

Development of Johnston's organ in *Drosophila*

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ABSTRACT Hearing is a specialized mechanosensory modality that is refined during evolution to meet the particular requirements of different organisms. In the fruitfly, *Drosophila*, hearing is mediated by Johnston's organ, a large chordotonal organ in the antenna that is exquisitely sensitive to the near-field acoustic signal of courtship songs generated by male wing vibration. We summarize recent progress in understanding the molecular genetic determinants of Johnston's organ development and discuss surprising differences from other chordotonal organs that likely facilitate hearing. We outline novel discoveries of active processes that generate motion of the antenna for acute sensitivity to the stimulus. Finally, we discuss further research directions that would probe remaining questions in understanding Johnston's organ development, function and evolution.

KEY WORDS: *audition, hearing, scolopidia, chordotonal organ, active mechanics*

Introduction

Practically the entire progress in genetic and molecular elucidation of hearing mechanisms in the fruitfly, *Drosophila melanogaster* has occurred in the last decade. The Johnston's organ (JO), located in the fly's antenna, formally has been confirmed as the major auditory organ and mutations in many genes required for hearing have been identified using a variety of approaches. These include mutagenesis screens, gene expression patterns and other candidate gene approaches including homology to known human hereditary hearing loss genes—almost all of which have been identified over the same time period. Thus, *Drosophila* hearing research has metamorphosed into an exciting research field that ties together intricate physiological and mechanical mechanisms with complex developmental biology. Several reviews have summarized aspects of this progress (Eberl, 1999, Caldwell and Eberl, 2002, Jarman, 2002, Robert and Göpfert, 2002, Todi *et al.*, 2004, Boekhoff-Falk, 2005). In this paper, we first focus on advances that reinforce the emerging conclusion that the JO is much more than a mere recapitulation of the canonical larval chordotonal sense organ type. Instead, the *Drosophila* JO represents an evolution to a highly specialized organ for hearing, clearly chordotonal in nature, but with some features as different from ancestral proprioceptors as the butterfly is from the caterpillar. Second, we highlight some important questions raised by these considerations, the answers to which will represent significant advances in the decade(s) to come.

Drosophila chordotonal organs and their functions

Selection pressures on the functions of specific sense organs have long-term effects on whether those functions will be maintained and further perfected, whether functions will be attenuated, even lost, or whether novel functions will arise. The diverse chordotonal organs of *Drosophila* almost certainly derive from a common ancestral mechanosensor whose developmental genetic program has been modified in multiple ways to generate chordotonal organs of distinct sizes and functions.

In the larva, the eight scolopidia (five of them shown in Figure 1) per abdominal hemisegment provide touch sensitivity and during locomotion they provide sensory feedback to the locomotor circuit (Kernan *et al.*, 1994, Caldwell *et al.*, 2003). In the adult, the femoral chordotonal organs (fCHO) comprise three clusters totaling about 74 scolopidia (Figure 2) per leg (Shanbhag *et al.*, 1992) and provide proprioceptive feedback. In the absence of this feedback, chordotonal mutant flies are somewhat ataxic (Eberl *et al.*, 2000). The fCHO has been shown to participate in a resistance reflex (Reddy *et al.*, 1997). The wing and haltere chordotonal organs (Miller, 1950) provide proprioceptive feedback during flight. This feedback is essential, as strong chordotonal mutants are flightless (Eberl, unpublished). Wing chordotonal organs also provide feedback during singing. Males generate courtship songs by unilateral wing extension and vibration and the courtship songs

Abbreviations used in this paper: fCHO, femoral chordotonal organs; JO, Johnston's organ.

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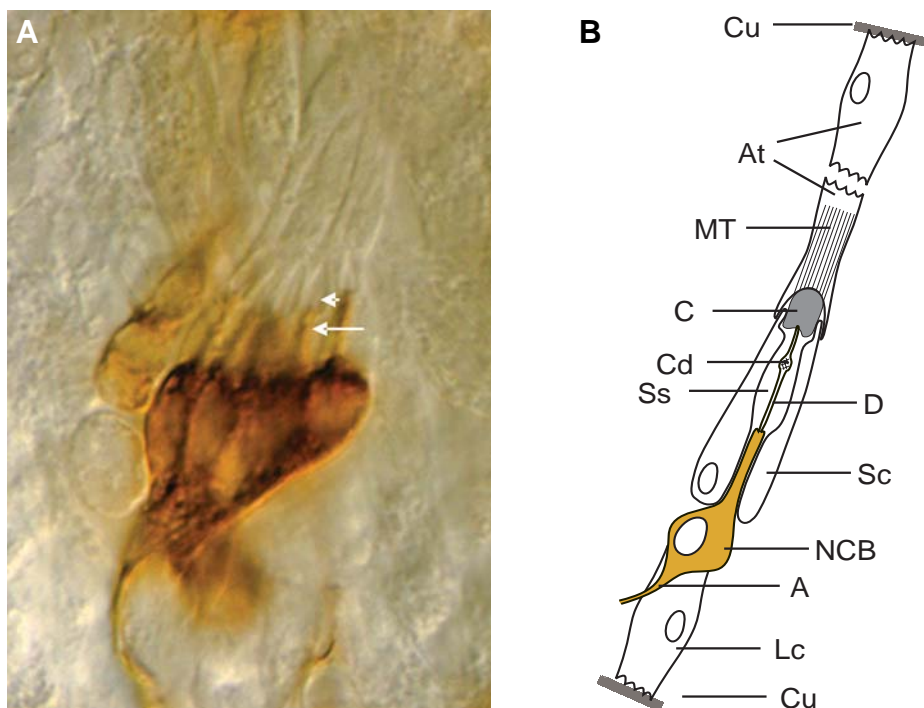


