyneins that are expressed in female as well as male flies [76] are probably present in sensory cilia, as these contain the only axonemes in the fly other than sperm flagella. Ciliary motility may be abolished in *touch-insensitive larva B* (*tilB*) mutants, which specifically affect chordotonal organs and sperm flagella. Their sensory cilia show no evident structural defects, but mutant sperm flagella are fragmented and lack dynein arms [26].

The scolopale that encapsulates the cilia first appears as a cleft, which expands into two concentric cavities that ultimately coalesce into a membrane-lined space [13, 14], marked as apical by the Crumbs protein [46]. The cavity walls are reinforced by thick, cytoskeletal rods composed of filamentous actin and tropomyosin with some embedded microtubules [106]. The scolopale rods fuse together at the apex of the cavity where they converge on the dendritic cap; at their other end, they are anchored to the dendritic

inner segment and the ciliary rootlet by an electron-dense "necklace" and adherens junctions. These junctions seal off the scolopale cavity so that it can maintain a receptor lymph separate from the hemolymph [14], but it is not known if this fluid has a high $K^+$ concentration as in es organs, or if a TEP exists. Chordotonal organs do not express the NOMPH cytochrome that is needed for the tormogen to differentiate and generate the TEP in an es organ, and *nompH* mutants have normal chordotonal transduction [103].

Extracellular and cytoskeletal structures and intercellular junctions hold the scolopale and cilium in tension between the apical and basal attachment sites; the mechanical integrity of the unit is essential for transduction. The scolopale cell secretes the NOMPA matrix protein at its apical end to form the dendritic cap (Fig. 4) [17], but rather than ensheathing the entire outer segment, this is attached only at the tip of the cilium, distal to the ciliary dilation. *nompA* mutations, which disconnect the dendritic cap from the cilia, eliminate chordotonal responses [17], as do IFT and other mutations affecting ciliary integrity [8, 41, 79]. Apically, the dendritic cap is attached to a cap cell that is specialized as a tensile connective: It is filled with densely packed, aligned microtubules, and elongates greatly during larval growth. Mutations of cytoskeletal proteins including the microtubule plus-end-binding EB1 protein [29], the actin-microtubule linker and EB1 interactor Shortstop/Kakapo [74], or myosin VIIA [93], disrupt scolopidial organization, particularly at the apical attachment sites, reducing or eliminating chordotonal responses. Basally, the scolopale rods are connected via adherens junctions to the inner segment of the neuron, at the level of the proximal basal body. A long, striated ciliary rootlet or axial filament extends from the basal bodies through the neuronal cell body and into a basal process that is attached to a ligament or epidermal cell [42, 105, 106]. Striated rootlets are a general feature of ciliated cells and some are capable of calcium-dependent contraction [78]. Rootlet components include isoforms of centrin/caltractin, a calmodulin-related protein that regulates rootlet contractility [105, 106], and rootletin, a large coiled-coil protein required to maintain mammalian sensory cilia [109, 110]. *Drosophila* has genes encoding two centrin isoforms and a rootletin homolog. No mutations affecting either of these proteins have been identified, but the rootlet as well as the cilium is absent in *Rfx* mutants [25], so some RFX transcriptional targets must be involved in its assembly.

Chordotonal ion channels and transduction: *nompC*, *nan*, and *iav*

Because stimulus forces are distributed across the scolopidium, there are several sites where they could act on neuronal mechanogated channels to generate a receptor potential. These include the attachments of ciliary tips to

the dendritic cap, which is subject to stretch and/or shear under stimulation; the axonemal section, which may actively twist or flex when stimulated; the ciliary dilation; and the base of the cilium. Indeed, chordotonal mechanotransduction involves at least two TRP superfamily channels, which may be located at different sites: The TRPN channel encoded by *nompC* and a chordotonal-specific TRPV channel (Fig. 4).

*nompC* null mutants, which lack bristle receptor potentials show a substantial reduction in antennal sound-evoked potentials [26]. This could reflect a partial reduction in the activity of all chordotonal neurons, or a complete loss of response in a subset of neurons or of scolopidia. *nompC* is expressed in chordotonal organs [99] but its subcellular location is unknown. A likely location is the tip of the cilium, which contacts the matrix of the dendritic cap and so approximates the ensheathed es outer segment (Fig. 4). However, the expected stimulus force at this site tension or shear between the cap and the ciliary membrane—is different from the compression that is thought to stimulate the es outer segment: Perhaps, the channels open on a stimulus offset when tension is released.

