Using *Drosophila* to study mechanisms of hereditary hearing loss

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**ABSTRACT**

Johnston’s organ – the hearing organ of *Drosophila* – has a very different structure and morphology to that of the hearing organs of vertebrates. Nevertheless, it is becoming clear that vertebrate and invertebrate auditory organs share many physiological, molecular and genetic similarities. Here, we compare the molecular and cellular features of hearing organs in *Drosophila* with those of vertebrates, and discuss recent evidence concerning the functional conservation of Usher proteins between flies and mammals. Mutations in Usher genes cause Usher syndrome, the leading cause of human deafness and blindness. In *Drosophila*, some Usher syndrome proteins appear to physically interact in protein complexes that are similar to those described in mammals. This functional conservation highlights a rational role for *Drosophila* as a model for studying hearing, and for investigating the evolution of auditory organs, with the aim of advancing our understanding of the genes that regulate human hearing and the pathogenic mechanisms that lead to deafness.

**KEY WORDS:** Cochlea, Deafness, *Drosophila*, Hair cells, Hearing, Usher syndrome

**Introduction**

Hearing loss is one of the most common neurological disorders in humans, affecting 2 to 3 of every 1000 children in the USA (Intizai et al., 2016; Naz et al., 2017). Approximately 50% of congenital hearing loss is caused by genetic mutations, with a variety of other factors, particularly infectious disease, contributing to the remaining forms of congenital deafness (Korver et al., 2017). Genetic mutations affect many components of the auditory pathway, and can affect only hearing (nonsyndromic deafness), or other organs and tissues as well (syndromic deafness). Of the genetic mutations that cause nonsyndromic deafness, ~30% are autosomal dominant, ~70% are autosomal recessive, and five are X-linked (hereditary hearingloss.org). Among the many forms of syndromic hearing loss are Alport syndrome, Pendred syndrome, Branchio-oto-renal syndrome, Stickler syndrome and Usher syndrome (Korver et al., 2017). Usher syndrome is the leading cause of human deafness and blindness, with a prevalence of 1-4 per 25,000 people in the USA (Boughman et al., 1983; Hartong et al., 2006; Keats and Corey, 1999; Kimberling et al., 2010; Mathur and Yang, 2015). It is an autosomal recessive genetic disease, characterized by varying degrees of deafness and retinitis pigmentosa-induced vision loss. Although our understanding of genetic hearing loss has advanced greatly over the past 20 years (Vona et al., 2015), there is a pressing need for experimental systems to understand the function of the proteins encoded by deafness genes. The mouse is well established as a model for studying human genetic deafness (Brown et al., 2008), but other model organisms, such as the fruit fly *Drosophila*, might also provide convenient and more rapid ways to assay the function of candidate deafness genes.

In mammals, mechanosensitive hair cells reside in a specialized epithelial structure, the organ of Corti (see Glossary, Box 1), that runs along the length of the cochlear duct (Box 1) of the inner ear (Basch et al., 2016). Vibration of the organ of Corti in response to sound waves that are captured and amplified by the external and middle ears causes a deflection of the hair-like stereocilia of hair cells (Box 1). This deflection opens ion channels, leading to the release of neurotransmitters by hair cells and the activation of the first neurons in the auditory pathway (Fettiplace and Hackney, 2006). *Drosophila* also have a hearing organ – Johnston’s organ (Box 1) – located in the antenna, the second segment of which contains several hundred mechanosensitive units called scolopidia (Eberl and Boekhoff-Falk, 2007). Bulk air displacements caused by near-field sound stimuli such as courtship song rotate the distal antennal segments, stretching the scolopidia (Boekhoff-Falk and Eberl, 2014). Accumulating evidence from *Drosophila* suggests that there are many physiological, molecular and genetic similarities between vertebrate and invertebrate auditory organs (Albert and Göpfert, 2015; Bokolia and Mishra, 2015; Jarman and Groves, 2013; Lu et al., 2009; Senthilan et al., 2012). For example, vertebrates and invertebrates share homologous transcription factors that specify the development of their sensory cells, the analogous accessory structures to capture and transduce sound energy, and the use of analogous systems to transduce and amplify mechanical force into electrical energy.

Here, we review the evidence that the genes, cells and molecular networks that specify the *Drosophila* hearing organ have mammalian counterparts that function in the differentiated cells of the cochlea and are required for hearing in mammals. In particular, we focus on recent findings that Usher syndrome proteins in *Drosophila* appear to physically interact in protein complexes that are similar to those described in mammals. We also describe recent work that indicates that some Usher syndrome proteins interact physically with Myosin II, the variants of which can cause *MYH9*-related diseases, including deafness. Various approaches, including forward genetic screens in *Drosophila* (Box 1) to identify mutants with behavioral defects associated with mechanosensory transduction (Eberl et al., 1997; Kernan et al., 1994), and microarray screens to identify transcripts enriched in Johnston’s organ and regulated by *atonal*, have revealed striking similarities between the genes that are implicated in human deafness and those that affect the function and development of Johnston’s organ in
Drosophila. We also review the recently developed genetic toolkits in Drosophila that can characterize the function of novel genes in Drosophila hearing. It is estimated that several hundred genes involved in hearing still await discovery on the basis of mouse deafness mutants that have been isolated in mutagenesis and knockout screens (Steel, 2014). We suggest that by improving our understanding of the mechanisms of hearing in Drosophila, we could discover new genes required for hair cell function in vertebrates, better understand the evolution of auditory organs, and gain new insights into the pathogenic mechanisms of some deafness-related genes.