Fig. 1. *Drosophila* embryonic chordotonal organs. (A) The pentascolopodial organ (*lch5*) in the embryonic lateral body wall stained with monoclonal antibody 22C10, visualized with DAB and viewed with Nomarski optics. This antibody stains the Futsch protein in the membranes of all neurons. In chordotonal neurons it stains the inner dendritic segment (arrow) but not the cilium (arrowhead). The scolopale space is visible in relief surrounding the cilium. **(B)** Schematic of embryonic chordotonal organ structure. Abbreviations: A, axon; At, attachment cell; C, dendritic cap; Cd, ciliary dilation; Cu, cuticle; D, dendritic outer segment; Lc, ligament cell; MT, microtubules; NCB, nerve cell body; Sc, scolopale cell; SS, scolopale space.

and wing positioning of chordotonal mutant males are aberrant (Tauber and Eberl, 2001), likely because direct feedback of the wing chordotonal organ onto the singing motor circuit is lacking. Chordotonal organs of the bilateral Wheeler's organs, each with about 20 scolopidia in the first two abdominal sternites (Miller, 1950, Bodmer *et al.*, 1987, Jarman *et al.*, 1993, Elliott *et al.*, 2005) have not been functionally characterized, but are likely to have a sensory function either in abdominal position or abdominal distension. A prothoracic chordotonal organ with about 20 scolopidia (Hertweck, 1931, Power, 1948, Miller, 1950, Jarman *et al.*, 1993, Phillis *et al.*, 1996), connecting the prothoracic sternites to the sternal apodeme, has not been tested functionally in *Drosophila*, but in the blowfly, *Calliphora*, has been shown to respond to changes in head position (Milde *et al.*, 1987, Strausfeld *et al.*, 1987).

Interestingly, a homolog of this chordotonal organ appears to have evolved into a tympanal auditory organ in two independent dipteran lineages: tachinid and sarcophagid flies (Lakes-Harlan *et al.*, 1999). These authors also show that in a related non-hearing sarcophagid, the prosternal chordotonal organ is present and responds physiologically to direct mechanical stimulation of the prosternal cuticle but not to airborne sound presented in the same stimulus patterns. Thus, cuticular thinning appears to be a major prerequisite for efficient acousto-mechanical transformation in tympanal organs; nevertheless, molecular and cellular

modifications in the sensory organs also are likely to be necessary.

JO is the largest chordotonal organ in the fly, with about 227 scolopidia (Kamikouchi *et al.*, 2006) in the second antennal segment (a2) (Figure 3). Electrophysiological analysis was used to demonstrate that JO mediates hearing (Eberl *et al.*, 2000). In insects such as mosquitoes, auditory function can be even more highly elaborated, as evidenced in several ways. First, numbers of scolopidia are very high—over 7000 in male *Aedes aegypti*—resulting in auditory organs as prominent as the eyes (Boo and Richards, 1975a, Boo and Richards, 1975b). Secondly, the morphological diversity appears greater, with as many as four clear morphological types (Boo and Richards, 1975a, Boo and Richards, 1975b). Thirdly, flagellar size, shape and branching arrangement are more extensive in mosquitoes. Fourthly and perhaps complementing this, the auditory physiological mechanisms in the mosquito antenna include more complex dynamics, including frequency matching during courtship and more complex non-linearities in the antennal mechanics (Göpfert and Robert, 2001a, Gibson and Russell, 2006, Jackson and Robert, 2006).

While hearing is the best-characterized function of JO in *Drosophila*, this organ also appears to be important for gravitactic behavior (Armstrong *et al.*, 2006). A third function of JO, by homology to other Diptera (Burkhardt, 1960, Gronenberg and Strausfeld, 1990), likely involves detection of wind currents, either during flight or during walking. Indeed, *Drosophila* shows a strong anemotactic response in flight (Budick and Dickinson, 2006) and possibly when walking (Johnston, 1982), though in neither of these studies was the identity of the sense organ addressed. In other insects that use the antenna as a tactile organ, such as the green stink bug *Nezara viridula* (Jeram and Pabst, 1996), JO contributes to touch sensitivity as well. These different functions and any additional functions not yet discovered, are likely to impose certain conflicting constraints on the genetic architecture by which chordotonal organs develop and by which they diversify.

Comparative analysis of *Drosophila* chordotonal organ morphology and development

In this section we will discuss morphological and developmental similarities and differences between JO and other chordotonal organs in *Drosophila*. In addition to JO (Figure 3), two other well-studied *Drosophila* CHOs are the pentascolopodial organ (*lch5*) of the larval abdominal body wall (Figure 1) and the femoral chordotonal organ (fCHO) of the adult leg (Figure 2). Our understanding of most developmental events in the formation of JO has

been inferred by extrapolation from detailed studies in these other two organs. However, growing evidence of differences in developmental genetic mechanisms among *Drosophila* CHOs makes direct analysis of JO imperative. These differences also underscore the uniqueness of JO and provide evidence of genomic plasticity in ontogeny and evolution.