A role for TRPV channels in chordotonal organs was revealed when targeted deletions of a predicted *Drosophila* TRPV subunit gene yielded mutant flies with mild uncoordination and reduced locomotion, similar to the behavior of the deaf *btv* and *tilB* mutants; these *nanchung* (*nan*) mutants indeed completely lack sound-evoked potentials [55]. Positional cloning of another behavioral mutation, *inactive* (*iav*), showed that it affects the only other TRPV gene in *Drosophila*, and *iav* mutants were also found to be deaf [34]. Both NAN and IAV have the five N-terminal ankyrin repeats and transmembrane topology characteristic of TRPV subunits; NAN has an unusually long extracellular loop between the first and second transmembrane regions. Both proteins are specifically expressed in chordotonal organs and localized to the cilia; each subunit fails to appear in mutants lacking the other, suggesting that they form a heteromeric channel [34]. A similar interdependence is seen in nematodes where coexpression of the IAV ortholog, OSM-9, with a NAN-related OCR subunit, determines their ciliary colocalization and sensory function [92].

A functional GFP-tagged IAV subunit locates the *Drosophila* channel on the proximal part of the cilium, up to, but not beyond, the ciliary dilation [34], so it cannot be activated by direct interaction with the dendritic cap, which contacts the cilium only distal to this point (Fig. 4). The extracellular, agrin-related protein Eyes shut, which is secreted into the scolopale cavity and concentrated over the middle part of the cilium [46], could possibly interact with the TRPV channel, but its distribution is not altered in *nan* or *iav* mutants (our unpublished data). Alternatively,

the NAN/IAV channel could be activated by tension in the ciliary membrane or by movements of the axoneme. Many TRPV channel proteins are activated by physical stimuli, including heat and hypotonic stress [70]. Either NAN or IAV, when individually expressed in cell culture, gives rise to hypotonically induced cation currents and calcium spikes [34, 55], but these currents, which develop over seconds to minutes of exposure to hypotonic stress, and do not require coexpression of both subunits, may not necessarily reflect the much faster sensory gating of the channel in situ.

## Johnston's organ: active mechanics in the fly's antennal ear

Johnston's organ, the large chordotonal organ in the second antennal segment, has long been identified as the insect transducer of near-field sounds, such as are produced by the mosquito's wing beat [48], the honeybee's dance [56], or the *Drosophila* courtship song [10]. These are detected as vibrations of the distal antennal segments. Bulk air particle movement close to a sound source deflects the arista, the feathery distal part of the antenna, and rotates the third segment on its vertical axis (Fig. 5). A hooked cuticular stalk forms the sole connection between the third and second segments, terminating at a flexible, annular joint enclosed in a socket. The stalk and socket walls are stiffened so that all movement is translated into a rocking of the ring-joint on its vertical axis [37]. Formation of this specialized organ from epidermal cells requires the redundant zinc-finger proteins encoded by *spalt* (*sal*) and *spalt-related* (*salr*): *sal salr* double mutants have fused segments and disorganized scolopidia and are completely deaf [23].

