Int. J. Dev. Biol. 51: 663-678 (2007)
doi: 10.1387/ijdb.072367bf

Molecular evolution of the vertebrate mechanosensory cell and ear

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ABSTRACT The molecular basis of mechanosensation, mechanosensory cell development and mechanosensory organ development is reviewed with an emphasis on its evolution. In contrast to eye evolution and development, which apparently modified a genetic program through intercalation of genes between the master control genes on the top *(Pax6, Eya1, Six1)* of the hierarchy and the structural genes *(rhodopsin)* at the bottom, the as yet molecularly unknown mechanosensory channel precludes such a firm conclusion for mechanosensors. However, recent years have seen the identification of several structural genes which are involved in mechanosensory tethering and several transcription factors controlling mechanosensory cell and organ development; these warrant the interpretation of available data in very much the same fashion as for eye evolution: molecular homology combined with potential morphological parallelism. This assertion of molecular homology is strongly supported by recent findings of a highly conserved set of microRNAs that appear to be associated with mechanosensory cell development across phyla. The conservation of transcription factors and their regulators fits very well to the known or presumed mechanosensory specializations which can be mostly grouped as variations of a common cellular theme. Given the widespread distribution of the molecular ability to form mechanosensory cells, it comes as no surprise that structurally different mechanosensory organs evolved in different phyla, presenting a variation of a common theme specified by a conserved set of transcription factors in their cellular development. Within vertebrates and arthropods, some mechanosensory organs evolved into auditory organs, greatly increasing sensitivity to sound through modifications of accessory structures to direct sound to the specific sensory epithelia. However, while great attention has been paid to the evolution of these accessory structures in vertebrate fossils, comparatively less attention has been spent on the evolution of the inner ear and the central auditory system. Recent advances in our molecular understanding of ear and brain development provide novel avenues to this neglected aspect of auditory neurosensoy evolution.

KEY WORDS: *ear evolution, hair cell evolution, otic placode evolution, auditory system evolution*

Introduction

Next to the eye, the ear, with its ability to convert sound into meaningful signals that help mediate spoken communications, is the most important sense for human social interactions. Understanding how these two senses evolved and become the dominant input for everyday interactions is thus an important aspect for evolution of human. In the visual system, two insights have helped stimulate molecular evolutionary research:

A) A small set of genes (e.g., *Pax6*, *Atoh7/atonal*, *Eya1*) produce essential transcription factors for the development of eyes no matter what shape or form (Gehring, 2005).

B) All rhodopsins mediating photic stimulation are related at the molecular level thus aligning diversely shaped photoreceptors through a common molecular basis (Arendt *et al.,* 2004).

Thus, it has been proposed that molecular evolution of receptor, receptor cell and receptor organ all are tightly interrelated through a nested set of conserved genes necessary to specify the expression of rhodopsins in receptor cells and to ensure that the cells are properly placed within an organ to direct light to the cells that contain the highest concentration of rhodopsin. This view of photoreceptor evolution reflects the simple fact that even single celled organisms have specialized photoreceptor areas and that multicellular organisms have photic reception without specialized

Abbreviations used in this paper: bHLH, basic helix-loop-helix.

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0214-6282/2007/$30.00
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Printed in Spain
www.intjdevbiol.com

cells or with specialized cells that do not form a distinct organ. Subsequent evolution of the various eye types may have occurred by the modification of an original genetic program through intercalation of genes between the master control genes on the top of the hierarchy and the structural genes like rhodopsin at the bottom (Gehring, 2005) or other means (Alonso and Wilkins, 2005). In essence, what appears to be panoptical diversity of structurally dissimilar eyes may in fact be permutations of an ancient theme. In fact, some of the molecules associated with eyes are also conserved in ears (Fritzsch *et al.,* 2005b). Most importantly, despite an emerging and possibly unifying molecular evolutionary theme for these major sensory systems, shear structural diversity has been viewed as an insurmountable obstacle to align ear evolution across phyla.

In part this is so because evolution of terrestrial hearing has long been investigated from the point of view of evolution of three middle ear ossicles and the tympanic membrane owing to readily available but difficult to interpret fossils (Brazeau and Ahlberg, 2006, Clack, 2002, Fritzsch, 1992, Fritzsch, 1999, Massa *et al.,* 2006). However, the evolutionary changes in the inner ear that allow the selective perception of sound in a specialized organ have received much less attention despite the fact that any adaptive change in the middle ear requires the prior existence of an inner ear to make use of those changes in terms of sound perception (Fritzsch, 1992, Fritzsch *et al.,* 2006c). This lack of consideration of the inner ear relates to the near complete absence of fossil evidence of inner ear evolution, the contentious interpretation of existing data on extant taxa, the incomplete understanding and diverse interpretation of taxonomic relationships of key species and an incomplete understanding and implementation of the physics of sound in water and on land toward interpretation of the putative receivers. This review will focus exclusively on the inner ear aspect of vertebrate ear evolution, highlighting the necessary developmental change required to alter existing molecular programs to generate a novel, adaptive outcome.

Logically, the starting point for considerations on the evolution of sound perception has to be the hair cell and its mechanotransduction apparatus, the stereocilia with their tip links to open and close a still unknown channel. This channel allows ionic currents to change the resting potential to reflect sound mediated stimulations. We will first review the evolution of mechanosensation at the molecular and cellular level. Accessory structures and grouping of hair cells into organs that permit perception of distinct stimuli is the next logical step in evolution and the molecular evidence of how this might have happened will be reviewed next. Among vertebrates, it has long been recognized that a purely vestibular ear, dedicated to the perception of gravistatic and angular stimuli, predates the evolution of whole epithelia or their parts to dedicated sound receivers. We will therefore next review the evolution of the vertebrate ear, including the molecular evolution of placode formation and the molecular basis for morphogenesis to form the vertebrate labyrinth. Finally, evolution of an auditory system requires alterations in the developmental program of the ear to generate a sensory epithelium dedicated to sound, a set of sensory neurons dedicated to conducting the sound elicited information to the brain and a central target area dedicated to the reception of sound. In this part of our analysis we will review how a separate epithelium can arise, how it can be transformed into a sound perceiving epithelium through specific interactions with the adjacent periotic mesoderm and how separate sensory neurons can be generated and specified to project to a different central target than nearby vestibular neurons.

Molecular evolution suggests conservation of tethers, but not of channels

Mechanosensation refers to a mechanical stimulus-driven opening and closing of an ion channel to change the resting potential of the sensory cell. Despite extensive efforts, the mechanosensory equivalent of the phototransduction protein rhodopsin has not yet been found. Molecular evolution of mechanosensation thus cannot be understood the same way rhodopsin evolution contributes to understanding vision (Arendt *et al.,* 2004, Gehring, 2005). Comparison across phyla shows that mechanosensation comes in two basic principles:

1) Stretch within the membrane opens a channel.

2) Stretch on intracellular and/or extracellular tethers open a channel (Fig. 1).

Obviously, membrane stretch exists already in unicellular organisms whereas mechanosensation with tethers requires cellular specializations such as specific stiffness inside a cell (assembly of microtubules, intermediate filaments or actin filaments) or connections outside a cell (such as other cells, other protruding parts of the same cell or extracellular matrix) to be properly organized (Fig. 1). Interestingly, some of the molecules now known to be associated with such tethers show some degree of conservation. For example, the rare myosin VIIa is associated with hearing loss in both vertebrates and flies (Todi *et al.,* 2005), VLGR mutations cause hearing loss in humans and the gene exists in many Deuterostomes including sea urchins and protocadherins are found in most metazoan phyla (Burke *et al.,* 2006, Sodergren *et al.,* 2006).

In contrast to this apparent conservation and function as a part of the mechanotransduction process of molecules known or suspected in tethering, there is no candidate gene representing a conserved channel. Indeed, all candidates of putative vertebrate channel genes that have been mutated to date have resulted in minor effects but no loss of mechanosensation (Kwan *et al.,* 2006, Lin and Corey, 2005). The only known channel genes are those of the worm, *C. elegans* (O'Hagan *et al.,* 2005). These genes belong to the *ENaC/BNaC* family of genes, but have no orthologue in vertebrates. Nevertheless, the most likely candidates for mechanosensory channels are among the *ENac/BNaC* and *Trp* channel genes (Sukharev and Corey, 2004). Detailed sequence comparisons using existing databases show only limited conservation of a few genes across vertebrates that all share the same mechanosensory cell, the hair cell (Beisel *et al.,* 2007). Indeed, one gene identified as being crucial in mechanosensory function of zebrafish does not exist in mammals (Gillespie *et al.,* 2005, Sidi *et al.,* 2003). Combined, these data suggest that mechanosensory channel proteins might not be conserved and might actually be organized of several different components that form a heteromultimer. Such multimers with variable functions were recently described for the *TRPV* thermosensitive channels (Cheng *et al.,* 2007). If this is also true for

