Studies of mechanosensation using the fly

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Mechanosensation requires the transduction of mechanical stimuli into neuronal impulses. It encompasses not only the sense of touch but also proprioception and hearing. In contrast to sight, smell and taste, relatively little is known about the molecular machinery of mechanosensation. It is already clear, however, that important aspects are conserved across phyla, from Caenorhabditis elegans to humans. Drosophila melanogaster is well placed to make a significant contribution to this field. Its advantages include a sequenced genome allied with powerful genetic techniques, and the ability to conduct electrophysiological recording from mechanoreceptor neurons. For human geneticists, it is expected that Drosophila studies will provide a source of candidate genes whose human homologues can be examined for roles in mechanosensory development, function and disease.

ANATOMY OF MECHANOSENSATION

As an arthropod, Drosophila has a tough, mechanically resistant exoskeleton, which is not conducive to sensation of touch, and so this modality is largely detected via a battery of specialised mechanoreceptive sense organs inserted in the cuticle (external sense organs) (1). These are most noticeable as the sensory bristles that cover the fly (Fig. 1A). These organs are modifications of a basic structure that is also used for gustatory and olfactory sense organs. Each one consists of one or a few ciliated sensory neurons (type I neurons) innervating a structure formed by three specialised support cells, such as the bristle in its socket (Fig. 1B). Characteristically the neuronal dendrite has an axonemal segment with a $9 + 2$ arrangement of microtubules.

A distinct version of the type I sense organ is the chordotonal organ. In these internal proprioceptors, the neuron is typically suspended via its support cells between two areas of cuticle to allow detection of the relative movement of different cuticular structures; the dendrite is encased in a rigid scolopale structure (Fig. 1C and F). Unlike external sense organs, chordotonal organs are normally in organised arrays. A particularly large array within the antenna (called Johnston's organ) is adapted to detect sound transduced via vibration of the antennal capsule, as well as attitude with respect to gravity (2). Developmental and comparative studies suggest that external sense organs and chordotonal organs have a common evolutionary origin (3). It is probable that each mechanoreceptor type arose from exaggerations or reductions of different structural features found in a more generic primordial sense organ.

Although this brief review concentrates on type I sensory neurons, Drosophila also has non-ciliated sensory neurons (type II multiple dendritic neurons), which are not associated with specialised sensory structures. These neurons may be pain receptors, but they also have certain similarities with the major non-ciliated touch neurons in C. elegans and so may be touch sensitive (for instance, innervating the gut or the flexible larval cuticle).

Drosophila type I sensory neurons are strongly reminiscent of ciliated mechanosensitive cells in other organisms, including amphid neurons in C. elegans and cells associated with hearing in vertebrates (hair cells). In particular there is interest in the possibility that they represent a model of hair cell structure and function. There are many differences between these cells as well as intriguing similarities and it is not yet clear which of these is the more significant. Hair cells transduce sound via groups of stereocilia, which are not true axonemal cilia but are actin-rich outgrowths from microvilli; however these cells also have a true kinocilium with a $9 + 2$ arrangement of microtubules.

PHYSIOLOGY OF MECHANOSENSATION

Significantly, the type I sensory dendrite and hair cell stereocilia are both bathed in a characteristic high $K^+$, low $Ca^{2+}$ endolymph secreted by the supporting cells (4). For type I neurons it is envisaged that movement of the ciliated dendrite relative to surrounding structures causes the opening of cation channels and an influx of $K^+$, leading to neuronal depolarisation. This can readily be recorded during the deflection of individual adult bristles (5). Interestingly, depolarisation latencies of $\sim 200 \mu s$ have been recorded in a recent study, which is far too fast for any known second messenger cascade (6). Thus, unlike gustation, olfaction and photo-reception, mechanotransduction may involve the direct gating of the ion...
channels by mechanical force. This has been taken to suggest that mechanotransduction conforms to the gating-spring model proposed for the action of hair cell stereocilia (7,8). This model posits the existence of an ion channel anchored under tension between the dendritic cytoskeleton and the extracellular dendritic cap (an ECM structure secreted by the scolopale/sheath cell) (6) (Fig. 1D). Movement of one anchor relative to the other would ‘pull’ the channel open. Such a model also incorporates an explanation of adaptation—the process by which sensory receptors can ‘learn’ to ignore a sustained pre-existing stimulus and adjust their dynamic range to respond to a new or additional stimulus. Adaptation to a sustained deflection has been demonstrated for bristles by electrophysiology (6). In terms of the gating-spring model, adaptation is perceived as a sliding of the intracellular channel anchor along the cytoskeleton to ‘reset’ the tension on the channel, and it is easy to envisage how a molecular motor could be involved. Interestingly, differences in adaptation speed could provide a mechanism for sensory specialisation among different chordotonal organs. Electrophysiological studies on larger insects have provided evidence for different chordotonal neurons responding to stretch position (activated throughout the duration of the stretch) or velocity (activated only during the stretching movement), or even acceleration (activated only at the start of a stretching movement) (e.g. ref. 9). A mechanism could be that these simply reflect differing speeds of channel sliding during adaptation (slow for position-dependent, fast for velocity-dependent).