**Functional similarities between Drosophila and mammalian hearing organs**

Johnston’s organ, the largest chordotonal organ (Box 1) in Drosophila, is located in the second antennal segment (Box 1) and consists of ~200 functional units or scolopidia (Kernan, 2007). Each scolopidium is suspended between the cuticle of the second antennal segment and the insertion of the third antennal segment (Fig. 1A,B) (Todi et al., 2004). During courtship, male flies beat their wings, causing the air to move, which in turn causes the female-like arista (Box 1) on the third antennal segment of the female to vibrate. The resulting movement of the third antennal segment applies force to the scolopidia as the a2/a3 joint rotates (Boekhoff-Falk and Eberl, 2014; Eberl and Boekhoff-Falk, 2007). This stretch is transmitted to the mechanosensory cilia of sensory neurons, opening stretch-gated mechanotransduction channels (Kernan et al., 1994; Kernan, 2007). A transient receptor potential (TRP) channel – TRPN1 (also known as NompC) – was first identified as a candidate component of a mechanosensitive channel in a genetic screen in Drosophila (Walker et al., 2000), and was subsequently shown to be expressed in the distal region of the ciliary dendrite of Johnston’s organ neurons (Lee et al., 2010). Mutation of Drosophila nompC attenuates, but does not completely abolish, sound-evoked potentials (Eberl et al., 2000; Effertz et al., 2012, 2011; Göpfert et al., 2006; Lee et al., 2010; Liang et al., 2011). Two TRPV family members, Nanchung (Nan) and Inactive (Iav) are also expressed in Johnston’s organ neurons, although in a more proximal region of the ciliated dendrite than NompC (Cheng et al., 2010; Gong et al., 2004; Lee et al., 2010; Liang et al., 2011). Nan and Iav are also required for the generation of sound-evoked potentials in the antennal nerve (Gong et al., 2004; Kim, 2007; Kim et al., 2003). It is less clear whether Nan and Iav can be directly gated by mechanical force, as studies in which either or both channels were expressed in heterologous cells reached different conclusions over the degree to which the channels could be opened by osmotic stress [compare Gong et al. (2004) and Kim et al., (2003) with Nesterov et al. (2015)]. The physical separation of the TRPN and TRPV channels in the dendrite of Johnston’s organ neurons, together with the effects of these mutations on sound-evoked potentials and on active amplification, has led to the idea that NompC plays a direct role in mechanotransduction (Effertz et al., 2012; Göpfert et al., 2006), whereas Nan and Iav, which can form heteromers (Gong et al., 2004), are thought to amplify weak signals transmitted from the NompC complex (Gong et al., 2004; Göpfert et al., 2006; Kamikouchi et al., 2009; Kim et al., 2003).

Despite the progress made in characterizing the properties of these channels, it has been hard to resolve the relative contributions of these three channels to mechanotransduction, in part because of the technical challenges involved in recording from single auditory neurons in Johnston’s organ. A recent innovation by Wilson and colleagues (Lehnert et al., 2013) exploited the fact that auditory neurons terminate on, and are dye-coupled to, a single giant fiber neuron in the brain (Kamikouchi et al., 2009; Tootoonian et al., 2012). Recording the activity of this neuron allows currents from Johnston’s organ neurons to be indirectly measured. Results from
this approach suggest that Nan and Iav are required to respond to sound, whereas NompC is required for the modulation and amplification of force generated during sound reception (Lehnert et al., 2013). It is notable that NompC contains numerous ankyrin repeats (Cheng et al., 2010; Liang et al., 2013; Zhang et al., 2015) that might interact with motile components of the ciliated dendrite to modulate force, an idea that is supported circumstantially by the displacement of the antennal arista, which induces the third antennal segment to rotate (arrow). This acoustic, stimulus-induced rotation applies force to the cilia of mechanosensory neurons in the scolopidia, resulting in the firing of action potentials along the antennal nerve. A single scolopodium is highlighted in the light green box. Each scolopodium is attached to the antenna cuticle (arrows) by a cap cell (red) and a ligament cell (gray), as detailed in C. (C) Cellular components of an individual scolopium. Two to three mechanosensory neurons (two neurons are shown) have their ciliary dendrites enclosed by an actin-rich scolopale cell. Mechanosensory neurons express several ion channels that regulate mechanosensation, including Nan/Iav (green) and NompC (yellow). Scolopale cells form septate junctions with the ligament and cap cells at the basal and apical ends of the scolopodium, respectively, ensuring the formation of an enclosed scolopale space between the scolopale cell and neuronal cilia. The ciliary dendrites of each neuron insert into an acellular cap that contains the NompA glycoprotein (red), which is anchored to the A2/A3 joint by a cap cell. The scolopodium is attached to the A2 cuticle at its proximal end by a ligament cell. Approximately 200 scolopidia are located in Johnston’s organ.

Unlike the Drosophila hearing organ, which responds optimally to a narrow frequency range of the beating male wing, the mammalian inner ear has evolved to respond to a wide range of sound frequencies. The hearing organ of the cochlea, the organ of Corti, sits on a thin basilar membrane that runs the length of the cochlear duct (Fig. 1B). Both the thickness and width of the basilar membrane change along the length of the cochlear duct (Dallos, 1996); therefore, it can resonate in response to many different frequencies, allowing the basilar membrane to transform complex sounds into their component sound frequencies (Reichenbach and Hudspeth, 2014). The rising and falling oscillation of the basilar membrane in response to mechanical waves traveling along the cochlea is transmitted to the mechanosensory hair cells of the organ of Corti (Fettiplace and Hackney, 2006). One row of inner hair cells runs the length of the organ of Corti, and these cells form synapses with sensory afferent neurons of the acoustic (spiral) ganglion (Goodrich, 2016), while three rows of outer hair cells receive mostly efferent input from the brainstem (Busch et al., 2016; Yu and Goodrich, 2014). Hair cells bear elongated microvilli or stereocilia on their apical surface that insert into an acellular tectorial membrane above them (Fig. 2). As the basilar membrane vibrates, the motion of the organ of Corti causes the stereociliary bundles of the hair cells to be displaced by contact with the tectorial membrane (outer hair cells) or fluid flow (inner hair cells). This opens mechanically gated ion channels in the tips of the stereocilia (Corey and Holt, 2016; Fettiplace, 2016; Wu and Muller, 2016). Adjacent stereocilia connect to each other close to their tips by a tip link protein complex consisting of protocadherin 15 and cadherin 23 (Pepermans and Petit, 2015). This complex allows the opening of mechanotransduction channels to be coordinated as the hair bundle is displaced (Hudspeth, 1997, 2008). As in Drosophila, the identity of the mechanosensitive ion channels in these hair cells is still being investigated and debated (Corey and Holt, 2016; Fettiplace, 2016; Wu and Muller, 2016). Current work is focused on four transmembrane proteins, transmembrane inner ear (TMIE), tetraspan membrane protein of hair cell stereocilia/lipoma HMGIC fusion partner-like 5 (TMHS; Lhfpl5) and transmembrane channel-like 1 and 2 (TMC1/2), all of which are
mechanically gated. We first compare the axonless hair cells of accessory cells and proteins that allow these receptor cells to be similar sets of transcription factors, and have similarities in the cochlea (Nayak et al., 2007). Among invertebrates, only insects and they mature, including inner and outer hair cells of the mammalian cilium, the kinocilium, which disappears in some hair cell types as addition to their apical stereociliary bundle, they also have a true sensory neurons that also send processes to brainstem nuclei. In neither axons nor dendrites but are innervated by axons of bipolar cells. Inner and outer hair cells receive afferent and efferent innervation, respectively. Scolopidia located on the apical surface of hair cells are embedded into the tectorial membrane (outer hair cells) or lie just beneath the membrane (inner hair cells). During sound reception, the sound-induced vibration of the basilar membrane applies mechanical force to the tip links of the stereocilia, causing an influx of potassium and calcium and the depolarization of hair cells. The potassium gradient in the cochlear duct endolymph is maintained by the stria vasculosa in the lateral wall of the cochlear duct.

necessary for hearing in mice (Kawashima et al., 2011; Kurima et al., 2015; Xiong et al., 2012; Zhao et al., 2014). It is possible that other proteins, such as the large transmembrane protein Piezo2, might also contribute to mechanotransduction in other parts of the hair cell (Wu et al., 2017).