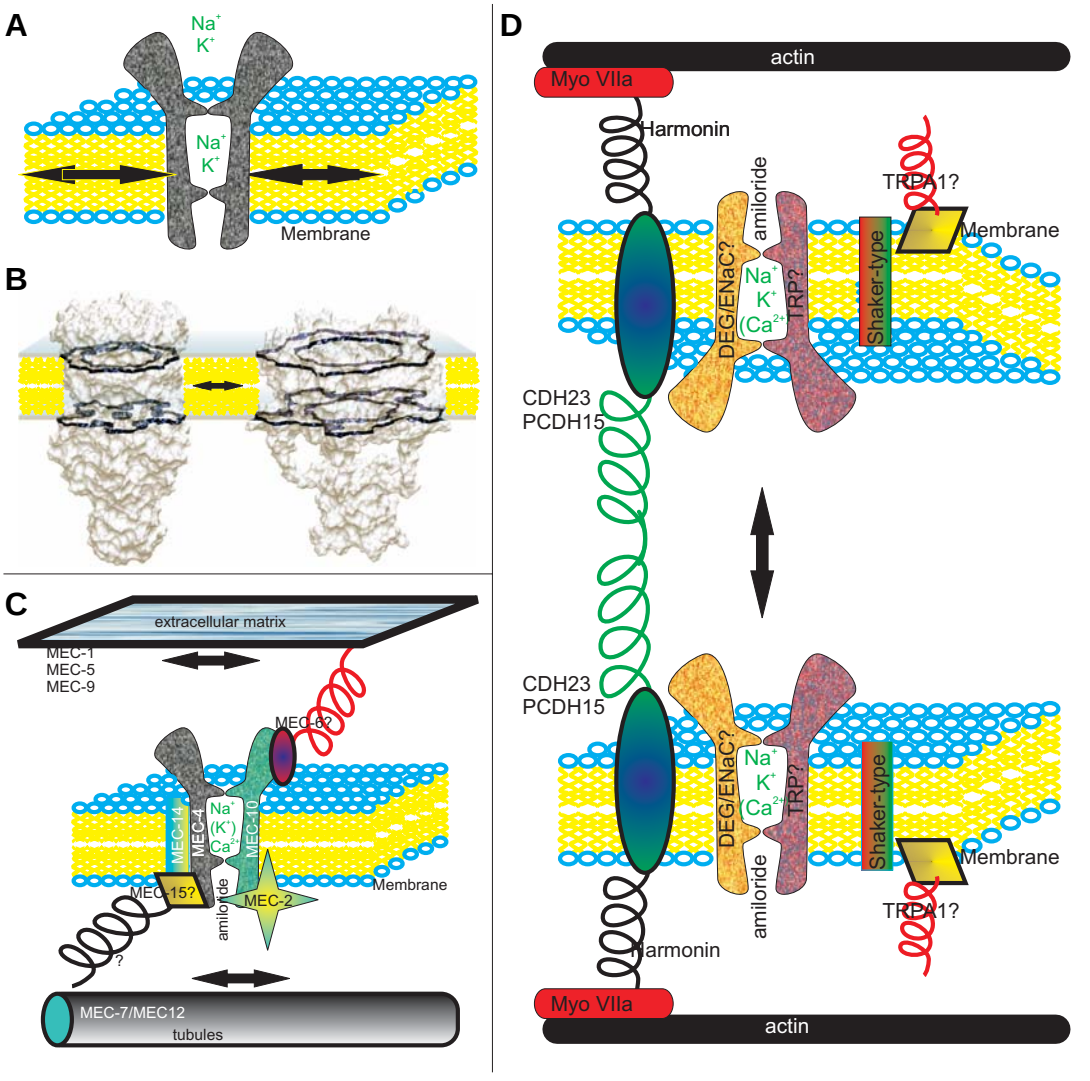

Fig. 1. Various channels that respond to changes in turgor resulting in stretch of the membranes (double arrow in A, B) have been found in single cell organisms. *Detailed models of the pentameric mechanosensitive channel of bacteria suggests an iris-like opening upon tension acting in the plane of the membrane (B). It is possible that such mechanosensitive channels were modified in the unicellular ancestor of metazoans through extracellular or intracellular matrix attachments to provide increased sensitivity for shearing forces. Molecular evidence suggests that, across metazoans, only members of two cation channel families are candidates for mechanosensitive channels* **(C,D)**. *It thus remains possible that metazoan ancestors evolved either or both families for specific properties that allow increased sensitivity to detect mechanical stimulations. What such properties could be remains unknown in the absence of any model of sensitive mechanosensory channel in any metazoan taxon. Mutational analysis has identified several genes that are essential for the function in nematodes* **(C)** *and vertebrates* **(D)**. *In nematodes, fine touch is lost when either specific molecules of the extracellular matrix to which the channel is anchored are lost (Mec-1, 5, 9), or if specific components of the channel complex are lost (Mec-2, 4, 10, 14). However, loss to the intracellular tubules (Mec-7, 12) may lead only to a reduced sensitivity, not a complete loss of sensation. In contrast, in vertebrates there is no extracellular matrix or cuticular connection. Instead, two stereocilia are interconnected presumably by Cdh23 that is hypothesized to be anchored to MyoVIIa via harmonin. Loss of any of these genes results in deafness indicating that in vertebrates mechanosensation requires relative movement against the actin core of the stereocilia. Additional connections exist between Cdh23 and Myo1c but no knockout data support the claimed function as an adaptor. It is speculated that MyoVIIa transports the still unknown amiloride sensitive mechanosensory channel to the tip but it is unclear whether this connection remains past development. In nematodes at least two essential subunits of the mechanosensitive channel are known whereas it is not clear what the vertebrate channel is composed of. Certain candidates have been excluded as mutants in, for example, TRPA1 do hear excluding an essential role of this protein in mechanosensory transduction. Note that both nematode and vertebrate have a shaker-type channel associated with the mechanosensory channel, but details are unknown. Modified after (Bryant et al., 2005, Chiang et al., 2004, Syntichaki and Tavernarakis, 2004).*

mechanosensory channels, eliminating a single gene will only result in measurable effects if the gene happens to provide the major or only component of the multimeric mechanosensory channel.

It is thus likely that the only aspect that is conserved across phyla for such channel proteins is an ability to attach to the apparently conserved tethering proteins. The constraints imposed by this basic principle are few and thus would allow for a multitude of cellular solutions to ensure that the membrane bound channel can be driven by mechanical stimuli. Comparative analysis of mechanosensory cells and organs thus show, expectedly, a rich variety of specializations suspected or known to function in the context of mechanosensation. The coordinated expression of apparently multiple genes in a given mechanosensory cell to assemble the mechanosensory complex requires higher level transcription factors that have the capacity to regulate the spatio-temporal expression of all those proteins within a cell and, in case of cell-cell connections, between neighboring cells. We therefore will next analyze the evolution of cellular transcription factors known or suspected

to play a role in this process across phyla.

Conserved transcription factors regulate mechanosensory cell development

Cellular differentiation of neurons and sensory cells requires bHLH genes (Bertrand *et al.,* 2002, Kageyama *et al.,* 2005) a highly conserved set of genes that evolved in single cell organisms where they functioned in regulating metabolism (Simionato *et al.,* 2007). Two of those bHLH gene families, the achaete-scute and the atonal family of genes appear to be associated with mechano-, chemo- and photoreceptor development across phyla. Of these genes, the atonal family of genes is more closely associated with photo- and mechanosensation (Fig. 2). Specifically, atonal genes evolved with multicellular organisms (Seipel *et al.,* 2004) and are among a few protein coding genes that are structurally and functionally conserved to the extent that orthologues of fly and mouse, *atonal* and *Atoh1*, can be mutually exchanged and show compensatory function in distant organisms (Wang *et al.,* 2002). The bHLH family shows expansion consistent with the requirement of additional members to regulate differentiation of developmentally and physically connected cells in vertebrates, the sensory neurons and hair cells (Fritzsch *et al.,* 2000, Fritzsch *et al.,* 2006b). The best known downstream factor of atonal genes is *Barlh*, a gene necessary to maintain hair cells which are progressively lost in null mutantsÄ(Li *et al.,* 2002). It remains unclear how *Atoh1* is affecting overall development of hair cells, but only undifferentiated precursors form in *Atoh1* null mice that eventually degenerate (Bermingham *et al.,* 1999, Chen *et al.,* 2002, Fritzsch *et al.,* 2005a). Likewise, *atonal* null mutant flies lose most of their mechanosensory proprioception, including hearing (Caldwell and Eberl, 2002). Atonal-like genes seem to have a somewhat broader function in coelenterates where they specify sensory neurons as well as myosensory cells (Seipel *et al.,* 2004).

In addition to bHLH genes, Pou domain factors are essential and conserved transcription factors for cellular differentiation, including the hair cells of the ear (Erkman *et al.,* 1996, O'Brien and Degnan, 2002) which initially form but soon degenerate in *Pou4f3* null mice (Hertzano *et al.,* 2004, Xiang *et al.,* 2003). Downstream to *Pou4f3* is another essential gene for sensory development, the zinc-finger protein *Gfi1* (Hertzano *et al.,* 2004, Wallis *et al.,* 2003). Hair cells form in the absence of *Gfi1* but degenerate over time (Hertzano *et al.,* 2004). The orthologue of *Gfi1* in insects (*senseless*) is needed for differentiation of sensory cells and can even substitute for atonal in insects (Jafar-Nejad *et al.,* 2003). Interactions of *senseless* and bHLH genes appear to function as a binary switch to promote or suppress sensory fate and thus to enhance other associated processes such as delta-notch lateral inhibition (Daudet and Lewis, 2005, Kageyama *et al.,* 2007). It appears that mechanosensory cell development is governed by a nested expression of transcription factors that cooperate to ensure complete differentiation and maintenance of such cells. Neither the individual functions nor interactions of transcription factors required to achieve the desired outcome are completely understood (Fritzsch *et al.,* 2006a, Kelley, 2006a). However, it is clear that these genes are ancestral to triploblasts and possibly metazoans and this may form the basis of mechanosensory cell evolution.