GENETICS OF MECHANOSENSATION

A prime advantage of Drosophila as a model of mechanosensation is the potential for genetic screens to discover molecular components. This was demonstrated by the behavioural screens for mutants that exhibited either touch insensitivity as larvae or adult uncoordination (indicative of gross mechanosensory deficit) (10). If one is interested specifically in mechanotransduction, the downside of these kinds of assay is that they could detect mutations that alter the development or function of any step in the touch pathway (from sensory structure, to sensory transduction, to CNS processing,
to generation of effector motor signals). Nevertheless, many of the mutations isolated were clearly demonstrated to lack bristle mechanoreceptor potentials, pointing to a primary sensory defect (10). These include uncoordinated, uncoordinated-like, and no mechanoreceptor potential (nompA–D). Most of them remain to be characterised molecularly.

Interestingly, all but one of the mutations that had reduced or absent bristle mechanoreceptor potentials were later shown to have defective hearing too (lack of sound-evoked antennal potentials), suggesting that the genes encode components common to the mechanotransduction apparatus of both bristles and chordotonal organs (11), thereby supporting the strong structural and functional connection between these sense organs. One mutation showed a chordotonal-specific transduction defect. This mutation, touch-insensitive-larva B (ttiB) was originally characterised through a reduced larval response to touch (10), even though it generates normal bristle mechanoreceptor potentials.

Eberl et al. carried out a separate specific screen for mutant flies defective in hearing (12). To screen for hearing loss, these authors assayed a behaviour that relies on the wing-generated auditory cue that male Drosophila use during courtship (so-called love song). Male Drosophila vigorously court one another if experimentally presented with a recording of the courtship song, thereby giving a rapid and non-invasive assay of mutagenised flies. Many of the isolated ‘courtship defective’ mutants showed normal sound-evoked antennal potentials, suggestive of defects neurally downstream of mechanotransduction (11). One mutation lacked sound-evoked potentials and was named, inevitably, beethoven (btv) (11). This deaf mutant has ultrastructural defects in the dendritic cilia of chordotonal organs, but its molecular identity is not yet known.

These screens have demonstrated the utility of the genetic approach, but have so far only scratched at surface. For instance, both the adult uncoordination and the hearing screens have only covered the second chromosome (10,12). In addition more sophisticated genetic approaches will undoubtedly be used in the future, such as using a sensitised system in genetic modifier screens.

MOLECULES OF MECHANOSENSATION

Only a few mechanosensation protein components have been characterised so far, but these few are very significant. As intimated above, the nomp genes are now known to encode mechanotransduction molecules required in both bristles and chordotonal organs. The nompC gene product is a cation channel belonging to the TRP family, with six transmembrane domains and a ion pore loop. Mechanoreceptor potentials are strongly reduced in the null nompC mutant, making it a candidate for the spring-gated channel. This is notably supported by the presence of 29 ankyrin repeats in the NompC protein intracellular domain – the largest number known of any protein (6) and giving plenty of scope for interaction with cytoskeleton and/or intracellular adaptation machinery (Fig. 1D). But NompC has little extracellular domain and it is suggested that another unidentified protein in a multimeric channel complex could provide the platform for the extracellular link. Another possibility is that NompC is a secondary (downstream) channel activated in response to the (unknown) mechanically gated channel, particularly since there remains some residual channel activity in a nompC null mutant (6), especially in chordotonal organs (11). Important unresolved questions include the cellular location of NompC, which will give a clue as to its function. It is encouraging that a C. elegans NompC homologue has been located at low resolution in the ciliated dendrites of amphid neurons (6). Finding the proteins with which NompC interacts extra- and intracellularly will be crucial. It is notable that the predicted vertebrate spring-gated channel has not yet been identified, and so the many vertebrate TRP members are candidates.

