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Evolution of vertebrate mechanosensory hair cells and inner ears: toward identifying stimuli that select mutation driven altered morphologies

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Abstract

Among the major distance senses of vertebrates, the ear is unique in its complex morphological changes during evolution. Conceivably, these changes enable the ear to adapt toward sensing various physically well-characterized stimuli. This review develops a scenario that integrates sensory cell with organ evolution. We propose that molecular and cellular evolution of the vertebrate hair cells occurred prior to the formation of the vertebrate ear. We previously proposed that the genes driving hair cell differentiation, were aggregated in the otic region through developmental re-patterning that generated a unique vertebrate embryonic structure, the otic placode. In agreement with the presence of graviceptive receptors in many vertebrate outgroups, it is likely that the vertebrate ear originally functioned as a simple gravity-sensing organ. Based on the rare occurrence of angular acceleration receptors in vertebrate outgroups, we further propose that the canal system evolved with a more sophisticated ear morphogenesis. This evolving morphogenesis obviously turned the initial otocyst into a complex set of canals and recesses, harboring multiple sensory epithelia each adapted to the acquisition of a specific aspect of a given physical stimulus. As support for this evolutionary progression, we provide several details of the molecular basis of ear development.

Keywords

hair cell; evolution; molecular evolution; organ formation; ear morphology evolution

1. Introduction

Evolution of senses, in particular the eye and ear, should adapt those organs through mutational changes to perceive stimuli necessary for the survival of a species. Those adaptations will change the temporal bandwidth of the sensory stimuli that the animal experiences for optimized data extraction appropriate for the needs of a given species. This is particularly obvious for the well-characterized physical parameters (Lewis et al. 1985) underlying the sensation of body motion in space. The temporal spectrum of motion sensitivity of vestibular sense organs must cover the dynamic range of the animal's locomotor performance. During self-motion, the activation of adequate gaze- and posture-stabilizing motor reactions are indispensable and mediated by short-latency pathways between inner ear (vestibular) sensory organs and respective motor effectors. From this

premise, it follows that vestibular sense organs and their dynamic bandwidth co-evolved with the locomotor capacity of animals: maneuverability/speed on the motor side. The dynamic range of motion and motion sensing with the vestibular system co-adapted. A good example of this co-adaptation of sensor and motor capacities is the evolution of a horizontal semicircular canal along with the reorganization of eye muscles and their innervation during the evolution of jawed vertebrates (Fritzsche 1998).

However, the evolution of senses is also an adaptationist's nightmare (Northcutt 1988b) as one can easily fall prey to a circular reasoning, correlating specific features of an organ's evolution with a subset of the physical parameters of the received stimuli to generate 'adaptive traits'. The ultimate target of the evolutionary outcome is only in certain cases unquestionably linked to the underlying physics. For example, three distinct receptors are required to have trichromatic color vision. Unfortunately, for complex sensors such as the vertebrate eye, we have little data of intermediate forms that can explain how this partial functional eye can be modified through selection of novel changes in the genome to improve the receiver function (Lamb 2013). In fact, Darwin conceded that an explanation of eye evolution out of simple beginnings is difficult to imagine (Darwin 1859). Yet, we now understand that all eyes, despite their morphological differences, may be derived from the same simple anlagen through a selection of highly different developmental events to turn ancestral sensory cells into a fly, cephalopod or vertebrate eye (Gehring 2011; Lamb 2011) possibly by diversifying an ancestral prototype photoreceptor cell into a complex organ (Arendt et al. 2009).