Despite the clear importance of such transcription factors for mechanosensory cell development, their regulation remains unclear except that *Pou4f3* regulates *Gfi1* and that *Atoh1* regulates *Barlh*. It appears that in mammals *Sox2* regulates, directly or indirectly, expression of *Atoh1* as no *Atoh1* expression has been reported in *Sox2* hypomorphs (Kiernan *et al.,* 2005). How *Sox2* expression is regulated, however, remains unclear despite tremendous insights into the *Sox2* promoter region (Uchikawa *et al.,* 2003). Moreover, in insects there is no evidence for the expression of Sox genes in neurosensory precursors (McKimmie *et al.,* 2005), suggesting that *Sox2* in the PNS is a vertebrate acquisition that may relate to the clonal expansion of neurosensory precursors required to form large sensory arrays such as the sensory epithelia of the ear (Pauley *et al.,* 2005). In contrast, flies seem to use EGFr for somewhat similar clonal expansion (Eberl and Boekhof-Falk, this volume). How *Pou4f3* expression is regulated is even less

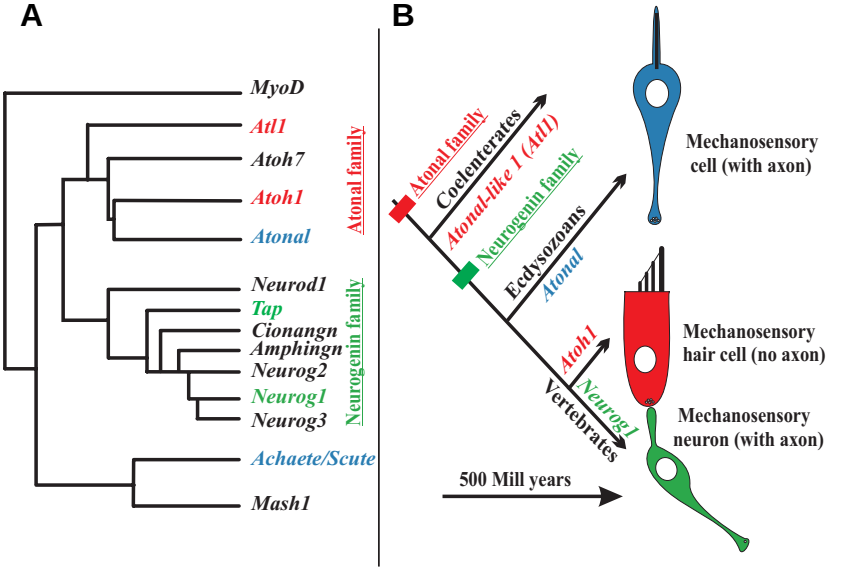

Fig. 2. The evolution of the atonal and neurogenin families of bHLH genes (A) and the evolution of the mechanosensory cells and their associated neurons that require those family members for cellular development (B) is shown. *Note that atonal and achaete/scute family evolved already in coelenterates. However, the neurogenin family may have evolved only in triploblasts. Evolution of a pair of cells (a secondary mechanosensory cell without an axon and a mechanosensory neuron connecting the cell to the brain) evolved out of a primary mechanosensory cell (with an axon) only after the neurogenin family had evolved. Whether atonal family members are always associated with mechanosensory cells in triploblasts and whether neurons associated with mechanosensory cells in other triploblasts require neurogenin for development is unknown. Modified after (Fritzsch and Beisel, 2004, Furlong and Graham, 2005).*

clear and it remains equally unclear how independent its expression is of *Atoh1*.

In addition to the regulation of molecular components that possibly govern the organization of the mechanosensory apparatus, these early expressed transcription factors also need to ensure that a mechanosensory cell develops through suppression of alternative fates such as supporting cell or generalized ectoderm. It appears that the former is predominantly regulated through the ubiquitous delta/notch system for lateral inhibition of cell fate (Daudet and Lewis, 2005, Fritzsch *et al.,* 2006a, Kageyama *et al.,* 2007, Lanford and Kelley, 2005) whereas the latter might require the presence of specific inhibitors of transcription and/or translation to eliminate mRNA that favors an alternate fate. In addition to hair cell specific mRNA for transcription factors that positively regulate mechanosensory cell development, the microRNAs (miRNAs) known to be selectively expressed in mechanosensory cells (Weston *et al.,* 2006) can repress specific existing mRNAs and thus might ensure a secure transition from one presumptive cell fate to another. Most importantly, these mechanisms likely evolved prior to the formation of mechanosensory organs. It is conceivable that, in analogy to the evolution of photoreceptor organs, a rich variety of organs can evolve using the highly conserved set of genes currently characterized for mechanosensory development across phyla (Fritzsch and Beisel, 2004, Todi *et al.,* 2005). Among these conserved set of genes, miRNAs are the most conserved. Indeed, not a single base may be different across entire phyla. Such conservation might eventually enable us to understand the molecular evolution of mechanosensory cell development through an understanding of the genes targeted for repression by miRNAs. Such genes are expected to antagonize *Atoh1/atonal* signaling and stabilize the default development as ectodermal/skin cells.

Cellular transitions in fate will bear the risk for cells that novel gene expression patterns interfere with the existing profile. Minimizing such risk requires a molecular means to stabilize precursors from cell death to enable such transitions. A set of molecules recently identified to have this capacity are the Pax genes (Bouchard *et al.,* 2002, Torban *et al.,* 2000). As with eye development, *Pax2/5/8* expression has been associated with mechanosensory development across phyla (Czerny *et al.,* 1997, Kozmik *et al.,* 2003), possibly providing this

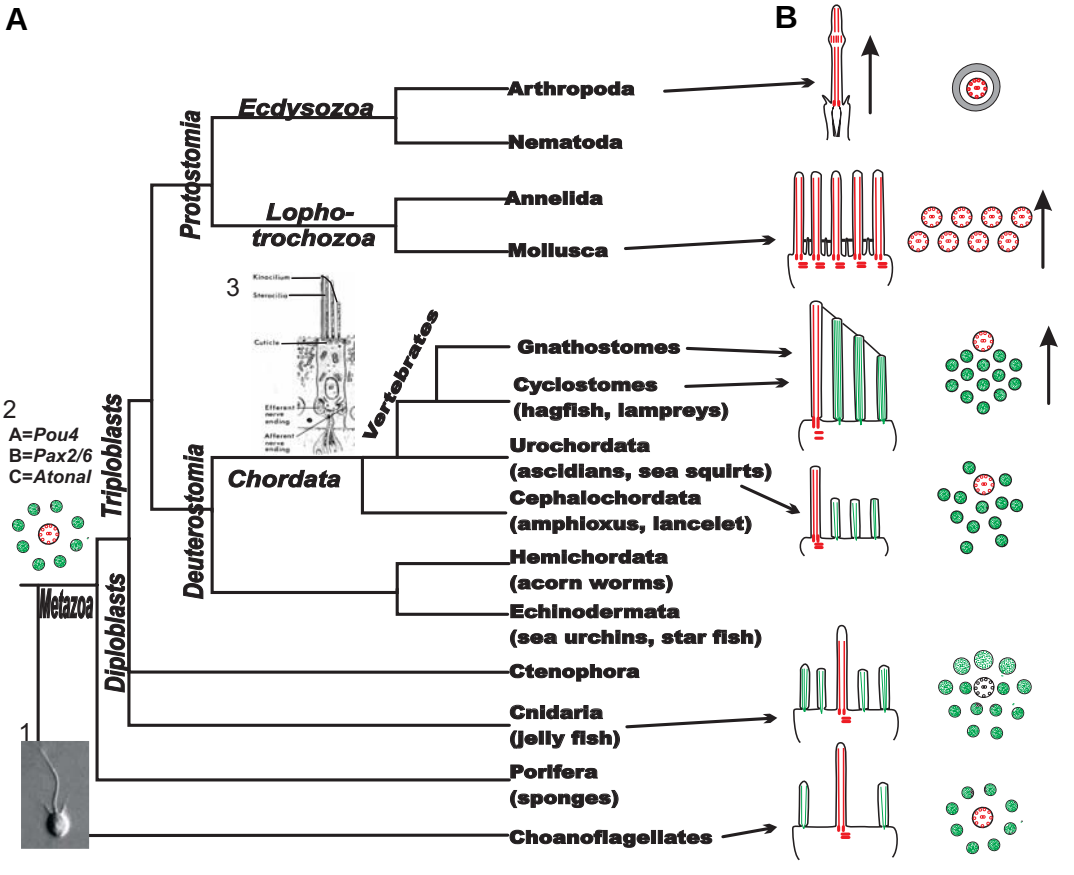

Fig. 3. Evolution of mechanosensory cells. *Kinocilia (red) and microvilli (light blue) of known or suspected mechanosensory cells in various eukaryotic unicellular (1) and multicellular (3) organisms are shown. Orthologues of structural genes relevant for mechanosensation or for development of polarity such as actin, tubulin, rare myosins, cadherins, espin, β-catenin and Wnt genes and several transcription factors are known in protists, Diploblasts (2) and various triploblasts and are thus ancestral to vertebrates. Note that the single celled ancestor of all multicellular animals, the choanoflagellates (1), has a single, actively beating kinocilium surrounded by microvilli that carry an actin core* **(A)**. *In some diploblasts the central kinocilium is surrounded by an asymmetric assembly of microvilli of various diameters, potentially providing directional sensitivity* **(B)**. *Among deuterostomes, urochordates have various presumed mechanosensory cells that have a kinocilium with asymmetrically arranged microvilli. Vertebrates are unique in that a highly polarized, organ-pipe assembly of actin rich stereocilia is attached via tip links with each other and the asymmetrically placed kinocilium. Among protostomes, mollusks may have numerous, interconnected kinocilia on mechanosensory cells. Ecdysozoans have either mechanosensory cells with cilia or have a kinocilium that is stretched by the stimulus. Arrows indicate the direction of stimulation. Statocysts are known for many taxa of metazoans, but a lateral line system is restricted to few. Modified after (Arkett* et al., *1988, Budelmann, 1989, Burighel* et al., *2003, Fritzsch* et al., *2006b, Jorgensen, 1989, Steenkamp* et al., *2006, Todi* et al., *2005).*