Although the appearance and organization of mammalian and Drosophila hearing organs are clearly very different, they share a number of genes that regulate their formation and cellular functions. We describe some of these in the following section.

Cellular and molecular similarities between Drosophila and mammalian hearing organs

Despite being separated by several hundred million years of evolutionary time, the mechanosensitive cells and supporting cells of mammalian and Drosophila hearing organs are specified by similar sets of transcription factors, and have similarities in the accessory cells and proteins that allow these receptor cells to be mechanically gated. We first compare the axonless hair cells of vertebrates with the mechanosensitive neurons of Drosophila and then describe similarities in how these cells are formed and how they function.

Vertebrate hair cells are secondary receptor cells: they elaborate neither axons nor dendrites but are innervated by axons of bipolar sensory neurons that also send processes to brainstem nuclei. In addition to their apical stereociliary bundle, they also have a true cilium, the kinocilium, which disappears in some hair cell types as they mature, including inner and outer hair cells of the mammalian cochlea (Nayak et al., 2007). Among invertebrates, only insects and crustaceans have true hearing; however, other invertebrate groups possess mechanoreceptor cells with similar apical specializations that can detect gravity and acceleration. These receptor cells are also sometimes referred to as hair cells, although their homology to true vertebrate hair cells is still under debate (Burighel et al., 2011, 2008, 2003; Fritzsch and Straka, 2014; Muñoz and Ladher, 2007). These mechanosensitive sensory neuron represents the ancestral cell type from which hair cells and sensory neurons emerged as sister cell types in multiple taxa, or whether secondary mechanosensitive cells were lost in the arthropod lineage (Arendt et al., 2016).

Despite the structural differences between insect and vertebrate sound-detecting cells, molecular evidence suggests that certain aspects of the development of the various cell types found in auditory organs are conserved between invertebrates and vertebrates.
(Bechstedt and Howard, 2008; Bokolia and Mishra, 2015; Caldwell and Eberl, 2002; Lewis and Steel, 2012) (Table 1). The *atonic (ato)* basic helix-loop-helix (bHLH) transcription factor was discovered in *Drosophila* as a proneural gene required for the formation of photoreceptors and some mechanoreceptors (Jarman et al., 1993, 1994). In *Drosophila*, the loss of *ato* leads to loss of all chordotonal

| Table 1. Molecular and cellular conservation between *Drosophila* and mammalian auditory organs |
|---------------------------------------------|---------------------------------|-----------------------------------------------|
| **Drosophila** | **Mammals** | **References** |
| Transcription factors specifying hearing organ cells | Ato | Atoh1 | Necessary and sufficient for differentiation and survival of hair cells. Fly Atoh1 rescues mouse Atoh1 mutant phenotype. |
| | Sens | Gfi1 | Required for the survival of hair cells in mouse. |
| | Pros | Prox1 | Initially expressed in the progenitors of hair cells and supporting cells, but becomes restricted to supporting cells as the sensory epithelium differentiates. |
| | Pax2 (Sv) | Pax2 | Initially expressed in the progenitors of the entire inner ear but becomes restricted to the stria vascularis of the cochlear duct. |
| Structural proteins required for mechanosensation | NompA | Produced and secreted by supporting cells, they constitute the major structural proteins in the acellular tectorial membrane that lies above the hair cells. |
| | Myosin VIIA | MYO7A (USH1B) | Recessive mutations cause Usher syndrome and nonsyndromic deafness in humans; physically interacts with other Usher proteins in a protein complex to form stereocilia tip links. |
| | Cad99C | PCDH19 (USH1F) | Recessive mutations cause Usher syndrome in humans; complexes with other Usher proteins to form stereocilia tip links. |
| | Sans | SANS | Recessive mutations cause Usher syndrome in humans; physically interacts with other Usher proteins in a protein complex to form stereocilia tip links. |
| | Myosin II | MYH9 | Dominant mutations cause MYH9-related diseases and nonsyndromic deafness disorder, DFNA17. |
| | TMC | TMC1/2 | Required for auditory mechanosensation. |
| Conserved cells and accessory structures | Accessory cells | The scolopale, cap and ligament cells of Johnston’s organ scolopidia. | Deiters’, pillar, inner phalangeal, border and Hensen’s cells of the organ of Corti. |
| | Scolopale fluid | Potassium-rich fluid secreted by scolopale cells, bathing the ciliary dendrites of mechanosensitive neurons. | Potassium-rich fluid secreted by the stria vascularis of the cochlear duct, bathing the apical surface of sensory hair cells. |
| | Dendritic cap | Secreted extracellular structure that anchors neuronal cilia and transfers force to them. Composed of the zona pellucida (ZP) domain glycoprotein NompA. | Secreted extracellular structure that lies above hair cell stereocilia and applies force to the hair bundles of outer hair cells. Major components include the ZP domain glycoproteins α- and β-tectorin. |
organisms, including Johnston’s organ, while its misexpression results in the formation of ectopic chordotonal organs (Jarman et al., 1993). Similarly, the vertebrate homolog of ato, Atoh1, is both necessary and sufficient for the differentiation and survival of hair cells (Bermingham et al., 1999; Cai et al., 2013). Remarkably, Drosophila ato and mouse Atoh1 can functionally replace each other in loss-of-function mutants (Ben-Arie et al., 2000; Wang et al., 2002), and are both regulated through conserved post-translational regulation (Quan et al., 2016), even though they share little homology outside the bHLH domain. A conserved post-translational control of the temporal dynamics of ato/Atoh1 expression during neurogenesis further supports the functional similarities between ato and Atoh1 (Quan et al., 2016). The conservation between Drosophila ato and the vertebrate Atoh1 gene regulatory networks is reflected by conservation of some of their downstream target genes. The zinc finger transcription factor senseless (sens)/Gfi1, which can be regulated by ato/Atoh1, is required for the development of the chordotonal organ in Drosophila embryos and for the survival of hair cells in the mouse (Acar et al., 2006; Jafar-Nejad et al., 2003, 2006; Nolo et al., 2000; Wallis et al., 2003; Wang et al., 2002). Regulatory factor X (Rfx) is another downstream transcription factor activated by Atonal (Jarman and Groves, 2013; Laurençon et al., 2007) that regulates Rfx1 and Rfx3; both genes are necessary for the generation of inner ear neurons (Dubruille et al., 2002; Vandaele et al., 2001). A recent study showed that combined loss of Rfx1 and Rfx3 in mice leads to a rapid loss of initially well-formed outer hair cells in the mouse cochlea and causes deafness (Elkon et al., 2015).