The eye of vertebrates is a comparatively simple device that only generates a detailed, two-dimensional image of a still or slow moving object in black and white or a variable number of colors. Compared to the eye, the terrestrial vertebrate inner ear is a more complex sensory array that harbors three distinct sensory functions: perception of sound for hearing, perception of linear acceleration for gravitational/translational motion detection, and perception of angular acceleration generated by active or passive head/body movements. All three sensors are part of the complicated labyrinth (Lewis et al. 1985; Retzius 1881; Lewis and Narins 1999; Kopecky et al. 2012) that decomposes three-dimensional body movements in space into separate motion vectors and separates sound into its spectral components. For more than 2000 years the ear was exclusively considered a hearing organ (Haeckel 1877). However, it is now clear that the detector for airborne sound, the mammalian organ of Corti and the tetrapod basilar papilla, are evolutionary new additions to the 'vestibular' ear. Work in the last 80 years established that the terrestrial hearing organ evolved in tetrapod ancestors from vestibular precursors (Fritzsche et al. 2013), supporting the 'vestibular first' hypothesis (Baird 1974; Duncan and Fritzsche 2012). Consistent with this hypothesis, comparative work shows frequent occurrence of complex gravity sensors among crown groups of metazoans (Bullock and Horridge 1965; Budelmann 1988). This frequent occurrence of gravistatic receptors in metazoans suggests that the original vertebrate ear may have served a similar graviceptive function. In contrast to the multiplicity of gravity receptors, angular acceleration detection is found in vertebrates and very few non-vertebrate species. Where angular acceleration detection is present, it appears to be a derived feature that likely evolved along with an increase in locomotor performance and maneuverability, for example in squids (Koppl et al. 2000). These data suggest that the vertebrate ear as an organ differs from the vertebrate eye (Fritzsche and Glover 2007) in that ear precursors with different sets of receptors that extract different physical parameters from stimuli can be found among extant vertebrates. Within vertebrates, one can thus trace the molecular and morphological evolution of ear diversification toward a novel adaptation to perceive a physically well-characterized new stimulus such as angular acceleration or airborne sound.

Orientation in space in any free moving animal requires senses that can at least tell bottom from top (Bullock and Horridge 1965). The simplest way to obtain this information is by accessing the ubiquitously present earth gravity, assisted by the periodically present light provided by the sun (or moon). Tapping into these stimuli for orientation purpose is possible even with intracellular organelles since response to light and gravity have been detected in single celled organisms as gravitaxis and phototaxis (Hader et al. 2003; Gehring 2011; Budelmann 1988). Understanding the selection pressure operating on sensory evolution requires an appreciation of the needs of any free-swimming organism to orient itself in space. With this in mind, it makes perfect sense that most free-swimming marine multicellular organisms have evolved complex organs that allow detection of gravity to facilitate orientation in space (Markl 1974). Many of these free-swimming organisms also have evolved eyes (Bullock and Horridge 1965; Von Salvini-Plawen and Mayr 1977) allowing both synchronization of internal clocks to external stimuli and in many cases sophisticated patterned vision (Gehring 2011). Notably, while gravity-sensing with specialized organs is widespread in free-swimming, multicellular organisms, eyes evolved in both sessile and free-swimming forms (Von Salvini-Plawen and Mayr 1977). However, both eyes and gravity sensing organs are found together in marine animals able to actively obtain a desired position in space such as vertebrates, cephalopods and crustaceans. In addition, such mobility requires a monitoring and computation of self-induced locomotion to compensate for unpredicted disturbances (Chagnaud et al. 2012). Moreover, when both eyes and gravity sensors exist in free-swimming organisms that have a sophisticated nervous system and active motion, gravity-sensing organs have evolved further to perceive also angular acceleration to stabilize the visual field while moving or following moving objects. For example, cephalopods have evolved a semicircular canal like duct systems to apparently guide compensatory eye movement (Budelmann 1992) comparable to vertebrates (Straka 2010).

This review will analyze ‘chance and necessity’ (Gehring 2011) in the evolution of graviceptive organ evolution. Specifically, we will first review the morphological and molecular evolution of mechanosensory cells, which form the basis of all graviceptive organs. In the second part of this review we will elaborate how these cells might become aggregated to form an ear. Finally, we will explore how the molecular evolution of the ear can diversify a simple gravity sensing organ into a complex labyrinth able to perceive angular acceleration and, in land vertebrates, airborne sound.