important function for cell survival through the molecular transition throughout evolution. Additional factors enabling the proneurosensory transformation and subsequent clonal expansion are *Eya1, Six1* and *Gata3* (Karis *et al.,* 2001, Schlosser, 2006, Zou *et al.,* 2006). Combined, these factors form a core for organ development and their absence is invariably associated with severe reduction if not entire loss of specific sensory development.

Mechanosensory cells may represent an evolutionary variation of a generalized cellular theme

The above analysis suggests that mechanosensation as a cellular function could have evolved in single cell organisms, provided they have proper organization of cell protuberances to tether channels or to tether channels to a surrounding matrix. Indeed, the accepted single-cell origins of animals are protists that have a central kinocilium surrounded by microvilli that can be encased into a mineralized matrix, the choanoflagellates. The various apical specializations in metazoan cells that are suspected to play a role in mechanosensation can be organized into variations on a common theme (Fig. 3). In particular the deuterostome phylum resembles closely the organization of coelenterates and shows a variation of the common theme, a central kinocilium surrounded by microvilli. The hair cell of vertebrates always develops with a central kinocilium surrounded by microvilli and forms the asymmetric organization of the kinocilium with the staircase pattern of stereocilia under the influence of planar polarizing factors (Jones and Chen, 2007, Montcouquiol *et al.,* 2006, Wang and Nathans, 2007). In contrast, most sensory systems of protostomia appear to be derived through reduction of stereocilia/microvilli at the expense of kinocilia. Arthropod scolopidial organs appear to be the most derived where stress along, rather across the kinocilium is the appropriate stimulation (Todi *et al.,* 2005). Putative mechanosensors of less derived protostomia need to be investigated to understand the relationship of the apparently more derived features in this phylum with those found in Coelenterates and Deuterostomes.

Combined with the strong evidence of evolutionary ancestry of many cellular transcription factors and possibly certain components for the tethering of the mechanosensory transducer channel outlined above, it appears possible that hair cells of chordates represent a uniquely derived feature of an ancestral theme of mechanosensory cells and can be traced back to mechanosensory cells of coelenterates that also show some degree of asymmetric development (Fritzsch *et al.,* 2006b). The grouping of these molecular and anatomical features make it increasingly less likely that mechanosensory cells arose through independent evolutionary events as previously suggested based on fewer data (Coffin *et al.,* 2004). This grouping is also consistent with recent molecular data which suggests a close affinity between coelenterates and deuterostomes (Putnam et al., 2007).

Evolution of mechanosensory organs: grouping single mechanosensory cells into arrays and organs for specialized signal perception

It is a dogma of evolutionary biology that single celled organisms evolved before the more complex multicellular organisms. However, as far as sensory organ evolution is concerned, it appears to be conceptually difficult to accept the equivalence of mechanosensors represented either by single, distributed cells across the body or as a well organized sensory cell patch with a narrowly defined function of an epithelium in the vertebrate ear. However, recent years have shown that an ancestral global patterning of the body exists combined with a diffuse nerve net and singly distributed sensory cells (Burke *et al.,* 2006, Lowe *et al.,* 2003). Moreover, the basiepithelial nerve net or skin brain (Holland, 2003) maybe the ancestral condition in deuterostomes out of which the central nervous system evolved through concentration in a specific region (Fritzsch and Glover, 2006, Lowe *et al.,* 2003)}. It appears that such focal generation of the nervous system might be accomplished through altered patterns of gastrulation (Meinhardt, 2004). Most importantly, the molecular basis of this concentration process to form a central nervous system is molecularly distinct between protostomia and deuterostomia: the former require Fgf signaling, the latter do not (Bertrand *et al.,* 2003, Delaune *et al.,* 2005, Fritzsch and Glover, 2006). Comparable to this focal formation of a central nervous system is the local formation of all major sensory organs in chordates. Notably, as with the development of the central nervous system, development of the ear requires an epithelial transition that uses many of the same transcription factor families also used for central nervous system formation (Bailey *et al.,* 2006, Fritzsch *et al.,* 2006a, Litsiou *et al.,* 2005). As much as the embryonic precursor of the brain is the neural plate, so is the embryonic precursor of the ear the otic placode. In contrast, the evolutionary precursor of the brain is the diffuse nerve net whereas the evolutionary precursors of the ear might be single, diffusely distributed mechanosensory cells.

If we accept this interpretation, it then follows that evolution of placodes must be interpreted as an embryonic adaptation to ensure development of mechanosensors only in distinct places as compared to ubiquitous distribution over the entire skin. Indeed, in many chordates and also in amphioxus there is distribution of single sensory cells in the skin, suggesting that the capacity of the skin to generate diffusely distributed sensory cells is not fully suppressed in these species or may be the only sensory arrangement (Fritzsch *et al.,* 2006b, Holland, 2005). Moreover, it appears that such concentration of sensory precursors evolved independently several times in metazoans but might use only a limited set of conserved transcription factors to do so thus leading to a false impression of homology that is largely based on the common and ancestral, cellular development regulating transcription factors. In the following we want to discuss one specific transcription factor that is involved in ear development and has been thoroughly analyzed across several relevant taxa.

In vertebrates, *Pax8* is among the earliest genes unequivocally expressed in the developing otic placode of fish and mice (Nornes *et al.,* 1990, Pfeffer *et al.,* 1998) and appears to be largely co-expressed with *Pax2* in the mouse ear (Bouchard *et al.,* 2002, Zou *et al.,* 2006). Several papers have at least partially characterized the effects of *Pax2* null in the mouse ear (Burton *et al.,* 2004, Favor *et al.,* 1996, Torres *et al.,* 1996, Zou *et al.,* 2006) and show that *Pax2* function is essential for development of the cochlea in mice and human. Although the cochlea is a mammalian novelty (Fritzsch *et al.,* 2006b, Fritzsch *et al.,* 2006c), there is expression of *Pax2* in the ear of bony fish that have no cochlea (Pfeffer *et al.,* 1998,

Riley, 2003). *Pax2* expression in mammals is thus unlikely to reflect the ancestral function of *Pax2* in vertebrate ear development.

In addition, the functional analysis of *d-Pax2*, the fly *Pax2/5/8* orthologue, has shown the involvement of this gene in the bristle selection process and, most importantly, in the differentiation of the shaft and sheath cells (Kavaler *et al.,* 1999). However, whether *d-Pax2* plays any role in chordotonal organ formation, in particular in the Johnston hearing organ development of flies, is unknown. At a cellular level, several genes suggest homology between chordotonal organs of flies and hair cells in mammals, but much less evidence exists for surface mechanoreceptors related to bristles (Caldwell and Eberl, 2002, Fritzsch and Beisel, 2004, Todi *et al.,* 2005). Vertebrate *Pax8* is highly expressed in both neurosensory (including sensory neurons) and non-sensory areas, resembling thereby the expression of *d-Pax2* in the fly mechanosensory bristle development. *Pax2/5/8* is also associated with the statocyst of mollusks, suggesting some more specific association with mechanosensation in some Protostomes (O'Brien and Degnan, 2003). The issues around *Pax2/5/8* are equally unclear in Deuterostomes. Problematic for any attempt to use *Pax2* to establish organ homology is the widespread expression of *Pax2* in the tube feet of sea urchins (Czerny *et al.,* 1997) which have no know mechanosensory organ in those structures (Burke *et al.,* 2006) and the absence of *Pax2* expression in the ectoderm of amphioxus (Kozmik *et al.,* 1999) which are known to have single sensory cells in part suspected to be mechanosensitive (Holland, 2005). Combined with the apparent absence of function of *d-Pax2* in fly chordotonal organ development this suggests that the association of the *Pax2* transcription factor with the formation of mechanosensory organs is more variable then the association of *Pax6* with photoreceptors and may suggest that *Pax2/5/8* may play no role in the development of single mechanosensory cells. Whether the association of *Pax2/8* with vertebrate ear formation stems from chance or necessity remains to be analyzed once the development of mechanosensory organs in additional taxa has been elucidated. It remains therefore unclear at which time in the evolution of the vertebrate otic placode *Pax2/8* became associated, but *Pax2/8* is now an integrated aspect of molecular development of the vertebrate ear.