The division of labor between mechanosensitive secondary receptor cells and their afferent sensory neurons in mammals and some invertebrate taxa is reflected by an analogous division of labor between the transcription factors that specify the neuronal and sensory lineages. Neurog1 is a bHLH factor closely related to Atoh1 and is expressed in the progenitor cells of the inner ear primordium shortly before they delaminate as neuroblasts (Zheng and Gao, 2000). Its expression is then extinguished and replaced by another closely related bHLH gene, Neurod1; both genes are necessary for the generation of inner ear neurons (Kim et al., 2001; Liu et al., 2000). The Atoh, Neurogenin and NeuroD bHLH transcription factor families are ancient, with members of each family observed in basal taxa, such as sponges (Simionato et al., 2008, 2007). Arthropods appear to have dispensed with the need for the Neurog genes in sensory development; the one identified member of this family in Drosophila, target of pos neuro (tap), appears to function in neurite outgrowth rather than in neuronal specification (Gautier et al., 1997; Yuan et al., 2016). In contrast, Drosophila ato has undergone duplication to give rise to two additional orthologs: absent multidentritic and olfactory sensilla (amos) and cousin of atonal (cato) (Jarman and Groves, 2013). Although the Atoh, Neurogenin and NeuroD bHLH families are more closely related to each other than to other bHLH genes, they nevertheless have extremely specific functions. For example, while Drosophila ato can functionally replace Atoh1 in mice, and vice versa (Ben-Arie et al., 2000; Wang et al., 2002), Neurog1 can only rescue loss of Atoh1 in mice to a very modest degree (Jahan et al., 2012, 2015), suggesting that Neurog1 is only able to activate a subset of Atoh1 target genes. Thus, these different bHLH factors can specify different cell fates – neuron or hair cell – depending on the binding affinities and range of their targets.

A common feature of sensory systems is the presence of accessory cells that surround, support, nourish or modulate the output of sensory cells. In each scolopidium of Johnston’s organ, neuronal cilia are enclosed by a tube-like scolopale cell (Fig. 1). The shape of the scolopale cell is thought to be maintained by a cage-like scolopale consisting of actin rods, microtubules and some microtubule-associated proteins (Todi et al., 2004; Wolfrum, 1997). Scolopale cells form septate junctions with the ligament and cap cells at the basal and apical ends of the scolopidium, respectively, ensuring the formation of an enclosed scolopale space between the scolopale cell and neuronal cilia (Boekhoff-Falk, 2005; Carlson et al., 1997; Eberl and Boekhoff-Falk, 2007; Todi et al., 2004). Although the ionic composition of the scolopale space has not been measured directly in Drosophila, studies of the fluid that bathes a variety of insect and crustacean mechanoreceptors have reported that this fluid has a positive potential of about +70 mV relative to the hemolymph (Thurm and Wessel, 1979). In addition, studies of blowfly mechanoreceptor organs have found such fluid to be high in potassium (Küppers, 1974), the concentration of which is actively maintained by transport ATPases (French, 1987; Thurm, 1974; Wieczorek, 1982). Scolopale cells express Na+/K+ ATPases that localize to their ablumenal plasma membrane and are required for hearing in Drosophila. These Na+ pumps function to actively transport K+ into the scolopale space to maintain ion homeostasis (Roy et al., 2013). In the mammalian organ of Corti, inner hair cells and outer hair cells are surrounded by different types of supporting cells: inner phalangeal and border cells surround the inner hair cells, while Deiters’ cells surround the outer hair cells (Fig. 2), and inner and outer pillar cells located between these two domains form the tunnel of Corti. During sound perception, sound-induced vibration of the basilar membrane applies force to the tip links of the stereocilia. This results in their deflection, in the opening of cation channels in the stereocilia and in the influx of K+ and Ca2+ from the surrounding potassium-enriched endolymph (Fettiplace and Hackney, 2006). Potassium homeostasis in the endolymph is crucial for the maintenance of the endocochlear potential and for hair cell function. The K+ that enters hair cells is recycled through the supporting cells and is ultimately transported back into the endolymph by cells in the stria vascularis of the lateral wall of the cochlear duct (Kikuchi et al., 2000). It is not clear whether the K+-rich extracellular fluid of the endolymph and scolopale space reflect a common or convergent evolutionary origin. For example, sensory hair cells of the lateral line organs of vertebrates are not enclosed, but open to the external fluid environment, and a number of studies measuring the endolymph-like fluid bathing cephalopod statocyst sensory organs have found it to have low levels of K+ compared with Na+ (reviewed in Vinnikov, 2011).

Some molecular and cellular similarities have been identified between scolopale cells and cochlear-supporting cells. prospero (pros), a homeodomain transcription factor, is specifically expressed by scolopale cells in Johnston’s organ (Boekhoff-Falk and Eberl, 2014). The mammalian homolog of pros, Prox1, is initially expressed in the progenitors of hair cells and supporting cells, but becomes restricted to supporting cells as the sensory epithelium differentiates (Bermingham-McDonogh et al., 2006). On the apical side of Johnston’s organ, cap cells participate in the coupling of neuronal cilia to the cuticle of the third antennal segment, and help impart mechanical force to the cilia during sound reception. nompA encodes a zona pellucida (ZP) domain glycoprotein that is produced and secreted by scolopale cells. It forms acellular filaments that connect the mechanosensory neuronal dendrites to the third antennal cuticle (Chung et al., 2001). Loss of NompA leads to the detachment of both the scolopidia from the third antennal segment cuticle and of the ciliary dendrites from the cuticular structures in other sensory organs, such as the external
sensory organs and other campaniform organs (Chung et al., 2001). Mammals also have ZP domain-containing proteins – α- and β-tectorin (Legan et al., 1997) – which are produced and secreted by supporting cells, and are the major structural proteins in the acellular tectorial membrane that lies above the hair cells, and that comes into contact with the hair bundles of the outer hair cells. Loss of tectorin in mice leads to the detachment of hair cells from the tectorial membranes (Legan et al., 2000; Xia et al., 2010). Intriguingly, a Caenorhabditis elegans ZP domain protein, DVF-7, resembles tectorins and anchors the tips of sensory dendrites to the body wall as the neuronal cell bodies in the developing amphibian migrate away (Heiman and Shalam, 2009). In addition, the transmembrane channel-like (TMC) proteins are required for auditory mechanosensation in mice and humans (Kawashima et al., 2015). Although it has yet to be shown that the Drosophila homolog of the TMC proteins functions directly in auditory mechanosensation, it has been shown to regulate mechanosensation in larval locomotion (Guo et al., 2016).