JO contains 477 ± 24 neurons in an estimated 227 scolopidial units (Kamikouchi *et al.*, 2006). This estimate is based on the observation that 10-15% of JO scolopidia include three neurons (Todi *et al.*, 2004), the remainder two neurons. All CHOs in which it has been investigated utilize the basic helix-loop-helix (bHLH) proneural transcription factor Atonal (Ato) for specification of their sense organ precursors (SOPs) (Jarman *et al.*, 1993, Jarman *et al.*, 1995). In the antennal imaginal disc at mid-third instar, there is a complete ring of *ato*-expressing cells in presumptive a2. This ring resolves by late third instar into stronger expression in the three clusters of presumptive SOPs that give rise to JO (Jarman *et al.*, 1993). JO neurons become recognizable by staining with the pan-neural antibody, 22C10, as early as three hours after pupariation and within the next five hours all JO neurons appear, loosely organized into three clusters, j1, j2 and je (Lienhard and Stocker, 1991). Such clustering is no longer evident in the fully differentiated adult antenna. Interestingly, the early neurons in the j1 cluster stain more lightly with 22C10 than those in j2 and je (Lienhard and Stocker, 1991). Understanding whether this stain-

ing pattern is related to the distinction between triply and doubly innervated neurons, or to the cytoplasmic differential electron density between the two neurons of a given scolopidium (Todi *et al.*, 2004, Yack, 2004), will require a more detailed characterization of the cell division patterns of JO precursors as well as a careful clonal analysis of all the cell types.

In contrast to JO, fCHO consists of ~71-74 scolopidial units (Shanbhag *et al.*, 1992). In the adult leg, these scolopidia are clustered into a large ventral group of about 32 scolopidia and two central groups of 14 and 25-28 scolopidia. While the ventral cluster makes distal attachments with the inner cuticle, the two central clusters attach apically to muscle group membranes instead of to cuticle (Shanbhag *et al.*, 1992). Each fCHO scolopidium contains two neurons, one being more electron-dense than the other, as also described for JO. However, no triply innervated scolopidia are seen. The genetic mechanisms in the early development of fCHO SOPs have been studied in some detail, though the post-specification events that give rise to the three separate clusters have yet to be scrutinized. In apparent contrast with the JO, the fCHO scolopidia arise through reiterative recruitment of SOPs from the relatively small patch of *ato*-expressing proneural cluster cells (zur Lage and Jarman, 1999, zur Lage *et al.*, 2004). This recruitment is achieved through EGF signaling by nascent SOPs to the overlying proneural cluster. This signaling antagonizes Notch-mediated lateral inhibition, permit-