A multilayered, conical array of over 200 scolopidia converges on the attachment between the second and third segments. A subset of these, present in many insects but overlooked in *Drosophila* until recently, has three ciliated neurons [94]; the third cilium is more like an es outer segment, with a non-axonemal, microtubule-filled distal part [108]. All of the antennal scolopidia have elongated dendritic caps that extend beyond the scolopales and are attached directly to the flexible membrane of the ring-joint so that they are alternately stretched and relaxed when it vibrates. The response to sine-wave stimuli, recorded as sound-evoked potentials in the antennal nerve, has a strong frequency-doubling component, suggesting that at least two different groups of neurons respond to different phases of a stimulus [26]. These may correspond to neurons innervating the scolopidia on either side of the ring, or the different sensory neurons in each scolopidium. In *Drosophila*, Johnston's organ is broadly tuned to frequencies up to several hundred Hz, and is most effective in the range of 160–250 Hz, the range of frequencies in courtship song. It
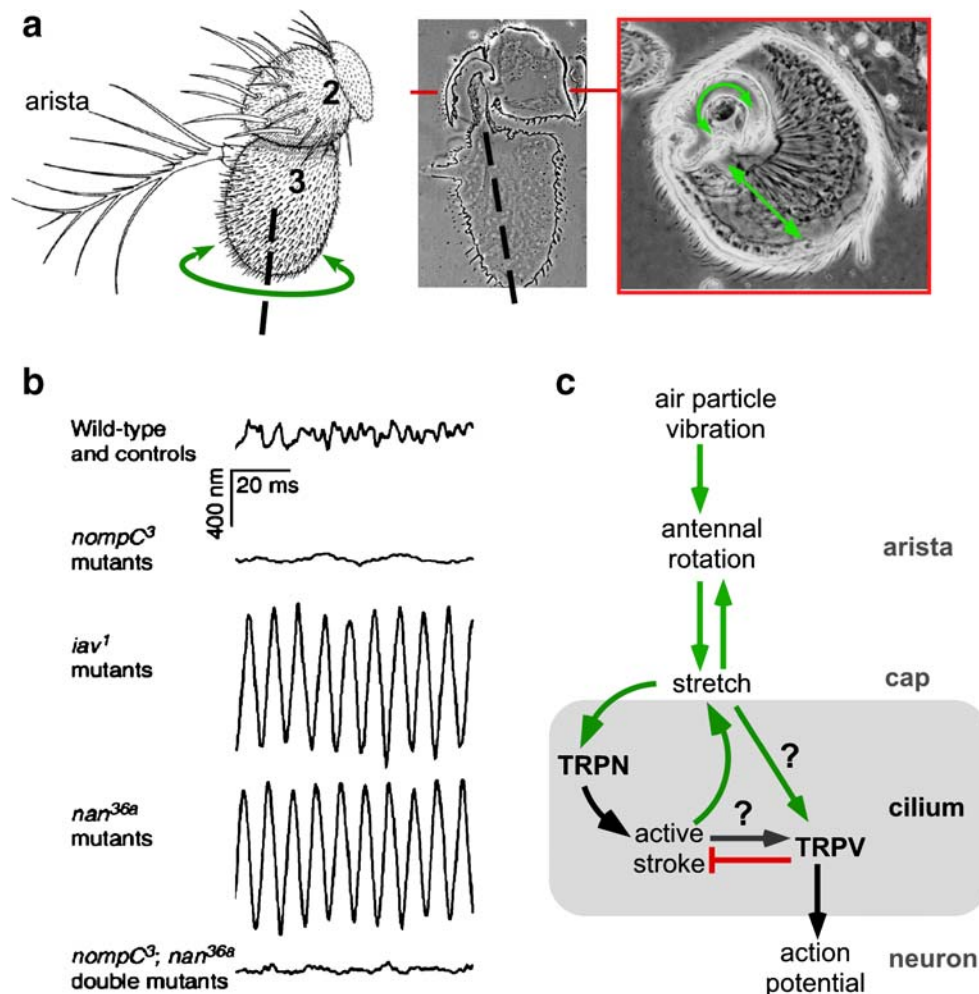
**Fig. 5** Laser vibrometry and active mechanics in Johnston's organ. **a** Antennal mechanics leading to transduction of near-field sound by Johnston's organ. Air-driven vibration of the arista rotates the third antennal segment [3] relative to the second [2]. *Dashed lines* indicate the axis of rotation. Rotation of the stalk connecting the segments stretches (*green arrow*) the dendritic caps attached to the articulating cuticle. Drawing by P. Bryant from Flybase; micrographs from [26]. **b** Spontaneous movements of the arista recorded by laser vibrometry in wild-type and mutant antennae. Wild type show active vibration of the arista in the absence of sound stimuli; these movements are abolished in *nompC* mutants but greatly increased in the TRPV channel mutants *iav* or *nan*. Data from Gopfert et al. [35]. **c** Proposed model [35] for the role of TRPN and TRPV channels in sound transduction and amplification based on vibrometry and electrophysiology data. Stretch transmitted by the dendritic caps to the cilia opens TRPN channels. This activates a ciliary stroke, further increasing tension on the transducers and, thus, forming a positive feedback loop. TRPV channels negatively regulate this feedback and are also required to depolarize the neuron to generate action potentials. The gating mode and stimulus that open the TRPV channels are unspecified

appears particularly sensitive to stimulus onsets or short pulses, consistent with the ability of female flies to discriminate species-specific, inter-pulse intervals in courtship song. Males as well as females can hear and respond to the song; there are no apparent differences between male and female *Drosophila* auditory anatomy or electrophysiology.