A striking illustration of the molecular and genetic similarities between Drosophila and mammalian hearing organs comes from the work of Göpfert and colleagues (Senthilan et al., 2012). Here, a microarray survey of genes expressed in Johnston’s organ of wild-type and ato mutant flies identified 274 genes expressed in this auditory organ. Approximately 20% of these genes had a human ortholog implicated in hearing loss. This suggests that the fruit fly might be a rich resource for identifying and characterizing genes involved in human deafness. In the next section, we describe recent work in which forward genetic screens identified a Drosophila gene that linked together two myosin genes previously implicated in human syndromic hearing loss.

Can Drosophila be used to model hereditary deafness and hearing loss?

The molecular and functional similarities between Johnston’s organ and the mammalian cochlea summarized above suggest that some genes implicated in human deafness might have similar functions in Johnston’s organ. Here, we focus on two particular forms of syndromic hearing loss in humans, Usher syndrome and MYH9-related disorders, as these well-characterized syndromes provide evidence of such functional similarity and highlight the potential advantages of using Drosophila as a model for studying hereditary deafness.

Conservation of Usher syndrome proteins and their interactions in Drosophila

Usher syndrome is an autosomal recessive genetic disease [Online Mendelian Inheritance in Man (OMIM) #276900, #276904, #601067, #276901, #605472, #611383] that is characterized by varying degrees of deafness and retinitis pigmentosa-induced vision loss, and is the leading cause of deaf-blindness in humans (Boughman et al., 1983; Hartong et al., 2006; Keats and Corey, 1999; Kimberling et al., 2010). Based on the severity of clinical symptoms in patients, Usher syndrome is subdivided into three types: USH1, 2 and 3. To date, 16 genetic loci have been associated with this syndrome: nine for USH1, three for USH2, two for USH3 and two that have yet to be identified (Cosgrove and Zalloccchi, 2014; Mathur and Yang, 2015). Some missense mutations of Usher (USH) genes that retain residual function are also associated with nonsyndromic forms of deafness that do not cause vision loss (see, for example, Schultz et al., 2011). USH proteins regulate a variety of cellular processes, including actin-based trafficking, cell adhesion, scaffolding, and G-protein- or Ca²⁺-mediated signaling (Table 2). Evidence from colocalization data, in vitro interaction studies, and mouse genetic studies suggest that USH proteins interact with each other to form multiprotein complexes in a variety of cell types (Fig. 3A) (Ahmed et al., 2013; Pan and Zhang, 2012). In mature mammalian hair cells, many Usher syndrome proteins localize to the tips of stereocilia where mechanotransduction takes place (Fig. 3A). Adjacent stereocilia are connected by complexes at their tips, called tip links, which are composed of cadherin 23 (USH1D; Cdh23) and protocadherin 15 (USH1F; Pcdh15). Protocadherin 15 is thought to interact with components of the mechanotransduction channel (TMHS), and both ends of the tip links are indirectly coupled to the actin core of the stereocilia through other Usher proteins, including myosin VIIA (USH1B; Myo7a), Sans (USH1G), harmonin (USH1C), Cib2 and whirlin (USH2D) (Ahmed et al., 2013; Cosgrove and Zalloccchi, 2014).

The Drosophila genome harbors homologs of several Usher genes and some of these have been characterized in Johnston’s organ (Table 2). The Drosophila homolog of myosin VIIA [myosin VIIA; also known as crinkled (ck)] is required for hearing: loss of ck abolishes a fly’s auditory responses and causes the scolopidia to detach from the cuticle of the a2/a3 antennal joint in Johnston’s organ (Li et al., 2016; Todi et al., 2005, 2008) (Fig. 4). Interestingly, Myosin VIIA is predominantly expressed in the actin-rich scolopale cells and is enriched at their apical tips close to where a scolopidium inserts into the cuticle (Li et al., 2016), a pattern that is superficially similar to the localization of myosin VIIA in the actin-rich stereocilia tips of mammalian hair cells. However, whereas myosin VIIA functions in hair cell stereocilia to mechanically couple mechanotransduction channels to the actin cytoskeleton, Myosin VIIA is unlikely to have the same function in scolopale cells, which ensheath the mechanosensitive cilia of the Johnston’s organ sensory neurons and secrete the dendritic cap that anchors them to the cap cell and cuticle. However, it is likely that myosin VIIA serves an anchoring role in both cell types, because it is a high duty ratio motor – i.e. it predominantly binds to actin filaments rather than processing along them (Haithcock et al., 2011; Watanabe et al., 2006; Yang et al., 2006). Pcdh15 and Sans also have Drosophila homologs, named Cad99C and Sans, respectively (D’Alterio et al., 2005; Demontis and Dahmann, 2009). Recent studies have shown that Cad99C, Sans and Myosin VIIA form a complex in Johnston’s organ and in the ovary of Drosophila (Glowinski et al., 2014; Li et al., 2016). In Johnston’s organ, a subtle, low-penetrant phenotype of scolopidal detachment can be observed in Cad99C mutants, resembling that of myosin VIIA mutants. Cad99C localizes to the apical tips of scolopodia, reminiscent of the localization of Pcdh15 to the tip link region in mammalian hair cells. In addition, myosin VIIA, Cad99C and Sans genetically interact with Ubr3, a regulator of Usher proteins in Johnston’s organ (Li et al., 2016).