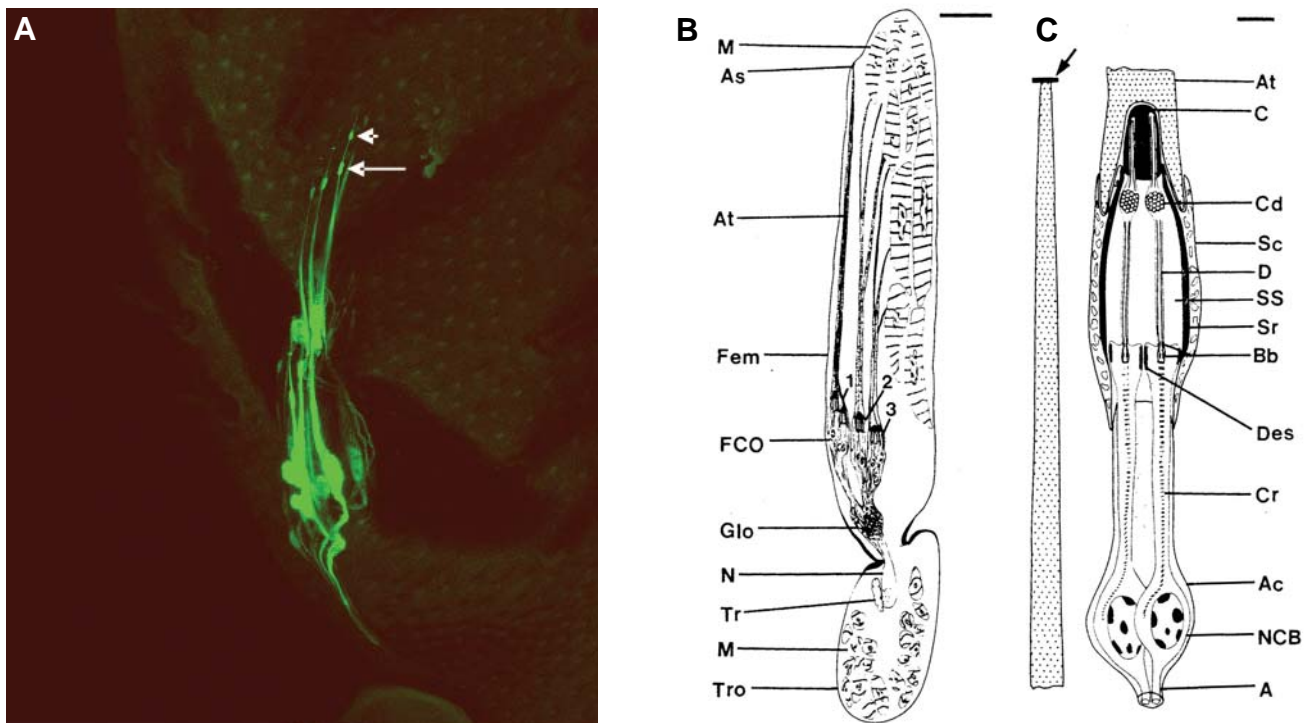


Fig. 2. *Drosophila* femoral chordotonal organ. (A) Proximal femur of a fly in which JO15-Gal4 drives UAS-GFP expression sporadically in a few neurons of fCHO. The inner dendritic segment ends at the basal body (arrow). The outer dendritic segment is a cilium extending from the basal body and includes the ciliary dilation (arrowhead). **(B)** Schematic drawing through the femur (Fem) and trochanter (Tro) of the leg, showing the arrangement of 3 groups of scolopidia. Scale bar, 50 microns. **(C)** Detailed drawing of a fCHO scolopidium from distal attachment (arrow) to the level of the axon (labeled A). The attachment cell fragment on the left is drawn at 1/4 scale compared to the rest of the scolopidium on the right. Abbreviations: A, axon; Ac, accessory cell; As, attachment site; At, attachment cell; Bb, basal bodies; C, dendritic cap; Cd, ciliary dilation; Cr, ciliary root; D, dendritic outer segment; Des, desmosome; Fem, femur; Glo, glomerulus; M, muscle; N, nerve; NCB, nerve cell body; Sc, scolopale cell; Sr, scolopale rod; SS, scolopale space; Tr, trachea; Tro, trochanter. Scale bar, 1 micron. (B,C) are reprinted from Shanbhag (1992) with permission from Elsevier.

ting persistent expression of the proneural gene *ato* within the cluster as well as facilitating recruitment of additional SOPs from the cluster. This elegant mechanism has not been described for JO, despite its larger numbers of scolopidia. If indeed there is EGF receptor mediated recruitment of SOPs in JO, it is likely to be much less extensive than in fCHO, as the a2 ring of *ato* expression in the imaginal disk is also broader at the outset, likely generating more SOPs, perhaps even all of them, during a single round of SOP specification. However, instead of increasing SOP number for JO, recruitment could underlie the multiple innervation of scolopidia. There is precedent for this in the olfactory sensillae in a3 where different neurons (some of which are derived from *ato*-expressing SOPs) within a sensillum are recruited as versus clonally derived (Sen *et al.*, 2003). Experiments that address the lineages and the possibility of recruitment in JO development are still lacking.

The larval lch5 consists of five scolopidial units. Not only do these CHOs differ from JO and fCHO in scolopidial number, but the cellular composition of these scolopidia differs as well. lch5 is the only *Drosophila* CHO for which the entire cell lineage and division patterns have been elucidated (Brewster and Bodmer, 1995). For lch5, each scolopidium consists of four cells derived by asymmetric cell division from a single sense organ precursor (SOP). These are: a neuron, a scolopale cell, a ligament cell and a cap cell. In addition, each SOP of lch5 gives rise to a single epidermal cell. Using clonal analysis, Brewster and Bodmer (1995) showed that one daughter cell produced by the first division of each lch5 SOP goes on to generate the cap cell and an

ectodermal cell, while the other daughter first spawns the ligament cell, followed by the scolopale cell and the neuron. A variation on this lineage pattern is seen in the ventral chordotonal pair, vchA and vchB, where the prospective neuron divides further to cast off an additional cell which becomes a multidendritic neuron that differentiates without further association with the mature scolopidium.

Using arguments of genetic and developmental homology in lineage relationships, Lai and Orgogozo (2004) suggest that sense organs are derived from an ancestral sense organ type and that the lineages of modern *Drosophila* sense organs show variations of this common pattern. Thus, the ectodermal cell that arises from the embryonic chordotonal lineage may correspond to the cell in external sensory lineages that makes the external structure—the hair shaft of bristle organs or dome of the campaniform sensillum. In this model, this cell in CHOs is involved in the epithelium in scolopidial attachment instead of elaborating an external structure.

From the discussion so far, it would seem that embryonic CHOs are singly innervated while adult CHOs are multiply innervated. Interestingly, however, the Wheeler's organ is an adult CHO whose scolopidia have only a single neuron (Elliott *et al.*, 2005). So the number of neurons per scolopidium is a flexible parameter in the adult that may, under selection, change to whatever confers maximal physiological advantages.

Furthermore, whether additional, non-CHO neurons arise out of CHO lineages in adults as they do in embryos has not been well studied. In a2 and femur, there have been no neurons described