Beyond this unclear situation of *Pax2/8*, placode evolution needs to be interpreted in the context of global pattern formation in the course of evolution of the dorsal central nervous system of chordates. Apparently, Deuterostomes such as amphioxus and sea squirts have the same central patterning process, but only sea squirts have *Pax2/5/8* expression outside the central nervous system in structures interpreted by some as being possible homologues of the vertebrate ear (Kozmik *et al.,* 1999, Krelova *et al.,* 2002, Mazet *et al.,* 2005, Mazet and Shimeld, 2005, Schlosser, 2006, Wada *et al.,* 1998). Such interpretations seem to be supported by the existence of cells in those areas that have apical specializations consistent with their possible function as mechanosensors (Burighel *et al.,* 2003) but others argue against this (Holland, 2005). We are just beginning to understand how various factors interact with each other to generate the otic placode out of a much larger protoplacode (Bailey *et al.,* 2006, Litsiou *et al.,* 2005, Schlosser, 2006, Torres and Giraldez, 1998) through conversion of ectodermal cells to a new fate, formation of neurosensory and non-neurosensory otic cells (Fritzsch *et al.,* 2006a). Clearly, resolution of this discussion requires the study of many more factors involved in global patterning (*Wnt, Bmp, Shh*) and genes that are expressed in the ear (*Sox2, Gata3, Neurog1, Atoh1, Prox1, Isl1, Foxg1, Six1, Eya1*) in more Deuterostomes (Fritzsch *et al.,* 2006a, Ohyama *et al.,* 2006, Schlosser, 2006) before a conclusion can be reached. It is entirely possible that the obvious, small set of similarities in mechanosensory organ specific genes across phyla might have come about by an evolutionary independent transformation of genes involved in mechanosensory cell formation and organization into a localized group of cells driven by the global patterning process. The likely ancestral set of genes necessary for mechanosensory cell development will make it difficult to solve this problem. It will therefore be critical to analyze the otocyst/statocyst development in such distant species as squids and cuboidal medusas to appreciate similarities in the molecular basis of organ development. Combined with a better understanding of molecular pathways, we will eventually be able to distinguish between chance and necessity of molecules in organ development.

In summary, the most likely evolutionary scenario for statocysts and chordate otocyst evolution is the transformation of widely distributed, ancestral single mechanosensory cells into mechanosensory organs (Fritzsch *et al.,* 2006b, Jorgensen, 1989). Much like the discussion in the evolution of the visual system (many structurally distinct eyes consist of photosensitive modules that have convergently been organized into organs) can be resolved with this approach (Gehring, 2005), the apparent molecular similarities in otherwise rather different organs can be viewed as multiple, convergent evolution of various statocysts, including the vertebrate ear, out of diffusely distributed single mechanosensory cells. Indeed, in certain sea stars the tube feet near the tip of an arm can differentiate into eye-like organs, showing within an organism how diffuse gene expression of *Pax6* can be reorganized in certain areas only to govern photoreceptor development.

Older ideas discussed the lateral line system as a precursor of the ear. However, comparisons of the prevalence of lateral line and statocysts among metazoans suggest that every aquatic free swimming animal has a statocyst but only a small number have evolved lateral line-like organs (Bleckmann *et al.,* 1991, Jorgensen, 1989). Evolving both hydrodynamic and gravistatic organs out of an ancestral single mechanosensory cell indicates that this controversial discussion might essentially be obsolete as both might have evolved as a consequence of the global patterning change that led to the formation of a central nervous system and otic placodes in chordates (Fritzsch and Glover, 2006, Lowe *et al.,* 2003, Meinhardt, 2004).

Ear morphogenesis requires conserved genes for branching morphogenesis

Many, if not all, of the transcription factors used in ear development existed in chordate ancestors and apparently evolved as a nested set of genes for various aspects of branching morphogenesis, including intrusion or extrusion of lungs, glands, tracheas and limbs. Ear evolution has apparently tapped into this readily available resource of genes as the basic module to govern invagination and formation of canals (Fig. 4). However, while branching morphogenesis is typically a process of mesenchym/

ectoderm interaction, factors typically expressed in the mesoderm are expressed in the sensory epithelia in the ear. For example, in lung and lacrimal gland development, *Fgf10* is expressed in the mesoderm near the leading edge of the ectodermal invagination (Govindarajan *et al.,* 2000, Makarenkova *et al.,* 2000)). However, in the ear the expression of *Fgf10* moves rapidly into the ectoderm where it becomes focally expressed in the developing sensory organs (Pauley *et al.,* 2003, Wright *et al.,* 2003).

While involvement of the Fgf/branchless/breathless signaling pathway is not known to play a role in fly auditory organ development, there is a striking similarity in overall conservation of genes found to interact with each other in vertebrate ear and fly trachea development (Fig. 4). This impressive conservation of possibly functional interactions of genes, albeit in non-homologous organs, suggests a degree of molecular network stability that requires transposition of entire functional modules as components of developing systems. It is likely that the context of other genes expressed in the developing fly trachea and vertebrate ear will determine the outcome in terms of expression profiles of downstream genes. In analogy to the idea proposed for eye evolution, namely that only the top transcription regulator and the bottom functional genes may be conserved (Gehring, 2005), this example suggests that entire cascades of genes may be intercalated or added as functional modules to an existing program for overall similar and yet clearly distinct functions. Thus, branching morphogenesis in fly trachea and vertebrate ear have only very rudimentary developmental aspects in common, growth through proliferation and splitting of the growth plate into two parts that result in different outcomes. Most important for our consideration here is that the developmental program of otic vesicle of early chordates had already the potential to tap into an existing module that can drive specific aspects of morphogenesis. Effective use of this resource required the regulation of the expression of the entire module in the ear and modification of the signals to achieve new goals; namely, the formation of two or three canals instead of one, as in hagfish. While the outgroup comparison with fly trachea development strongly supports this model, demonstrating that hagfish have the same module but do not express it in the developing ear would provide even stronger support for this model. Limited evidence does support this idea for certain ear morphogenesis genes (Fritzsch *et al.,* 2001, Hammond *et al.,* 2002, Hammond and Whitfield, 2006).

Evolution of the vertebrate ear through segregation of epithelial patches into a multifunctional organ

Outgroup comparison suggests that the original vertebrate ear was likely a gravistatic organ not unlike the many statocysts found in metazoan animals. Evolution of an angular acceleration system mediated by semicircular canals appears to have occurred only among crustaceans, cephalopods and vertebrates (Budelmann,

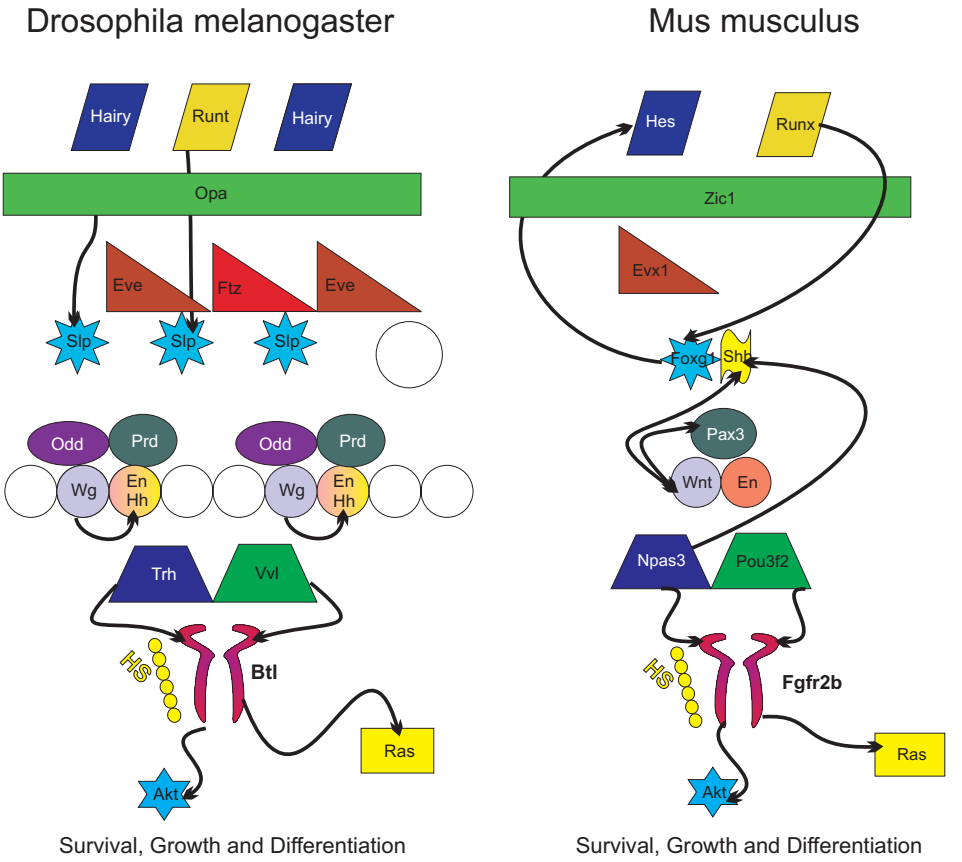

Fig. 4. Comparison of the genes involved in trachea formation in flies with inner ear development in mice. *Icons of the same shape and color represent homologous genes in* Drosophila m. *and* Mus m. *Known interactions between genes are demonstrated by the black arrows. Several homologous genes have been identified in these developmental pathways, but their interactions have not yet been fully described in either species. Putative sequence of activation is from the top with the fly and louse Fgf/Fgfr system providing the integration between patterning events outside the trachea/ear and intracellular signaling that leads to morphogenesis The striking resemblance of these genes as a complex interacting module supports a model in which not only are individual genes conserved across phyla, but the entire signaling network is conserved and utilized in the development of diverse structures, governing various aspects of branching morphogenesis. We recently tested the predictive value of this model and reported on the effect of* Foxg1, *the orthologue of* Slp1+2 *(Lee and Frasch, 2004, Mondal* et al., *2007), in mouse ear development (Pauley* et al., *2006). More of the listed genes should be tested for such conserved function to assess how much of the entire developmental module is invariable and thus needs to be inherited as an entire cascade of gene interactions with modifications to suit the need of the specific tissue in question. Modified after (Swantek and Gergen, 2004). Gene names follow the nomenclature as published in PubMed.*