These data suggest that USH1 proteins are not only conserved in Drosophila, but that their interactions are important for the development and function of the Drosophila auditory apparatus. USH1 proteins appear to interact in the scolopidial tips to link the scolopale cell and cap cell, which might stabilize the scolopidal unit during mechanotransduction where force is applied to the neuronal cilia through the extracellular dendritic cap (Fig. 3B,C). This function of USH proteins in Johnston’s organ – where the proteins appear to mediate intercellular interaction and attachment – is somewhat different to their functions in the mammalian hair cell, in which the USH complex acts intracellularly to anchor adjacent stereocilia.
Several other USH genes have *Drosophila* homologs, such as harmonin (*CG5921, Cib2 (cib2), whirlin (dyschronic) and clarin 1 (CG1103) (Table 2). Although *cib2* is expressed in the eye and is required for normal phototransduction in *Drosophila*, whether these homologs are also present in the scolopidia of Johnston’s organ and/or participate in hearing remains unclear. Other vertebrate USH proteins that are present in the ankle links that connect the base of stereocilia, such as usherin and VLGR1/GPR98 (Adgrv1), do not appear to have any obvious homologs in *Drosophila*.

### MYH9-related disease

Autosomal dominant MYH9-related diseases are caused by mutations in *MYH9*, which encodes nonmuscle myosin IIA. These diseases are characterized by large platelets and thrombocytopenia from birth, and are associated with a variable onset of progressive sensorineural hearing loss, presenile cataracts, elevated liver enzymes and renal disease, which initially manifests as a glomerular nephropathy (Heath et al., 2001). Prior to the identification of the underlying molecular cause, individuals with MYH9-related disease were variously diagnosed as having Epstein syndrome, Fechtner syndrome, May-Hegglin anomaly or Sebastian syndrome, which encode nonmuscle myosin IIA.

| USH type | Locus | Human gene | Human protein | Drosophila ortholog | Function | References |
|----------|-------|------------|---------------|---------------------|----------|------------|
| USH1     | USH1B | MYO7A      | myosin VIIA   | (crinkled) ck       | Cytoskeleton-associated motor protein | Li et al., 2016; Todi et al., 2005, 2008 |
|          | USH1C | USH1C      | harmonin      | CG5921*             | Scaffold protein | Ahmed et al., 2013; Cosgrove and Zalocchi, 2014; Pan and Zhang, 2012 |
|          | USH1D | CDH23      | CDH23         | No candidates identified | Cell-cell adhesion; tip link protein | Alagramam et al., 2011; Kazmierczak et al., 2007; Sollner et al., 2004 |
|          | USH1E | Unknown    | Unknown       | No candidates identified | Unknown | D’Altono et al., 2005; Li et al., 2016 |
|          | USH1F | PCDH15     | PCDH15        | Cad99C              | Cell-cell adhesion; tip link protein | D’Altono et al., 2005; Li et al., 2016 |
|          | USH1G | SANS       | SANS          | Sans                | Scaffold protein | Demontis and Dahmann, 2009; Glowinski et al., 2014; Li et al., 2016 |
|          | USH1H | Unknown    | Unknown       | No candidates identified | Unknown | Riazuddin et al., 2012 |
|          | USH1J | CIB2       | CIB2          | No candidates identified | Calcium and integrin binding protein | Riazuddin et al., 2012 |
|          | USH1K | Unknown    | Unknown       | No candidates identified | Unknown | Riazuddin et al., 2012 |
|          | USH2A | usherin    | usherin       | No candidates identified | Stereocilia links; extracellular matrix | Adato et al., 2005; Liu et al., 2007; Weston et al., 2000 |
|          | USH2B | Unknown    | Unknown       | No candidates identified | Unknown | Adato et al., 2005; Liu et al., 2007; Weston et al., 2000 |
|          | USH2C | GPR98      | VLGR1/GPR98   | No candidates identified | Stereocilia links; G-protein signaling | Schwartz et al., 2005; Weston et al., 2004 |
|          | USH2D | WHRN       | WHRN          | No candidates identified | Stereocilia links; G-protein signaling | Schwartz et al., 2005; Weston et al., 2004 |
|          | USH3A | CLRN1      | clarin 1      | CG1103*             | Extracellular matrix structure | Adato et al., 2002; Fields et al., 2002; Joensuu et al., 2001 |
|          | USH3B | HARS       | HARS          | Histidyl transfer RNA synthetase | Histidyl transfer RNA synthetase | Abbott et al., 2017; Puffenberger et al., 2012 |
|          | –     | PDZD7      | PDZD7         | No candidates identified | Scaffold protein with homology to harmonin and whirlin; interacts genetically with USH2A and GPR98 | Chen et al., 2014; Ebermann et al., 2010; Schneider et al., 2009 |

For human USH loci for which a causative gene has not been identified, we indicate the cytogenetic band in which the locus is situated. Asterisks indicate *Drosophila* homologs predicted by protein sequence homology that have yet to be functionally validated.
This suggests that MYH9-related diseases are not caused by MYH9 haplo-insufficiency. However, knock-in mice, in which one Myh9 allele has been modified to contain a specific dominant pathogenic Myh9 mutation, R702C, develop defects, including hearing loss and kidney disease, that are similar to symptoms associated with several MYH9-related syndromes such as May-Hegglin anomaly (Suzuki et al., 2013). Although Myh9 is expressed in hair cells and NMIIA localizes to the stereocilia and apical surface of the hair cell (Li et al., 2016; Meyer Zum Gottesberge and Hansen, 2014; Mhatre et al., 2006), NMIIA is also broadly expressed in many other parts of the cochlea. It is therefore possible that the deafness that occurs in MYH9-related diseases might not be directly caused by hair cell defects. Thus, the molecular mechanism by which MYH9 mutations cause hearing loss remains unclear.

The single Drosophila homolog of NMIIA is encoded by the myosin II gene, also known as zipper. This gene is expressed in Johnston’s organ, and Myosin II is enriched at the apical tips of stereocilia, adjacent to Myosin VIIA (Fig. 3B) (Li et al., 2016). Myosin II is also required for the normal apical attachment of stereocilia. Consistent with this, the gene encoding the regulatory light chain of Myosin II, spaghetti squash (sqh), genetically interacts with myosin VIIA in Johnston’s organ and affects hearing (Todi et al., 2008). Recent studies in Drosophila show that Myosin II physically interacts with Myosin VIIA, probably through the mono-ubiquitination of Myosin II, which is regulated by the E3 ligase, Ubr3 (Li et al., 2016). The myosin IIA-myosin VIIA interaction and the mono-ubiquitination of myosin IIA also occur in the mammalian cochlea (Li et al., 2016), although an interaction...
between these two myosins in stereocilia (Fig. 3C) remains speculative. The overexpression of different pathogenic variants of Myosin II in Johnston’s organ leads to the apical detachment of scolopidia, which also occurs in Drosophila USH mutants (Li et al., 2016). These data indicate that Myosin II functions in Drosophila hearing, together with Myosin VIIA and/or other USH1 proteins; this interaction might also underlie the pathogenic effects of some pathogenic variants of myosin IIA in MYH9-related disease. The clear cut and quantitative scolopidal detachment phenotype (Fig. 4) associated with myosin II and myosin VIIA mutations in Johnston’s organ provides a quick and easy assay with which to investigate the molecular function of Myosin II in hearing and the mechanisms by which pathogenic Myosin II variants cause hearing loss. In the next section, we describe new technology that exploits the Drosophila to human conservation of genes and their variants in Drosophila.