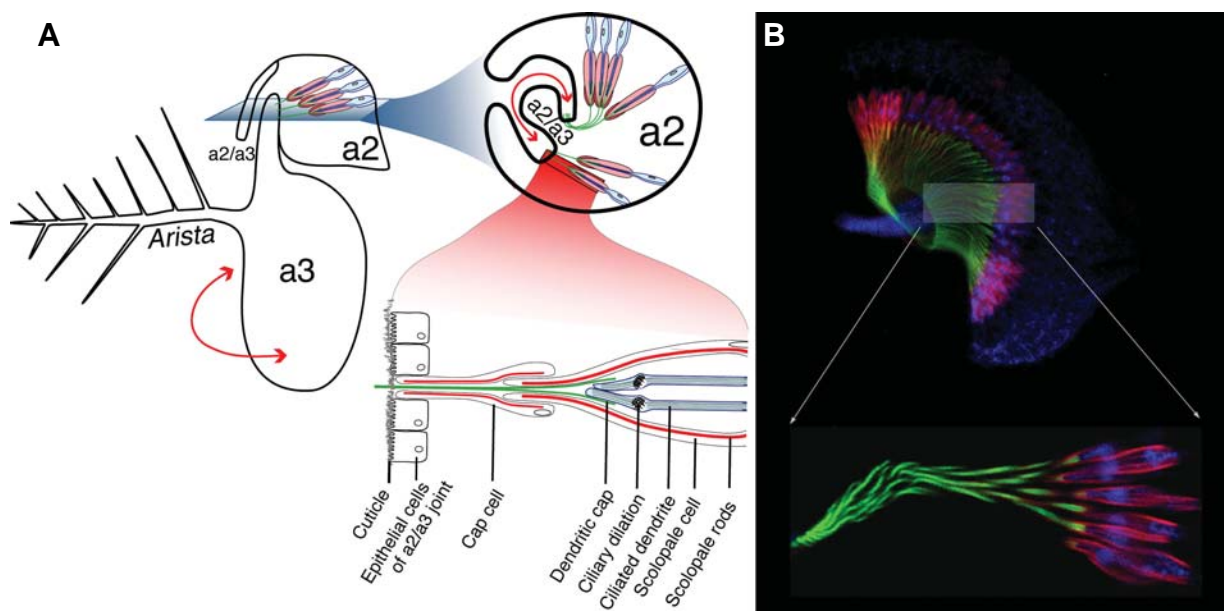


Fig. 3. Johnston's organ in *Drosophila*. (A) Schematic drawing of the *Drosophila* antenna and the sensory units of Johnston's organ. Acoustic activation of the arista causes rotation (doubled-headed red arrow) of antennal segment a3 relative to a2 at the a2/a3 joint. Cross-sectional view through a2 shows the arrangement of scolopidia with apical attachments at the joint. Expanded view of the distal half of one scolopidium shows the arrangement of cellular components in relation to the cuticle, the tubular structure of the extracellular dendritic cap (green) enclosing the tips of the ciliated outer dendritic segments of the neurons and the thick actin-based rods (red) in the scolopale and cap cells. (B) Confocal image of pupal antenna cells expressing the GFP-NompA protein in the dendritic cap (green), counterstained with Cy3-phalloidin (red) to reveal the scolopale rods in the scolopale cells and cap cells and stained with anti-horseradish peroxidase to label the neuronal membranes (blue). Inset shows higher magnification optical section of several scolopidia.

that would be obvious candidates, so this mechanism would require migration of such neurons to other parts of—or even out of—the limb. This is possible but not likely. However, development of the adult abdominal and thoracic CHOs has not been studied well enough to either favor or disfavor this mechanism. In any of these cases, clonal analysis would provide the answers.

The apical ends of chordotonal neurons are associated with a dendritic cap, an extracellular matrix structure whose morphology can take one of two major forms (reviewed by Field and Matheson, 1998, Yack, 2004). In one form, the mononematic type, the major connection between the cuticular (or muscle membrane) attachment site and the cilium is a microtubule-rich attachment cell. The electron-dense extracellular material is compact and localizes primarily at the junction between the cilium and the attachment cell. In *Drosophila*, the lch5 scolopidia are of the mononematic type, as are the fCHO (Shanbhag *et al.*, 1992). In the second, amphinematic, type, the extracellular material of the cap forms a long hollow tube that encloses the ciliary tips and extends all the way into the cuticle at the attachment site. The *Drosophila* JO scolopidia are clearly amphinematic by this criterion. The attachment cell, or cap cell, in this case contains some microtubules, but also contains actin-rich “scolopale rods” like those present in the scolopale cell. The functional significance of the morphological differences between mononematic and amphinematic scolopidia is not clear but it has been suggested that they confer different viscoelastic properties upon the sensory units (Moulins, 1976, Yack, 2004).

Formation of cuticular elements at the JO scolopidial attachment sites at the a2/a3 joint is reported to be defective in the *ato* mutant (Göpfert *et al.*, 2002). These cuticular elements may depend directly on *ato*-mediated specification, for example by secretion from putative ectodermal cells that may arise from the chordotonal lineage. Alternatively, these elements may depend on *ato* indirectly and be induced, genetically or even mechanically, by the associated scolopidia. These issues may be clarified by careful lineage analysis together with investigation of mutants that affect this process more specifically (such as perhaps *ct* and *salm/sal*).

JO is used for hearing, while fCHO and lch5 are proprioceptive. However, it is worth noting that in some insects a subset of fCHOs or other CHOs are modified for hearing. This developmental and evolutionary flexibility of different CHO substrates to become adapted for hearing is exemplified by the fact that insect auditory organs, whether flagellar or tympanal, have been described in a wide variety of anatomical locations (reviewed by Hoy, 1998, Yager, 1999 and others). Comparative studies that examine properties of transition from proprioceptive to auditory function suggest that these changes can occur relatively quickly in narrow lineages (see for example Boyan, 1993, Boyan, 1998, van Staaden and Römer, 1998).