2. Transforming single celled ancestors into vertebrate mechanosensory hair cells

Research over the last 20 years demonstrated that all multicellular organisms derive from single cell or colonial choanoflagellates. These single cells have a central motile kinocilium that drives water and thus particles through a surrounding collar of interconnected microvilli (Fairclough et al. 2013; Fritzsche et al. 2010). Particles, such as bacteria, caught with this interconnected microvilli ‘net’ are digested by the cell (Fig. 1). A similar organization exists in sponges with the choanoflagellate-like choanocytes driving fluid through chambers (Fig. 2). In some cnidarians and most prominently in deuterostomes, there is a tendency for this basic metazoan feature to be transformed into a more complex arrangement of an eccentric kinocilium and asymmetrically arranged microvilli of variable thickness (Fritzsche et al. 2007). This is particularly diverse in ascidians where multiple configurations of what appears to be eccentric assemblies of ‘stereovilli’ occur (Burighel et al. 2011; Jorgensen 1989). Unfortunately, there is no electrophysiological evidence that many of these cells in non-chordates are mechanosensitive. Nevertheless, the rich diversity of cell types among

multicellular organisms illustrate the variations on the common theme of a central cilium surrounded by microvilli implying that transformation of an actively beating kinocilium for propagation into a passive, sensory kinocilium is a relatively simple step. In this context it is noteworthy that one vertebrate ear, the lamprey ear, has two large ciliated chambers that keep the endolymph flowing (de Burlet 1934), indicating that the vertebrate ear can have actively beating, non-sensory cells next to non-motile sensory hair cells in nearby areas that develop from the same ear anlage (Hagelin 1974). What is more complicated to understand, however, is the eccentric position of the kinocilium typical for vertebrate hair cells. While it is clear that this qualifies vertebrate hair cells with their stair-case arrangement of tip-link connected stereocilia as unique for vertebrates, it is also clear that these cells represent a transformation of the non-polarized cells of the choanoflagellate type (Figs. 1, 2).

In an apparent form of ontogenetic recapitulation, the hair cells of vertebrates develop through an ontogenetic stage, where a central kinocilium forms surrounded by microvilli, resembling closely the choanoflagellate and sponge arrangements (Fig. 2) but also many protonephridia in many metazoans. However, during development, the kinocilium moves into an eccentric position in a given cell. This movement is synchronized across epithelia by the planar cell polarity (PCP) pathway (Dabdoub and Kelley 2005), which otherwise seems to have little effect on the overall asymmetric stereocilia assembly. Thus, while the synchronization across sensory epithelia is guided by the PCP pathway, there is no data yet as to what molecular event moves the kinocilium into an eccentric position and transforms microvilli into stereovilli/stereocilia (Duncan and Fritzsche 2012; Schwander et al. 2010). What we do know, however, is that transforming microvilli into stereocilia is not simply bundling actin filaments but requires spacing the stereocilia, generating tip links, and maintaining actin assembly and disassembly to generate progressive alterations in length and diameter (Goodyear et al. 2012).

Given that microvilli are linked already in choanoflagellates (Mock et al. 2012) to form a net for prey capturing and signaling for prey ingestion, it appears possible that the variable links found among stereocilia of vertebrate hair cells (Goodyear and Richardson 2002) may be derived from those ancestral links, likely serving a different function in single celled organisms. Mammalian stereocilia would thus be distinguishable from ancestral microvilli by their graded height, dense core, tapering near the base (Kitajiri et al. 2010), and the molecularly so far unknown mechano-electric transducer channel (Warchol and Montcouquiol 2010).