The direct, parallel coupling of many individual scolopidia to the distal antennal segments means that deflections of the arista indicate the stimuli that impinge on the scolopidia, and conversely, that any synchronous mechanical response of the scolopidia will result in movement of the arista. Antennal movements can be tracked precisely and without a mechanical load by laser vibrometry (Fig. 5)

[37, 56]. A low-power laser is focused on the arista. Vibration causes a Doppler shift and alters the interference between the incident and reflected light, and the displacement and velocity of the arista can be derived from these signals. These measurements show the fly antenna to behave as a damped harmonic oscillator [37]. However, while dead or $CO_2$-anesthetized flies show the constant resonant frequency and linear stimulus–response relation of a passive oscillator, live wild-type antennae show an adaptive, non-linear behavior. At low stimulus intensities (air particle velocities) they become more compliant, and their resonant frequency decreases—a characteristic feature of active auditory systems [38]. Furthermore, wild-type

antennae can oscillate in the absence of any stimulus [36, 38]. Thus, some motor element adds mechanical energy to the system to boost sensitivity, a function similar to the outer hair cells of the mammalian cochlear amplifier.

No muscles are connected to the distal antennal segments; the most likely motor elements are the chordotonal cilia, and the effects of ciliary mutations on the antennal mechanics support this idea. *nompA* mutant antennae, in which the cilia are disconnected from the distal segments, show a constant, reduced resonant frequency at all stimulus intensities and do not oscillate. *btv* and *tilB* mutant antennae, in which the cilia remain connected to the dendritic caps but are likely non-motile, also show linearized antennal mechanics and lack active oscillations, but are stiffer than in *nompA* mutants. *nompC* mutants, in which sound-evoked potentials are reduced, show reduced non-linearity and no spontaneous oscillations (Fig. 5). Taken together, the results indicate that the active vibrations are triggered by mechanotransduction and generated by ciliary motility. Mutating the somatically expressed axonemal dyneins would be a good test of this hypothesis.

The effects of the *nan* and *iav* mutations on antennal mechanics give revealing insights into the roles of TRPN and TRPV channels in mechanotransduction and amplification. Although sound-evoked potentials are completely abolished in *nan* and *iav* mutants, both show *increased* nonlinear amplification of input stimuli and continuous, large-amplitude spontaneous oscillations, suggesting an uncontrolled positive mechanical feedback [35]. These effects were abolished in *nompC; nan* double mutant antennae, which showed linear, passive properties similar to those of *nompC* alone [35] (Fig. 5). These unexpected results have several implications. An initial transducer, sufficient to trigger mechanical feedback, must persist in the TRPV mutants, but any initial receptor potential must be undetectable by antennal nerve recording and insufficient to generate neuronal action potentials. The IAV/NAN channel now appears unlikely to be the primary mechanotransducer, but may amplify the initial receptor potential. It could still be mechanogated, perhaps by membrane tension or cytoskeletal forces generated during an active ciliary stroke, but must also negatively regulate the active antennal amplifier, possibly by calcium regulation of ciliary motors (Fig. 5). Both functions are consistent with the location of IAV and NAN along the proximal cilium.

The involvement of NOMPC in both transduction and amplification makes it the better candidate for a primary chordotonal transducer channel, especially if it turns out to be located at the tip of the cilium. But because *nompC* null mutants do not completely eliminate the Johnston's organ response, some other source must also contribute to an initial receptor potential: This could be IAV/NAN, or another, as yet unidentified channel.

If the TRPV and TRPN channels pass similar cation currents into the cilium from the scolopale cavity, how can they have opposing effects on antennal mechanics? They could be spatially separated so as to localize current and calcium influxes to differently responding sections of the axoneme; The ciliary dilation, which sets a distal limit to the TRPV channel location, could be a calcium barrier or other functional separator. Alternatively, activation of the channels at different phases of a periodic stimulus may have different effects on ciliary mechanics. However, the NOMPC and TRPV channels could also be expressed in different neurons, or different subsets of scolopidia, that act respectively to amplify and to damp antennal movement. Precise localization of NOMPC within the cilia and higher-resolution assays of *nan*, *iav*, and *nompC* expression and function in the different subsets of Johnston's organ neurons and scolopidia will help to answer this question.