From a developmental genetic perspective, JO exhibits both similarities to and differences from other CHOs. For instance, the SOPs of all *Drosophila* CHOs, including JO, are specified through the activity of the proneural gene *ato*. However, three genes that inhibit CHO differentiation elsewhere in the *Drosophila* body, *spalt-major* (*salm*), *spalt-related* (*sal*) and *cut* (*ct*), are required for normal development and function of JO.

Both *salm/sal* and *ct* mutants are completely deaf (Dong *et al.*, 2003, Ebacher *et al.*, 2007). The zinc-finger transcription factors

encoded by *salm* and *sal* normally are expressed in the oenocytes surrounding lch5. In the absence of *salm/sal* function, the number of scolopidia in lch5 increases at the expense of oenocytes. It has been proposed that *salm* and *sal* function in oenocyte precursors both downstream of and in parallel to EGF signaling. In the absence of *salm/sal* function, the EGF signal is interpreted as a differentiation cue for secondary lch5 SOPs. As described above, in fCHOs, EGF signaling also plays a critical role and is required for the recruitment of secondary SOPs. In marked contrast to their role in lch5 differentiation, *salm* and *sal* are essential to JO differentiation and maintenance (Dong *et al.*, 2003). In *salm/sal* mutant antennae, JO differentiates abnormally, then degenerates. *salm* and *sal* are expressed in both the epidermal precursors of a2 and the SOPs. In *salm/sal* mutant antennae, the a2 cuticle and the a2/a3 joint to which JO scolopidia attach are defective. It remains unknown whether the failure of JO scolopidia to make proper attachments leads to their degeneration in *salm/sal* mutants or whether there are intrinsic defects in the scolopidia themselves in these mutants.

ct encodes the *Drosophila* homolog of human CAAT-displacement protein, a cell cycle-regulated homeodomain transcription factor. In the embryo, *ct* has well-studied roles in multiple dendritic (md) neurons and in external sensory (ES) organs. In md neurons, the higher levels of *ct* expression correspond to more elaborate dendritic arborizations (Grueber *et al.*, 2003). In ES organs, *ct* plays a role in fate specification and in the absence of *ct* function, some ES organs are transformed to CHO fates (Bodmer *et al.*, 1987, Merritt, 1997). Furthermore, misexpression of *ct* in developing embryonic CHO transforms them into ES organs. It therefore is surprising that *ct* is required for normal JO differentiation and that in *ct* mutants the JO scolopidial units differentiate abnormally and subsequently degenerate (Ebacher *et al.*, 2007). Similar to *salm/sal*, *ct* is expressed in both the epidermal precursors of a2 and the JO SOPs and *ct* mutants exhibit defects in the a2/a3 joint cuticle. However, in the case of *ct*, specific subcellular defects have been observed in both JO neurons and scolopale cells by transmission electron microscopy (Eberl, unpublished). Because *ct* neither regulates nor is regulated by *ato*, *salm* and *sal* in the developing JO, it has been proposed that *ct* functions in conjunction with the transcription factors encoded by these genes to regulate as yet unidentified targets required for JO development, maintenance and function (Ebacher *et al.*, 2007).

There has long been evidence of diversity among lch5 and fCHO scolopidia, but insights into the molecular diversity among JO scolopidia have only recently come to light. For instance, a monoclonal antibody, 49C4, labels only the posterior four scolopidia of lch5, indicating the presence of a cellular antigen in those four that is missing from the most anterior scolopidium (Bodmer *et al.*, 1987). One lch5 scolopidium often remains in the *ato* mutant (Jarman *et al.*, 1993, zur Lage *et al.*, 1997); whether this is the 49C4 negative scolopidium is not clear. In addition, because two of the five units arise from EGFR-mediated signaling through the *rho* and *argos* genes (Okabe and Okano, 1997, zur Lage *et al.*, 1997), these two may be molecularly unique, even though no known markers label them differentially. Axonal projections from lch5 have been mapped and also show some diversity (Merritt and Whittington, 1995, Smith and Shepherd, 1996, Merritt, 1997, Schrader and Merritt, 2000). In the fCHO, several Gal4 lines (see

Brand and Perrimon (1993) and Duffy (2002) for explanation of these genetic tools) express in different subsets of neurons, with different projection patterns into the thoracic ganglia (Phillis *et al.*, 1996, Reddy *et al.*, 1997, Murphey *et al.*, 1999). It is not known whether any of these also express in JO. Before leaving the leg to enter the thoracic ganglia, the fCHO axons form a glomerulus (Shanbhag *et al.*, 1992), an arrangement that is not seen in JO. In JO, one enhancer trap line, *J21.17*, stains only a subset of neurons (Sharma *et al.*, 2002, Sivan-Loukianova and Eberl, 2005). This line, when converted into a Gal4 line (JO15), was shown to express in 145±2 neurons (Kamikouchi *et al.*, 2006). Another Gal4 line, identified in a screen for mutations that affect gravitaxis, inserted into the *yuri gagarin* gene, is reported to express in about 40 JO neurons (Armstrong *et al.*, 2006, Baker *et al.*, 2007). In a systematic Gal4 screen, Kamikouchi *et al.* (2006) characterized 17 Gal4 insertions with expression in JO. These express in several subset patterns of JO neurons and project into five major zones in the brain. Comprehensive analysis of these lines, as well as single cell labeled clones, defined 19 subregions of projections. Thus, in addition to the important contribution towards understanding molecular diversity in JO, the Kamikouchi study also systematically documents diversity in projection patterns of JO neurons using these different lines. This diversity points to one of the possible functional consequences of molecular diversity, namely finding diverse targets in the brain. The new projection map will set the stage for beginning to understand the relationship between the diversity of JO scolopidia, neural circuitry and behavioral biology.