New approaches for modeling human deafness in Drosophila

Mutagenesis-based screens in mice and Drosophila have identified candidate genes responsible for hearing loss and age-related hearing loss (Eberl et al., 1997; Hrabe de Angelis et al., 2000; Kernan et al., 1994; Nolan et al., 2000; Potter et al., 2016; Senthilan et al., 2012). Researchers used a large-scale lethal mosaic screen (Box 1) to isolate mutations that affect the Drosophila peripheral nervous system (Yamamoto et al., 2014). A secondary mosaic screen was then performed in this mutant collection by creating mutant clones in the antenna to identify mutants with an abnormal Johnston’s organ (Li et al., 2016). As discussed in previous sections, genes implicated in hearing in these Drosophila screens include those known to cause hearing loss in humans or mice. The convenience of Drosophila as a model organism, including its short life span and armory of genetic tools, makes it an attractive system to investigate the function of genes involved in hearing. Historically, overexpression experiments have been used to test gene function in Drosophila, either using the relevant fly gene or a conserved mammalian homolog. However, it is not always possible to unambiguously identify mammalian homologs of Drosophila genes based on sequence alone. Moreover, these approaches are not necessarily able to dissect why particular human gene variants are pathogenic, especially in the case of missense mutations. In addition, overexpressing a gene or gene variant at nonphysiological levels can lead to experimental artifacts. In recent years, advances in recombination-mediated cassette exchange (RMCE, Box 1) technology and the ability to perform homologous recombination in Drosophila using the CRISPR-Cas9 system (Box 1) have allowed researchers to rapidly test the function of human genes in Drosophila and to rapidly assay the effects of potentially pathogenic human variants far more quickly than in mice. In this section, we describe the components of this technology, which is summarized in Fig. 5.

Gene trap cassette integration and exchange

An identified ortholog of a human or mouse gene is a prerequisite for functional testing in Drosophila. Although not all candidate disease genes will have invertebrate orthologs, ∼60% of the protein-coding genes in the Drosophila genome are conserved in mammals, and Drosophila orthologs have been identified for about two-thirds of human disease genes (Chien et al., 2002; Hu et al., 2011; Reiter and Bier, 2002; Spradling et al., 2006). Numerous Drosophila Minos-mediated integration cassette (MiMIC) lines are available (Venken et al., 2011) that can be further modified using RMCE (Nagarkar-Jaiswal et al., 2015a,b). Using this technique, an artificial exon that serves as a gene trap – such as a GAL4 Trojan exon cassette (Diao et al., 2015) – can be inserted into a MiMIC line to create a premature termination signal that disrupts the function of the endogenous gene. If MiMIC lines are not available or suitable for a particular gene, it is now possible to insert MiMIC-like cassettes in Drosophila genes using CRISPR-mediated homology-directed repair (Port et al., 2014; Ren et al., 2013).

Green fluorescent protein tagging to characterize expression and function

Artificial exons can be created to have flanking sites that facilitate RMCE (Nagarkar-Jaiswal et al., 2015a,b; Venken et al., 2011). These exons allow fluorescent tags to be introduced into a gene of interest to reveal its cellular and subcellular expression pattern (Fig. 5). This approach has been used to modify hundreds of Drosophila genes, providing detailed expression data and with ∼75% of the genes retaining their function after incorporation of the internal exon-encoding green fluorescent protein (GFP), as shown by their ability to rescue null alleles (Nagarkar-Jaiswal et al., 2015b). To date, nearly 10,000 Drosophila genes have been GFP
Reverse genetics: study function of candidate genes

- Overexpress the human variants or ‘humanized’ version of fly genes
- Assess LOF phenotypes associated with T2A-GAL4 gene trap mutants
- Rescue with human cDNA; express human pathogenic variants using T2A-GAL4 (precise temporal and spatial expression)

Forward genetics: identify novel regulators in hearing

- Mutagenesis-based screens followed by mapping
- Knockdown of proteins through iGFPi or deGradFP (efficient knockdown; bypass mapping)

Expression pattern in auditory organs

- Microarray, RNA-seq, antibody staining, GAL4 reporter lines
- Characterize expression pattern and protein localization using GFP-tagged protein trap lines

Traditional strategies

New strategies

Fig. 5. Approaches to functionally test candidate mammalian deafness genes in *Drosophila*. (A) Candidate genes can include known deafness genes or mouse genes with deafness phenotypes. Their *Drosophila* homologs are predicted by protein sequence homology. (B) An artificial exon that acts as a gene trap can be inserted into a MiMIC line by recombination-mediated cassette exchange (RMCE; Venken et al., 2011) or by CRISPR-mediated homology-directed repair, which prematurely terminates transcription of the endogenous gene through a splice acceptor site (SA) and poly-adenylation signal (PolyA), and expresses the GAL4 transcriptional activator as part of the endogenous transcript through a picornavirus self-cleaving 2A peptide sequence (2A). (C) Gene trap/GAL4 lines are screened for hearing phenotypes. The GAL4 protein expressed by the endogenous regulatory elements of the gene of interest can also be used to drive the expression of transgenes under the control of the UAS DNA element inserted elsewhere in the fly genome. These transgenes can be wild type, or pathogenic human or mouse orthologs, and the insertion of GAL4 into the endogenous locus allows the transgene to be expressed in a spatiotemporal pattern that is identical to the endogenous *Drosophila* gene. RMCE through attP and attB sites (yellow triangles) can convert the Trojan exon into an exon containing GFP flanked by SA and splice donor (SD) sites, to reveal the cellular and subcellular localization of the trapped gene (Nagarkar-Jaiswal et al., 2015b). The GFP exon can also be used to knockdown the encoded transcript or protein by in vivo GFP interference (iGFPi) or the deGradFP system, respectively. LOF, loss of function; RNA-seq, RNA sequencing.

tagged in this way (Kanca et al., 2017). These lines also allow gene and protein function to be tested through the targeting of the GFP-encoding exon with RNA interference (RNAi) approaches (Neumuller et al., 2012; Pastor-Pareja and Xu, 2011). In the adult fly, however, the stability of some proteins in postmitotic adult cells can preclude efficient RNAi knockdown. To address this, a genetic system known as the deGradFP system can be used to directly degrade the GFP-tagged protein by using a modified form of the ubiquitin-proteasome pathway to specifically target and degrade GFP-tagged proteins (Caussinus et al., 2012, 2013) (Fig. 5).