1987) and can thus be regarded as a derived feature of statocysts. Viewed from this perspective, one of the major driving forces for ear evolution would be the possibility for segregation and morphological specialization of distinct epithelia dedicated to detect specific sensory stimuli. This can be achieved through the association with unique morphologies that filter specific components of the stimuli (semicircular canals and otoconia, for example). In vertebrates, this leads to the evolution of up to nine distinct sensory organs [three semicircular canal cristae, utricle, saccule, lagena, cochlea/basilar papilla, neglected papilla, amphibian papilla; gymnophionans (Fritzsch and Wake, 1988)] out of a single common macula and two canal cristae [hagfish (Lewis *et al.,* 1985)].

Segregation of sensory patches (Fig. 5) is related to increased numbers of hair cells, but the stereotyped segregation of sensory organs combined with the various ways of adding hair cells in vertebrates [continuous addition over a long time as in bony fish (Millimaki *et al.,* 2007, Riley, 2003), lampreys (Hammond and Whitfield, 2006)), sharks (O'Neill *et al.,* 2006)) or amphibians(Fritzsch *et al.,* 1988) or short burst of formation of all hair cells as in mammals (Matei *et al.,* 2005, Ruben, 1967)] suggests that the two processes are at a certain level independent. What drives segregation of sensory patches is still unclear (Fritzsch *et al.,* 2002) despite suggestions of possible involvement of certain molecules (Daudet and Lewis, 2005). More specifically, one has to distinguish genes necessary to *conduct* the separation from genes that actually *initiate* such segregation. While the delta/notch system clearly plays a role in this process (Daudet and Lewis, 2005) it may require interaction with other factors for the segregation, comparable to the *Hes7* activity in somite formation (Dequeant *et al.,* 2006). Outgroup comparison shows the existence of the delta/notch pathway already in flies and it therefore likely exists in hagfish and lampreys which do have a common macula that does not segregate into patches. In essence, one or more genes are needed that play this role as much as the *Sox2/Atoh1* genes play this role for hair cell formation [which also is reinforced by the delta/notch system (Fritzsch *et al.,* 2006a, Kelley, 2006b)].

One of the genes involved in segregation of sensory epithelia is *Otx1*. In null mutants of that gene there is incomplete segregation of utricle and saccule across the utriculo-saccular foramen and the horizontal crista may not segregate completely from other sensory patches (Fritzsch *et al.,* 2001, Morsli *et al.,* 1999). Indeed, lack of morphogenetic segregation in lamprey was recently attributed to the absence of *Otx1* (Hammond and Whitfield, 2006) as previously suggested (Fritzsch *et al.,* 2001). Data on *Otx1* mutants as well as others (see below) suggest that segregation and morphogenesis are linked, but the molecular basis of this link is not completely clear (Chang *et al.,* 2004a, Chang *et al.,* 2004b, Fritzsch *et al.,* 2006b, Fritzsch and Wake, 1988). More genes that function like *Otx* genes are needed to fully understand what drives sensory epithelial segregation; in particular the segregation of canal cristae from gravistatic organs.

Segregation allows sensory epithelia to develop unique molecular properties that initiate formation of, for example, acellular covering structures that permit acquisition of specific stimuli (canal crista have cupulae, gravistatic organs have otoconia/otoliths and auditory organs have tectorial membranes, except for bony fish) and have specific associated morphologies to direct stimuli to the endorgans (canal cristae are associated with semicircular canals for angular acceleration perception; gravistatic organs are in recesses that are oriented in different directions for horizontal and vertical stimulus perception; auditory organs are associated with specific sound conducting pathways that extend from the sound entry point (e.g., stapes footplate) to the sound exit point (e.g., round window).

It is obvious that sensory epithelia diversification goes hand in hand with histological and morphological alterations of the ear. In principle there are two ways through which coordinated morphogenesis and histogenesis could be achieved: either a set of genes is driving morphogenesis from the sensory epithelia primordia, or common upstream regulators exist that simultaneously regulate both histogenesis of the sensory epithelia and morphogenesis of surrounding accessory structure. Little evidence exists at the moment for the second way of regulating ear development and normal ear morphogenesis in *Atoh1* null mice (which lacks differentiated hair cells) suggests that it is not hair cells but sensory epithelia precursors that regulate ear morphogenesis (Fritzsch *et al.,* 2005a). In line with this assumption are the morphogenetic defects reported for *Sox2* hypomorphic mice, mice that do not even develop recognizable prosensory patches (Kiernan *et al.,* 2005).

Consistent with this assumption is also that mutants in several genes expressed in prosensory patches prior to hair cell formation such as *Foxg1*, *Fgf10* and *Jag1* null mice lack or have reduced prosensory canal cristae formation and all have canal formation deficits (Brooker *et al.,* 2006, Kiernan *et al.,* 2006, Pauley *et al.,* 2003, 2006). In contrast, when prosensory epithelial formation is disrupted before prosensory patches even form as in *Neurog1* null mice (Matei *et al.,* 2005) or *Tbx1* mutants (Raft *et al.,* 2004, Xu *et al.,* 2007), there is altered morphogenesis. Therefore, the sensory epithelia primordia / supporting cells, but not the hair cells, are critical for canal development (Matei *et al.,* 2005, Pauley *et al.,* 2003, Wright and Mansour, 2003). Below we will explore in some detail how *Fgf10* might achieve this effect.

Many crucial functions of FGFs are conserved across phyla. In contrast to vertebrates where FGFs play a crucial role in neural plate formation (Bertrand *et al.,* 2003), there is no evidence for FGF signaling in this context in fly development, suggesting that the FGF function in neuronal formation is a new acquisition of vertebrate *Fgfs* (Fritzsch and Glover, 2006). In *Drosophila*, these genes regulate cell migration and branching patterns in trachea and limb bud formation (Sutherland *et al.,* 1996) whereas EGFr signaling is performing signals associated with FGF signaling in vertebrates, most importantly in chordotonal organ development (Eberl and Boekhof-Falk, this volume). This role in branching morphogenesis is conserved in vertebrates and is most readily apparent in the *Fgf10* null mice which do not form lungs, glands or limbs. The FGFs known to be involved in mammalian inner ear development are *Fgf3, 8, 10*. Others of the over 20 FGFs still need to be investigated (Zhang *et al.,* 2006). As with the CNS, FGFs are not present in the insect mechanosensory system development, but play a major role in the development of the vertebrate inner ear. Specifically, *Fgf10* mutants show significant defects in canal outgrowth and development, particularly in the posterior canal system (Pauley *et al.,* 2003). FGF10 has the highest affinity for the B isoform of FGFR2 (Zhang *et al.,* 2006); and *Fgfr2b* null mice show agenesis of the ear through arrest of morphogenesis at the