How the *Drosophila* antenna works as an ear

Acoustic physics of insect songs and hearing have been described by Bennet-Clark (1971). Relevant particularly to insects like *Drosophila* that use their hearing only very close to the source, airborne sound has two energy components, particle velocity (PV, also called particle displacement) and sound pressure. At a large distance from the source (in the so-called acoustic far-field), these two components are in phase and most of the energy is in the pressure component. The design of tympanal ears such as those of crickets, katydids and cicadas, which use long-distance acoustic communication, are optimized to respond to the pressure component. In contrast, close to the sound source (in the acoustic near-field), the PV and pressure components become increasingly out of phase, reaching 90° out of phase at the source. Perhaps more importantly, in this range, PV predominates over the pressure component. Specifically, PV intensity decreases as a function of the cubic root of the distance from the source, while pressure falls off only with the square root of the distance (see also Kalmijn (1997) for a review of these principles in the context of aquatic animal hearing). Thus, auditory systems that respond to the PV component are most sensitive at close range. To respond to PV, a receiving structure should be easily displaced with the moving air mass; in *Drosophila* this structure is the arista. Sound-induced arista movement imposes a rotational force on the joint between antennal segments 2 and 3 (a2/a3 joint) (Eberl *et al.*, 2000, Göpfert and Robert, 2001b, Göpfert and Robert, 2002). Joint flexion is then transduced by JO (Eberl *et al.*, 2000).

Contrary to long-held views that sensory cilia, which lack the central pair of microtubules, are non-motile, JO not only senses the movements, but actively generates motion in the antenna (Göpfert

and Robert, 2003). This motion generation, which may be considered analogous to the mammalian cochlear amplifier, serves to optimize sensitivity to the frequency components in the courtship song, as well as to broaden the dynamic range of hearing so that low intensity sounds are amplified while high intensity sounds are damped. Measurement of the power output of the JO neurons has produced estimates of about 19 zJ (Göpfert *et al.*, 2005).

These considerations suggest that there is an important interplay between CHO physiological properties, cuticular elasticity and shape, as well as the size/shape/arrangement of distal antennal segments. These factors will impinge on the various component forces discussed by Todi *et al.* (2004) that maintain the JO as a tensioned system. When considering these factors for *Drosophila*, mosquito and honeybee (Tsujiuchi *et al.*, 2007), for example, there may emerge a relationship between number/diversity and physiology of scolopidia with acoustic sensitivity; however, many more species (and mutants) need to be studied and other interacting factors need to be measured quantitatively to understand their significance in a systematic way. Finally, while outside the scope of this review, it is important to remember that insects with tympanal ears, responding to far-field sound, face and solve the same spectrum of issues.

Despite much progress in *Drosophila* JO biology, the precise sensory mechanism is still not clear in molecular detail. That the sensory mechanism localizes to the cilium is supported by the fact that mutations in genes that affect JO ciliary development and morphology lead to deafness. These include components of intraflagellar transport (IFT) such as the anterograde kinesin motor subunits *kinesin-like protein 64D* (*Klp64D*) and the *kinesin associated protein* (*DmKAP*) (Sarpal *et al.*, 2003), the retrograde cytoplasmic dynein motor encoded by *beethoven* (*btl*) (Eberl *et al.*, 2000, Sharma *et al.*, in preparation), the anterograde IFT-B particle proteins *no mechanoreceptor potential B* (*nompB*) (Han *et al.*, 2003), *outer segment protein 2* (*oseg2*, also called *osm-1*) and *outer segment protein 5* (*oseg5*) (Avidor-Reiss *et al.*, 2004), the retrograde IFT-A particle proteins *oseg1* and *reduced mechanoreceptor potential A* (*rempA*; also called *oseg3*) (Avidor-Reiss *et al.*, 2004, Lee *et al.*, in preparation) and the ciliary transcription regulator *Drosophila regulatory factor X* (*dRfx*) (Durand *et al.*, 2000, Dubruille *et al.*, 2002). Mutations that appear to affect the ciliary axoneme also lead to deafness. These include *touch insensitive larva B* (*tilB*) and *smetana* (*smet*) (Kernan *et al.*, 1994, Eberl *et al.*, 2000, Todi *et al.*, 2004). Others, such as *uncoordinated* (*unc*) and the *Drosophila pericentrin-like protein* (*D-PLP*), affect basal bodies or centrioles and disrupt hearing (Baker *et al.*, 2004, Martinez-Campos *et al.*, 2004). Finally, mutations that disrupt the physical connection between the a2/a3 joint and the sensory cilia, including *no mechanoreceptor potential A* (*nompA*) (Chung *et al.*, 2001), a component of the extracellular dendritic cap tube necessary for association of the cilium with the cap, and the myosin VIIA motor protein encoded by *crinkled* (*ck*), which is required for association of the dendritic cap with the cuticle (Todi *et al.*, 2005), lead to deafness.