Obviously, the evolution of the hair cell stereocilia can be viewed as a series of transformations across phyla and may even be recapitulated during hair-cell ontogeny (Fig. 2). However, it remains unclear how this morphological change is regulated during development at a cellular and molecular level. An analysis of loss of hair cell function in mice and fish has revealed the bHLH transcription factor *Atoh1* to be essential for hair cell differentiation (Bermingham et al. 1999). Other genes have been identified that are also required for some or all hair cell differentiation (Duncan and Fritzsche 2013; Bourigault et al. 2013). The homologous bHLH transcription factor of insects, *atonal*, is likewise required for mechanosensory cell development of proprioceptors such as chordotonal organs (Hassan and Bellen 2000) with a distinctly different morphology. Replacing the fly *atonal* gene by the mammalian *Atoh1* gene rescues fly organ development and the *atonal* gene can rescue mouse hair cell development (Wang et al. 2002) indicating a high level of functional equivalence of this transcription factor. Atonal-like genes exist in coelenterates (Seipel et al. 2004) and other multicellular organisms (Pan et al. 2012). Whether or not these genes drive mechanosensory cell development in the statocysts or lateral line organs of these animals has not yet been studied. Apart from *atonal/Atoh1*, other homologous genes have been

identified that are apparently conserved for mechanosensory cell development across phyla. Foremost among these are the POU domain factor *Pou4f3*, the zinc-finger protein *Gfi1* (Wallis et al. 2003; Hertzano et al. 2004) and many other genes that relate to hearing loss in flies and mammals alike (Senthilan et al. 2012). Even sponges may already have molecular precursors of the appropriate kind of transcription factor as injection of such isolated transcription factors can result in the formation of neuron-like cells in *Xenopus* (Richards et al. 2008), a widely used model to show the function of newly isolated genes (Kim et al. 1997).

In addition to these highly conserved bHLH transcription factors (Fritzsche et al. 2000; Pan et al. 2012), a set of extremely conserved microRNAs has been identified that is expressed in known or suspected mechanosensory cells across phyla. For example, the miR-183 of flies and vertebrates differs only in a single nucleotide and thus can be used to identify putative homologous mechanosensory cells (Pierce et al. 2008; Candiani et al. 2011). While not as conserved as neuron-specific miR-124, which is identical in all neurons of triploblasts, the nearly 100% conservation of miR-183 supports the notion that cells expressing this micro RNA are channeled toward mechanosensory cell development, possibly enhancing the function of other genes expressed in developing mechanosensory cells such as *Atoh1*, *Pou4f3* and *Gfi1*. Most importantly, miR-183 is also found in single cells in the collar region of an outgroup of vertebrates (Fig. 2), the acorn worms (Pierce et al. 2008) and has recently been identified in chordates such as the lancelet (Candiani et al. 2011). In summary, the distribution of all these genes and their expression in single putative mechanosensory cells of protostomes and deuterostomes alike suggests that the molecular basis for mechanosensory cell development evolved with bilaterian triploblasts and possibly already with diploblastic animals. What needs to be worked out now is how these conserved molecules interact during the development of the various sensory cell types and how this interaction evolved to regulate approximately 1000 downstream genes of the transcription factor *Atoh1* (Klisch et al. 2011) to generate vertebrate hair cells.

3. Turning mechanosensory cells into organs to detect specific mechanical stimuli

All aquatic metazoans, with the exception of sponges, have distinct organs invoked or known for sensing mechanical stimuli (Bullock and Horridge 1965). They can be broadly distinguished as graviceptive (acoustic) sensors and lateral line-like organs. Whereas the former detect head/body position relative to the earth gravitational vector (graviception), near field particle motion of sound, or the inertia of the fluid in the inner ear ducts during head/body rotations (semicircular canals), lateral line-like organs are sensitive to hydrodynamic events at the body surface. In fact, nearly every free-swimming aquatic multicellular organism has a graviceptive organ or a statocyst (Markl 1974). In contrast, hydrodynamic event-detecting organs have only been identified in derived mollusks (Budelman and Bleckmann 1988), vertebrates (Northcutt 1988a), possibly in some chordate ancestors of vertebrates such as the lancelet (Fritzsche 1996), and perhaps ascidians (Burighel et al. 2011; Bullock and Horridge 1965). In general, non-vertebrate deuterostomes have single cells or small groups of cells that can be identified by signature genes known for vertebrate mechanosensory organs (Joyce Tang et al. 2013; Candiani et al. 2011; Pierce et al. 2008; Pan et al. 2012). However, most non-vertebrate deuterostomes lack clearly identifiable lateral line or inner ear equivalents (Short et al. 2012; Bouchard et al. 2010). Among coelenterates, corals have lateral line-like organs comparable to vertebrates with a high level of molecular similarity (Repass and Watson 2001). In contrast, free-swimming jelly fish have complex statocysts to provide, together with associated eyes, information