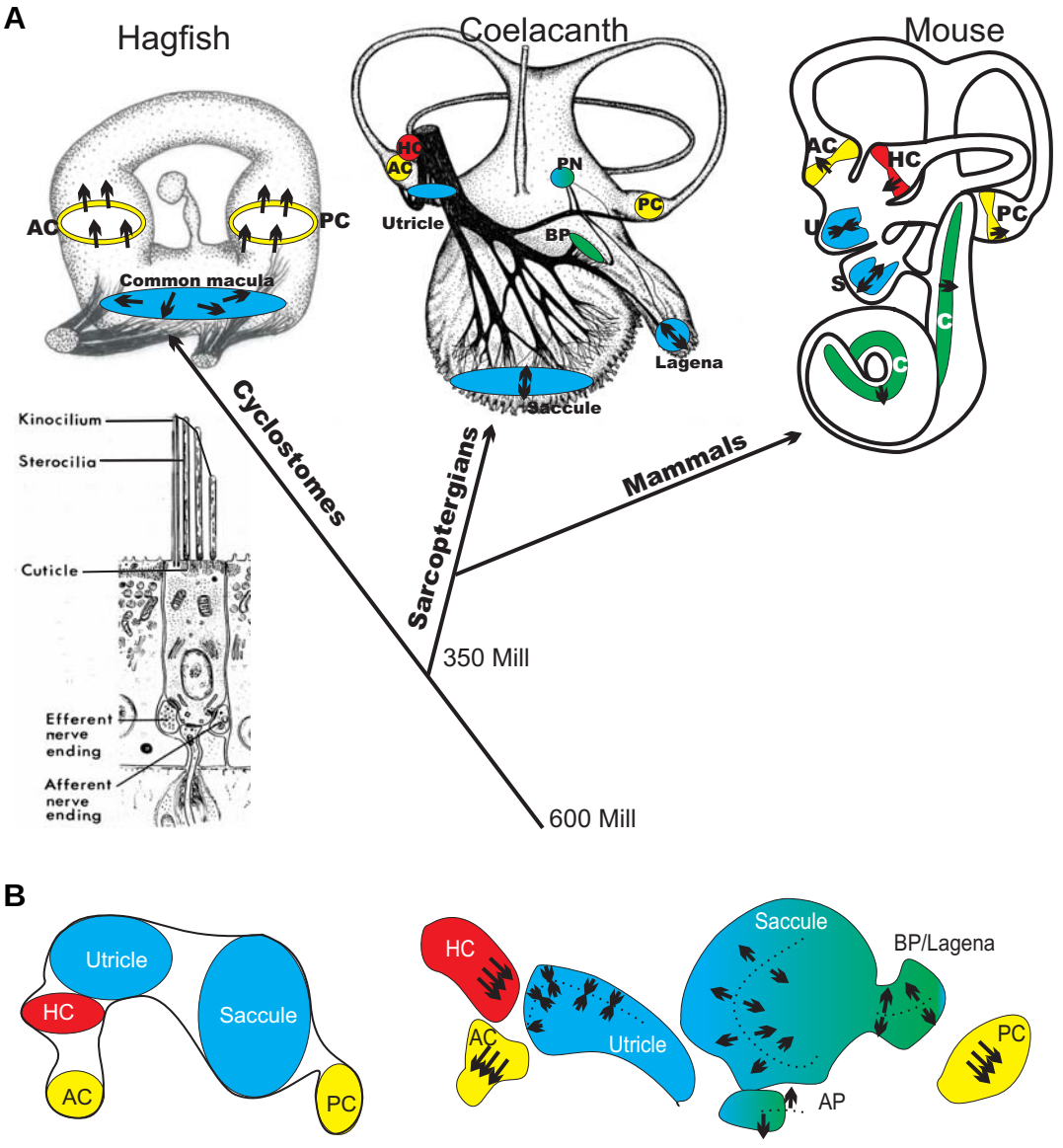

Fig. 5. Crucial steps in vertebrate ear evolution (A) and development (B) are depicted. *It is assumed that the vertebrate hair cells with afferents and efferents co-evolved with the ear (insert) some 600 million years ago. Note that the ears of the three depicted vertebrate species differ in the number of canal cristae (hagfish has two, coelacanth* [Latimeria] *and mouse have three), number of vestibular organs (hagfish has one [common macula], coelacanth has three [utricle, U; saccule, S; lagena, L] and mouse has two [utricle, U; saccule, S]) and number of organs near perilymphatic ducts (none in hagfish, two [basilar papilla, BP; papilla neglecta, PN] in coelacanth and one [organ of Corti of the cochlea, C] in mouse). Major morphological evolutionary changes are the addition of a horizontal canal in gnathostomes and the transformation of the utricle into several recesses containing the saccule, lagena and cochlea. It is suggested that the evolution of up to nine sensory organs of the vertebrate ear* **(A)** *comes about through ontogenetic segregation of a single primordium into multiple sensory patches* **(B)**. *After segregation, each sensory patch differentiates along a unique trajectory to form adult epithelia that perceive discrete aspects of the mechanical stimulation that reaches the ear (A). Development (B) therefore recapitulates the evolutionary segregation and differentiation of various epithelia from a common precursor. Integrated into this differentiation is the organization of different polarities of hair cells (arrows in B) that can be opposing (utricle, saccule, lagena) or one polarity (canal cristae). Note that the polarity of hair cells in the cristae is similar in anterior and posterior crista (away from the gravistatic organs), whereas the horizontal crista is polarized toward the gravistatic organs. How the ancestral molecular pathway to set up cellular polarity in the various sensory epithelia has been modified remains unclear. Modified after (Fritzsch and Beisel, 2004, Fritzsch* et al.*, 2002).*

otic vesicle stage (Pirvola *et al.,* 2000). Other members of the *Fgf* family are critical for ear development. *Fgf3/Fgf8* knockdown zebra fish do not develop otic vesicles (Maroon *et al.,* 2002, Riley, 2003) and the *Fgf3/Fgf8* combination is essential for placode formation in mice and chicken (Ladher *et al.,* 2005). Similarly, *Fgf3/Fgf10* double null mice show only limited formation of occasional micro vesicles (Alvarez *et al.,* 2003, Wright and Mansour, 2003). An ear does form in *Fgf3* mutant mice, but the size reduction of the ear is apparently different in the two *Fgf3* null lines, ranging from vesicle-like (Mansour, 1994) to apparently normal (Alvarez *et al.,* 2003).

Canal morphogenesis can be disrupted by a number of factors (Chang *et al.,* 2004a, Pauley *et al.,* 2006). FGF10 is known to interact with BMP4, which appears in the early development of the *Drosophila* mechanosensors and is part of the upstream regulation of cell fate determination. In the mammalian inner ear, *Fgf10* and *Bmp4* are both expressed in the presumptive sensory epithelia and they interact during canal morphogenesis (Chang *et al.,* 2004b). Further, *Bmp4* has been shown to reduce the size of prosensory patches, while *Noggin*, a BMP-inhibitor, increases the prosensory domain, thereby increasing the area of *Fgf10* expression (Pujades *et al.,* 2006).

We propose that *Fgf10*, consistent with its conserved function in branching morphogenesis (Fig. 4), acts as a central node in canal morphogenesis. We propose that prosensory crista size and the number of sensory epithelial precursor cells, determined by the molecular networks outlined above, determines the amount of *Fgf10* expression, which in turn regulates canal growth in interaction with other genes. Recently, genes known to affect ear growth

and morphogenesis have been found to bind the promoter region of the *Fgf10* gene. Among those genes are *Gata3*, a gene that affects ear morphogenesis (Karis *et al.,* 2001) and several genes known to affect canal formation such as *HoxA1, HoxB1, Dlx* and *Nkx* genes (Chang *et al.,* 2004a, Ohuchi *et al.,* 2005). Unfortunately, many of these genes have not been studied for their role in histogenesis or for expression of the key *Fgfs* or *Bmps* in the sensory epithelia. Further support for the idea that *Fgf10* is a central node of canal morphogenesis is the absence of all canal formation in *Fgf10* null mice (Ohuchi *et al.,* 2005, Pauley *et al.,* 2003). Another category of genes appears to act downstream of *Fgf10* and determines the fusion of the canal plate. One such gene is *Netrin* which appears to influence overall canal radius (Salminen *et al.,* 2000). *EphB2* and *Nor-1* are responsible for the diameter of the canals (Cowan *et al.,* 2000, Ponnio *et al.,* 2002). Further, the final diameter of the canals also depends on endolymph production and is enlarged in the *Foxi1* null mice due to endolymphatic hydrops (Hulander *et al.,* 2003). Importantly, loss of crista formation in *Jag1* or *Foxg1* null mice results in partial loss of canal formation (Brooker *et al.,* 2006, Kiernan *et al.,* 2006, Pauley *et al.,* 2006). In the *Fgf10* null, there is some 'rescue' of the anterior canal. This is likely mediated by *Fgf3*, which is expressed in the anterior portion of the developing otocyst (Wright *et al.,* 2003). This *Fgf3* expression may be too far away from the posterior crista to rescue its crista and canal development.

Our data show that *Foxg1* is at least one additional factor that cooperates with *Fgf10* to determine size of sensory epithelia and thereby the signal needed to interact with BMPs for proper canal growth (Chang *et al.,* 2004a) likely through interaction with Smad signaling (Massague *et al.,* 2005). Combining *Foxg1* with *Jag1* null mice should help to further clarify the complex interactions emerging in the NOTCH/HES/bHLH signaling system in the ear (Brooker *et al.,* 2006, Fritzsch *et al.,* 2006a, Matei *et al.,* 2005).

In addition to the FGFs and BMPs required for all canal development, novel genes have been recruited for the development of the horizontal crista. The horizontal canal system has some capacity to form independently of a horizontal crista, as demonstrated by the *Foxg1* null mouse in which the horizontal canal forms but the horizontal canal crista is lacking (Pauley *et al.,* 2006). Another gene that is critical to the horizontal canal system is the *Otx1* gene. *Otx1* null mice completely lack a horizontal canal, yet the horizontal canal crista forms and is properly innervated (Cantos *et al.,* 2000, Fritzsch *et al.,* 2001). These observations support the idea that the evolution of the horizontal canal system occurred in two phases: i) the formation of a separate sensory epithelia, likely by additional growth and splitting of an existing epithelial patch, mediated in part by *Foxg1*; and ii) the formation of a horizontal canal through the recruitment of *Otx1*. Combined these data point to the critical steps for canal morphogenesis in general and provide some insight into the evolution of the horizontal canal system. The expression of most of those genes prior to exit of hair cells from the cell cycle suggests that it is the size of the neurosensory precursor population and the expression of crucial genes such as *Fgf10* and *Bmp4* that ties neurosensory histogenesis into ear morphogenesis in the canal system. In contrast to the canal development, the cochlea grows through convergent extension (Lee *et al.,* 2006) but this process can also be compromised by eliminating some of the genes known to affect canal and gravistatic receptor morphogenesis (*Otx, Neurog1, Foxg1, Sox2, Delta, Notch*).