Testing auditory function in *Drosophila*

*Drosophila* mutants can be assessed for auditory function in several ways. In adult flies, a sound-evoked compound action potential can be recorded from the antennal nerve (Eberl et al., 2000; Todi et al., 2008), although this method is less sensitive if small clones of mutant cells are being assayed in an otherwise wild-type background (Li et al., 2016). Behavioral assays can also be used to detect hearing in response to stimuli such as mating songs (Eberl et al., 1997), or the response of flies to the force of gravity (Kamikouchi et al., 2009), although, once again, these suffer from a limited sensitivity. It is also possible to measure active movement of the antenna by laser vibrometry and to record the activity of single giant neurons in the *Drosophila* brain (Kamikouchi et al., 2009; Tootoonian et al., 2012). Finally, it is possible to examine the morphology of Johnston’s organ in different mutants using an array of antibodies and GAL4 lines (Box 1) that express fluorescent proteins in the cellular components of scolopidia.

Testing mammalian homologs and pathogenic variants

Having identified and characterized the function of *Drosophila* orthologs of mammalian genes in hearing, it is possible to test their functional conservation by rescuing a *Drosophila* mutation with one of its human or mouse orthologs. The use of GAL4-Trojan gene traps (Fig. 5) is particularly useful in this regard. In this approach, the GAL4 transcription factor is targeted to an endogenous *Drosophila* exon. By fusing the GAL4 sequence to a T2A peptide-coding sequence, the endogenous protein begins to be translated, but is then truncated and GAL4 protein is expressed instead. This transcriptional activator then activates the expression of a mammalian complementary DNA (cDNA) of interest under the control of a UAS promoter from a separate locus in the *Drosophila* genome (Fig. 5). Thus, it is possible to inactivate a *Drosophila* gene and simultaneously assay whether a mammalian homolog can functionally rescue the inactivated gene. Moreover, once it has been established that a mammalian cDNA can rescue its *Drosophila* ortholog, it is then possible to test variants of the mammalian gene to determine whether they are pathogenic, and if so, to evaluate the cellular or biochemical consequences of such pathogenic variants (Bellen and Yamamoto, 2015). The function of potentially dominant variants can also be assessed in this way by overexpressing the variant of interest in Johnston’s organ to see if it elicits a phenotype. For example, pathogenic variants of Myh9 known to cause deafness in humans also cause morphological defects in scolopidia when overexpressed in Johnston’s organ (Li et al., 2016).

The GAL4-Trojan system has several advantages over conventional methods to test gene function by overexpression. First, it can test the function of a mammalian gene in the context of a
null mutation for its *Drosophila* counterpart. Second, targeting the GAL4 cassette to the *Drosophila* gene of interest significantly increases the likelihood that the GAL4 activator will be expressed in the same spatial and temporal pattern as the endogenous *Drosophila* gene. Finally, the incorporation of DNA sequences in the Trojan cassette that permit RMCE allows the locus to be modified further with little effort. Although these advances are significant, they still have limitations. For example, this approach is only feasible if clear gene homologs can be identified between *Drosophila* and mammals. Moreover, although expression of the GAL4 transactivator might be faithful, the UAS promoter might drive expression of the mammalian gene at levels greater than those of the endogenous *Drosophila* gene.

**Conclusion**

The field of genetic hearing loss research has advanced significantly in the last several decades, but three challenges and opportunities remain. First, it is likely that many new genes that regulate hearing await discovery. The study of extended families, particularly those that feature consanguineous marriages, has revealed new deafness gene candidates, but following up these new candidates is hampered by the limited availability of these large families in restricted regions of the world. The National Institutes of Health (NIH)-funded Undiagnosed Diseases Network (http://undiagnosed.hms.harvard.edu) seeks to find possible genetic etiologies for rare human diseases (Wangler et al., 2017), some of which include hearing loss, and it is likely that this initiative will reveal new forms of syndromic hearing loss. In addition to the ongoing study of human families with hereditary deafness (Smith et al., 2014), there is currently a worldwide effort to create, characterize and curation mutations in every mouse gene coordinated through the International Mouse Phenotyping Consortium (IMPC) (www.mousephenotype.org), with the US effort coordinated through an NIH-funded Knockout Mouse Project (www.komp.org). Because auditory testing is part of the standard IMPC phenotyping pipeline, it is likely that many new mouse genes will be identified that can cause hearing loss in homozygous or heterozygous mutants, and this might support the identification of human variants that also cause hearing loss. Mutagenesis-based screens in mice have also revealed new genes responsible for hearing loss and age-related hearing loss (Hrabe de Angelis et al., 2000; Nolan et al., 2000; Potter et al., 2016).

Although over 100 deafness genes have been identified, a second challenge is to understand the function of these genes in hearing. While the cellular and biochemical functions of some of these genes have been extensively characterized, many other known deafness genes remain poorly characterized. Moreover, the pathogenic basis of many human deafness gene variants await elucidation. In this Review, we have suggested that the homologies between insect and mammals, in terms of the genes involved in hearing organ development and function, make *Drosophila* an attractive system for assaying the function of deafness-associated gene variants. We believe that the technology described in this Review will make it possible to perform medium- and high-throughput screens of candidate mammalian genes and known deafness-associated genes in *Drosophila*, in addition to identifying novel genes that are essential for hearing in *Drosophila* that might have mammalian homologs.

Once the pathogenic mechanisms of human deafness gene variants have been established, the final challenge is to develop therapies for particular forms of deafness that do not rely on hearing aids or cochlear implants. As with other forms of genetic disease, it is possible to improve function by pharmacological approaches that, to name but three, stabilize protein complexes, enhance the function of the affected protein(s) or proteins acting downstream of the affected proteins, and slow the aggregation of pathogenic protein variants. Once again, we suggest that *Drosophila* might offer a promising platform in which to rapidly screen for compounds or signaling pathways that improve auditory function in animals bearing pathogenic hearing gene variants.

**Competing interests**

The authors declare no competing or financial interests.

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