## Nociceptive mechanotransduction in type II neurons: *painless*

The non-ciliated type II neurons that innervate the larval and adult body wall have been proposed to be mechano-sensory, but in part, due to the difficulty of recording directly from individual md neurons, but there is little direct evidence for this due to the difficulty of recording directly from individual md neurons. Some show calcium responses in response to temperature shifts, as do larval chordotonal organs [63]. The ENaC channel Pickpocket1 (PPK1) is a member of the same epithelial sodium channel (ENaC) superfamily that includes the nematode body–touch transducer channels; other *Drosophila* ENaC channels have functions in tracheal fluid regulation, pheromone detection, and it is expressed in a set of the larval md neurons [1]. *ppk1* mutant larvae have an altered crawling pattern, making longer runs at increased speed, with fewer spontaneous stops and turns than wild-type larvae [2]. This suggests a defect in proprioception, but it is not known if the underlying defect is in fact mechanosensory.

A function for a type II neuron and a TRP superfamily channel in nociceptive touch was discovered by Tracey et al. [97] who found that larvae responded to noxious stimuli such as harsh touch, or contact with a high-temperature (<38°C) probe, with a writhing escape response distinct from the response to gentle touch. Three *painless* (*pain*) mutants that lacked the response to both heat and harsh touch all affect a gene encoding a TRPA channel subunit, which is expressed in a subset of larval md neurons with complex dendritic arbors (md–da class); the same channel has since been found also to be required in adults for thermal nociception [107] and avoidance of pungent isothiocyanates [3]. It is also expressed in

chordotonal organs, but as *atonal* mutants retain the writing response, chordotonal organs are apparently not required for nociception, nor are *pain* mutants defective in chordotonal transduction (our unpublished data). Antibody staining in md neurons showed the channel to be localized in discrete punctate associated with the dendrites; these are hypothesized to be specialized nociceptive structures [97]. Thus, this TRPA channel is sensitive to polymodal stimuli, including mechanical pain.

## Conclusion: progress and new challenges

From a state of complete molecular ignorance, genetic screens and targeted mutations have now identified many working parts of the fly's ciliary mechanotransducers—many of them novel and of such low abundance that it is difficult to see how else they could have been identified. However, the mechanotransducer "parts list" is by no means complete. Residual responses in *nompC* mutant bristles and chordotonal organs hint at another channel still to be discovered. Several other *nomp* loci remain to be identified. Moreover, the behavioral screens in which they were identified did not saturate the genome. Most of the identified proteins are large, and many are represented only by one or two mutant alleles; small mutagen targets, as well as redundant or viability-essential gene products probably remain to be found.

A tougher challenge is to understand how the identified channels interact with matrix and cytoskeletal elements to transduce, adapt to, and amplify stimuli. Defining the forces that impinge on the transducing proteins at the molecular level remains a problem. The gating of any mechanosensory channel is not yet characterized. If it requires channel subunits to interact with specific extracellular and cytoskeletal elements, reconstituting mechanotransduction ex vivo will require more complex expression systems than those currently used to assay channel function. However, functional dissection of the system in situ can still yield many insights. The first results from the vibrometry of wild-type and mutant antennae—currently, the only method to record the mechanical correlates of transduction in *Drosophila*—show the value of combining biomechanical measurements with the molecular information from genetic dissection. The adaptation mechanism in bristles seems particularly ripe for molecular and biomechanical analysis. If, according to current models, it involves the mechanical adjustment of gating tension in a channel-containing complex, screens for molecular interactions and genetic modifiers may be able to reveal some of the interacting components.

By offering the combination of molecular genetic tools with electrophysiological and biomechanical measurements, *Drosophila* mechanosensors provide a unique opportunity to manipulate identified sensory molecules and mechanisms. A truly molecular understanding of mechanotransduction will undoubtedly increase our understanding of the defects that underlie deafness and other mechanosensory disorders. But, ultimately, the joy and fascination of working with these exquisite sensilla is that, by bridging the scale gap between the macroscopic world of the experimenter and the molecular world of signal transduction, they give us a literal, not just a metaphorical, grasp on biological complexity.

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