Evolution of auditory receptors: from a simple sensory patch to complex morphogenesis

As outlined above, diversification of sensory function of the ear follows the well established principle of molecular evolution: duplicate, mutate and select the new gene for a new function using changes in the cis-regulatory elements (Sodergren *et al.,* 2006) while keeping the old for the ancestral function. There is uniform

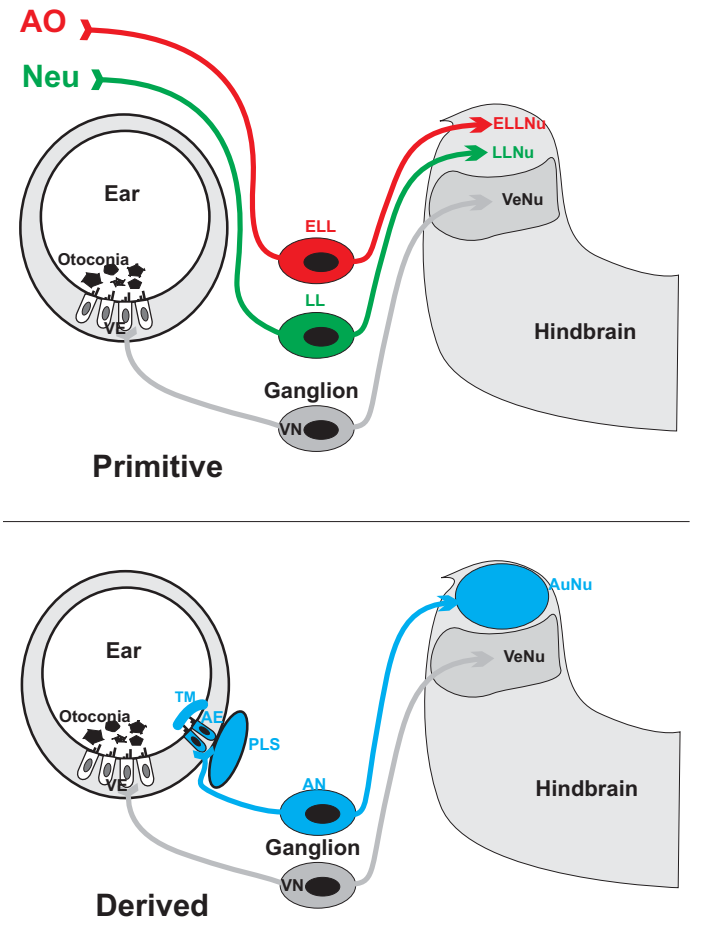

Fig. 6. The transformation of the ear, sensory neurons and brainstem from a non-auditory, primitive condition, into the derived condition, enabling a tetrapod vertebrate to hear, is shown. *The primitive ear has vestibular sensory epithelia (VE) that are connected with vestibular sensory neurons (VN) to the vestibular nuclei of the brainstem (VeNu). Additional sensory systems in primary aquatic vertebrates are the electroreceptive ampullary organs (AO) and the mechanosensory neuromasts of the lateral line (NEU). These organs are connected via specific sets of sensory neurons (ELL, LL) to specific brainstem nuclei (ELLNu, LLNu). Derived land vertebrates have lost these senses and have a sound pressure receiving sense, called hearing. This sense is characterized by the auditory epithelium (AE) that sits at or near a sound conducting perilymphatic system (PLS) and is covered with a tectorial membrane (TM). Auditory neurons (AN) conduct the information from the auditory epithelium to the auditory nuclei (AuNu) of the brainstem. This basic organization may have evolved in the aquatic ancestor of terrestrial vertebrates, but was modified in amphibians through the addition of the amphibian papilla and in amniotes through the formation of the cochlea. Modified after (Fritzsch and Neary, 1998).*

agreement that ear evolution started with a gravistatic and angular acceleration sensing vestibular ear (Fig. 6). As outlined above, formation of new functions in the vestibular system requires genes for formation of new sensory epithelia to associate with novel aspects of morphogenesis that may use different genes than those used for anterior and posterior canal morphogenesis. It appears that one of these events of sensory epithelia splitting may have already generated a novel epithelium in aquatic tetrapod ancestors (Fritzsch, 1992, Fritzsch, 2003). However, evolving this new sensory epithelium into a novel type of receptor, an auditory receptor, requires sophisticated reorganization of the periotic space to allow the formation of perilymphatic fluid filled space from the sound input to the sound output part of the ear, the oval and round window. It is conceivable, but not known at the molecular level that forming the cochlea sensory epithelia will help organize the perilymphatic space surrounding the cochlea. It is important to note that such perilymphatic specializations can evolve no matter which sensory epithelium is converted into an auditory system (Fritzsch, 1999), suggesting that the relevant genes are an ancestral module of ear development. Future research should focus on this important aspect of perilymphatic reorganization without which sound conduction from the tympanic membrane to the round window would be impossible. Unfortunately almost nothing is known on this subject as it has not been investigated in mutants with disrupted cochlea morphogenesis.

In addition to directing sound to this novel sensory epithelium, a novel set of neurons dedicated to conduct sound related information to the brain needed to evolve with molecular mechanisms to ensure a discrete connection to the sound processing sensory epithelium and a set of central neurons dedicated to receive this information rather then vestibular stimuli. In mammals we know some of those novel features. For example, a second neurotrophin, *Ntf3 (NT-3)* is expressed in the basal turn of the cochlea and avoids rerouting of vestibular fibers which can be experimentally induced in transgenic animals that express the neurotrophin *Bdnf* under control of the *Ntf3* promoter (Tessarollo *et al.,* 2004). In addition, mammals express the transcription factor *Gata3* in the spiral ganglion neurons and this factor may play a role in pathfinding (Karis *et al.,* 2001). Clearly, more needs to be known on this subject before we can begin to appreciate the steps taken to molecularly ensure proper routing of spiral ganglion cells and can use this information to enhance fiber growth during regeneration (Martinez-Monedero *et al.,* 2006).

Molecular understanding of cochlear nucleus development is moving ahead. In chicken and mice we now understand the rhombomeric orgin of cochlear nuclei (Cramer *et al.,* 2000, Farago *et al.,* 2006, Fritzsch *et al.,* 2006c) and also know that most neurons depend on the very same transcription factor that is essential for hair cell differentiation, *Atoh1* (Fritzsch *et al.,* 2006c, Wang *et al.,* 2005). These initial steps are certainly too crude to define the many neurons thus far recognized in the cochlear nuclei of vertebrates, but it is remarkable that the rhombomeric origin fits reasonably well to the recognized subdivisions of the cochlear nuclei in mice. Rhombomere 2 could generate the anterior subdivision of anterior ventral cochlear nucleus, rhombomere 3 could generate the posterior subdivision of the cochlear nucleus, rhombomere 4 could generate the neurons of the inferior division of the posterior ventral cochlear nucleus, rhombomere 5 could generate the octopus cells of the dorsal cochlear nucleus and rhombomere 6 could generate the remaining part of the dorsal cochlear nucleus (Ehret and Romand, 1997). More work using the approach of rhombomere specific expression of Cre (Farago *et al.,* 2006) combined with a floxed *Atoh1* or *Neurod1* could be used to selectively eliminate cochlear nucleus development in a rhombomere specific fashion and the effect on central neurons could be analyzed.

Summary and outlook

Within recent years it has become clear that evolution of senses is predominantly evolution at the receptor level while tapping into neuronal plasticity for information processing. For example, evolution of trichromatic vision has been reconstructed in mice through expression of a long wavelength opsin. Surprisingly, mice can process this new information through the apparently built in plasticity in their neuronal information processing system (Jacobs *et al.,* 2007). It has also been shown that Wnt induced formation of 'vestibular' hair cells in the chicken cochlea results in altered innervation with the possibility that these cells are connected to vestibular rather than auditory nuclei (Stevens *et al.,* 2003). It thus appears that we need to interpret evolution of mechanosensors and evolution of auditory organs out of vestibular organs primarily at the level of the receptor changes. It appears likely that the candidate receptor molecules evolved with multicellular organisms (Beisel *et al.,* 2007). When and how they were selected for the hair cells remains to be shown. Other issues such as formation of new sensory epithelia and segregated projections of innervating afferents to a distinct information processing part of the brain may be instantly sorted owing to the brain's capacity to plastically respond to altered sensory input. Of course, selection will ultimately drive complete segregation through picking developmental modules via changes in their promoter region. Such changes will accommodate more refined information processing through the evolution of accessory structures and the evolution of uniquely dedicated information processing pathways. While gathering information about such structures is informative in its own right, it is the receptor that primarily drives sensory evolution. In this context, it is important to reiterate that more work is needed to resolve open issues on the molecular basis of mechanoreceptor cell evolution to reconcile the apparent histological differences with the apparent conservation of certain cell fate determining genes.

Acknowledgements

This work was supported by a grant from NIH (RO1 DC005590; BF; DC005009; KB) and NASA (NAG 2-1611; BF, KB), COBRE (1P20RR018788-01; KB, BF; GS) and LB692 (BF, KB). This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number 1 C06 RR17417-01 from the National Center for Research Resources, National Institutes of Health. We acknowledge the use of the confocal microscope facility of the NCCB, supported by EPSCoR EPS-0346476 (CFD 47.076).

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