To complement these morphological studies, a subset of these mutations has been studied for their effects on non-linear mechanics and motion generation in the *Drosophila* antenna. All mutants tested affected the non-linear mechanics by partially or completely collapsing to linear mechanical systems (Göpfert and Robert, 2003). Interestingly, the resonant frequency at which the linearity

settles in each mutant is related to the stiffness of the mechanical system, a function of how much of the connection to the cilium is lost. Thus, *tilB* retains all major structures and connections and collapses to linearity at about 800 Hz, just as is seen in a dead wild-type fly; *biv* mutants, which lose much structural integrity (but not all connections) between cilia and dendritic caps, linearize at about 600 Hz; *nompA* mutants, in which all cilia are detached from the dendritic caps, linearize at about 400 Hz. These findings are consistent with a ciliary location for the mechanism(s) that detects movement and generates motion in the antenna.

Some details of the sensory mechanism of hearing have been elucidated recently at the molecular level. In particular, three members of the TRP channel family are involved in hearing, though their precise roles are not yet clear. The *nanchung* (*nan*) and *inactive* (*iav*) genes encode a pair of TRPV channels that are localized to the ciliary membrane and are mutually dependent for localization and absolutely required for hearing (Kim *et al.*, 2003, Gong *et al.*, 2004). The *no mechanoreceptor potential C* (*nompC*) gene, a member of the TRPN subfamily required for bristle organ mechanotransduction (Walker *et al.*, 2000), contributes to auditory sensitivity, but its absence does not completely abolish hearing (Eberl *et al.*, 2000), as measured by afferent auditory responses in the antennal nerve. Using measurements of non-linear mechanics in the antenna, Göpfert *et al.* (2006) argue that NompC is required both for a feedback loop in this non-linear amplification, but also for self-sustained spontaneous motion generation (analogous to spontaneous otoacoustic emissions in the mammalian ear). In contrast, *nan* and *iav* mutations increase the amplitudes of spontaneous oscillations of the antenna. Using double mutant combinations, in which both the sound-induced motions and the spontaneous oscillations of *nan* are suppressed by *nompC*, they present a model in which 1) Nan and Iav control the activity of the NompC-mediated amplificatory feedback and 2) NompC is a candidate mechanotransducer, but 3) one or more additional transducers must exist to explain residual afferent responses in the *nompC* mutants. In this model, the Nan/Iav channels are required for the propagation of the afferent sensory response, but not for the core mechanism of the amplificatory feedback mechanism (rather, only to modulate its gain). Further studies are required to test this model and to identify additional components in the amplification mechanism and the mechanosensory transduction mechanism, including the additional mechanotransducer channels. The findings to date suggest however that these two mechanisms are likely to be intricately and intimately intertwined.

What we don't know

In addition to the issues discussed above, other important questions remain about both JO development and JO function. For instance, it remains unclear why JO scolopidia possess multiple neurons and from where these neurons derive. Since fCHOs also have multiple neurons, increased neuron number is not correlated simply with a change from proprioception to audition. However, it is possible that possessing multiple neurons increases CHO sensitivity by increasing the likelihood of a unitary response. This would be useful for more precisely encoding sound intensity, particularly near thresholds. Alternatively, since increasing the number of neurons increases the density of cilia, the energy for the oscillatory mechanism would also be increased. That the number

of neurons in JO scolopidia is not uniform suggests that doubly and triply innervated scolopidia possess distinct functions. Whether these correlate cleanly with the functions currently attributed to JO—sensing wind currents, gravity sensation and hearing—remains to be seen, but seems unlikely.

What does seem likely is that the differently innervated scolopidia respond to different parameters of sound. Scolopidial tuning probably is significantly more refined than this, however, as illustrated by the striking gene expression differences and differences in electron density among JO neurons. The consequences of these gene expression differences and how they are achieved are likely to be fruitful avenues of future research. The nature of the organelles contributing to variation in electron density among JO neurons is likely to provide insight into JO function, as well as new directions for JO research.

Related issues are whether the various neurons of a particular scolopidium are clonally related, how JO neurons are related to other cells of the scolopidium such as the scolopale cell and the cap and whether there is recruitment of JO SOPs. Addressing these questions is essential to our understanding of JO specification and differentiation.

The developmental and functional consequences of genetic differences between JO and other CHOs remain unexplored and will undoubtedly be a significant area of future research. That genes involved in inhibiting CHO development elsewhere are required in JO is both astonishing and baffling. Whether genes such as *salm*, *salr* and *ct* regulate JO-specific targets is an important—and testable—question. If JO-specific target genes can be identified, this in turn would be likely to provide insights into how, at the cytoskeletal and/or organelle level, JO neurons differ from neurons in other sensory structures.

Finally, the evolutionary constraints on CHO plasticity due to the physical properties of sound may have important implications in terms of scolopidial specializations. In particular, the specializations that favor reception of near-field sound, such as JO, may be very different from those that favor performance of far-field sound detection in tympanal CHOs. Evolutionary selection is exerted via adaptive advantages conferred by performance enhancement. In this context, transformation between the far-field and near-field modes may be difficult without first losing function of the previous mode. Such constraints have been considered in vertebrate inner ear development and evolution during the transition from an aquatic to a terrestrial lifestyle (Fritzsche, 1999). The many advances toward understanding the molecular function and development of *Drosophila* auditory organ in the last ten years are starting to provide the tools to begin approaching such important evolutionary questions.

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