The nompA mutation has also been characterised recently (13). The structural basis for the lack of mechanoreceptor potential was found to be detachment of the dendrite from the bristle. Fittingly, NompA is a ZP domain-containing protein that is a key component of the dendritic caps of bristles and chordotonal organs (14) (Fig. 1E and F), and is a candidate for the extracellular component that tethers the ion channel (Fig. 1D).

Other components of the mechanosensation machinery are likely to include specialised cytoskeletal proteins (such as MAPs), motors, and cell junction molecules. Some such molecules are very important for hair cell cytoarchitecture, including a number of myosins (15). Will these be important in Drosophila sense organs, even though the cilium is tubulin-based? Preliminary evidence suggests that mutation of unconventional myosin VIIa (crinkled) causes complete deafness (S.V. Todi and D.F. Eberl, personal communication). The human homologue of this gene is responsible for the deafness syndrome, Usher Syndrome 1B.

Although little is known, type II non-ciliated neurons appear to have a distinct mechanism of mechanotransduction. Some are known to express a member of the DEG/EnaC family of Na+ channels (pickpocket) (16). Some vertebrate members of this family are implicated in touch, and members were identified (as degenerins) in the touch screens of C. elegans, which dissected the function of its non-ciliated body-touch neurons. Thus there may be two separate mechanisms for mechanosensation, both of which may be conserved in principle across several phyla.

DEVELOPMENTAL GENETICS OF MECHANOSENSATION

The precursor cells of all Drosophila type I sense organs are specified by the expression of transcription factors of the bHLH class (proneural proteins), including achaete-scute for external sense organs and atonal for chordotonal organs (17). Specification by variants of a single protein class supports the impression that sense organs differ from each other by relatively few gene products or even by quantitative differences rather than qualitative ones. An outstanding problem is that there is very little understanding of the genetic pathways that link these lofty regulatory factors with the ultimate components of sense organ structure and function. These pathways clearly involve the homeodomain transcription factor, Cut, which is activated by achaete-scute and inhibited by atonal (18). Cut is most strongly implicated in controlling support cell morphology,
which is the major influence on sense organ structure, but nothing is known of how it achieves this molecularly (3,19).

Is there an underlying developmental homology between sense organs (chordotonal organs particularly) and hair cells? A striking finding suggesting this might be so is that MATH1, one of atonal’s two closest relatives in mouse, is required for hair cell determination (20). MATH1 expression can efficiently rescue the atonal mutation in flies (21), suggesting that the biochemical characteristics of the Atonal/MATH1 proteins are conserved. There are also apparent differences between the phenotypes: atonal is required for precursors of the whole sense organ whereas MATH1 is not required for support cells or the sensory neurons, but this might be a rather superficial distinction. Does this suggest a common ancestor that had a primordial mechanoreceptor regulated by a proto-atonal? The answer at present is: primordial sense organ/neuron - possibly; primordial auditory receptor - probably not. Chordotonal organs are first and foremost proprioceptors, some of which have secondarily been adapted (or exapted) to detect sound. Moreover, the Drosophila proneural gene, amos, which is as similar to MATH1 as is atonal, is required for olfactory receptors rather than mechanoreceptors (22). It is human nature to pick out patterns and similarities among a host of information. In what way these patterns are meaningful is not yet clear. To go further, there is a major need to bridge the upstream developmental aspects and downstream structural/functional aspects of mechanosensation. Whilst the extent and level of anatomical and functional homology are debatable, it is clear that at least some common genetic pathways are involved. There is no doubt that Drosophila studies will make an impact on the search for human genes involved in mechanosensation.

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REFERENCES