about the direction of gravity and light as oppositely-oriented cues to orient in space (Arkett et al. 1988; Kozmik et al. 2003). Even more complex are the various organs found in arthropods (Markl 1974; Budelmann 1992) that will not be covered here.

How can we align this puzzling diversity of shape and position of organs and the structural diversity of cells with the apparent homology of certain essential transcription factors and the adaptive radiation of multicellular organisms? Obviously, there are two complementary approaches: one approach is using structural (Burighel et al. 2011) and molecular evidence (Fritzsche et al. 2007; Bourigault et al. 2013; Fritzsche et al. 2000) to test deep homology of all mechanosensory cells and organs and trace them back to the single-celled ancestor of metazoans (Fairclough et al. 2013). As a likely possibility, the molecular variations of a common theme might explain all structural differences that were selected in a given species based on the sensory requirements. Alternatively, the structural diversity of organs with a single common function of movement detection are considered to be examples of parallel evolution whereby the stimulus shaped the transducing cells and organs independent of their cellular or organ starting point (Markl 1974). The latter concept assumes that neither mechanosensory cells nor mechanosensory organs are closely related and rather illustrate a convergent evolution at the cellular and the organ level. In contrast, deep molecular homology (Shubin et al. 2009) indicates now that at least mechanosensory cells may be derived from an ancestral precursor (Chang et al. 2008).

A comparable problem of structural diversity was found for the eyes with some studies claiming multiple independent evolution of eyes (Von Salvini-Plawen and Mayr 1977). In contrast to this morphology-driven assumption, molecular evidence has clarified for various types of eyes that a single transcription factor, Pax6, is present in all eyes regardless of their receptor cell type (rhabdomeric or ciliogenic) or developmental origin (ectodermal or neuroectodermal) (Arendt 2003; Vopalensky et al. 2012; Rolle et al. 2000). This suggests that all complex eyes derive from a common ancestor (Gehring 2011) that likely consisted of a pair of clonally related sensory/pigment cells through 'division of labor' (Arendt et al. 2009). Below we explore a corresponding scenario for the evolution of mechanosensory organs.

All mechanosensors transform shearing forces with vectors parallel to the surface of single hair cells or organs into electric signals (Boide et al. 2013; Hudspeth 1989). At the level of the receptor cell, all multicellular organs are based on the same functional principle. In the vertebrate ear, the hair cell forms the core component that transduces displacements of the ciliary bundle into a neural response, independent of frequency and spatial direction of the mechanical stimulus (sound, angular acceleration, linear acceleration). The possibility to detect the different physical stimuli that generate the shearing force depends on the location (internally in the organism or on the surface) and the type and structure of the sensor (semicircular canal, otolith or papilla organ) in which the sensor cells are inserted. For example, dense otoconia or otoliths covering the hair cell epithelia can detect linear acceleration induced by tonic positional changes of the sensor with respect to gravity or by sound-induced vibration of the body in fish (Fig. 3) as in other vertebrates (Fritzsche 1999; Ladich and Popper 2004). In contrast, cupulae-covered semicircular canal cristae and fluid-filled ducts detect angular acceleration, while lateral line neuromasts on the body surface or inside the dermal duct system in aquatic amniotes serve as sensors of the water flow around the animal. For the vestibular component of the vertebrate ear, the quality of the stimulus is evident: gravity or linear translational acceleration (such as near-field sound particle motion) will displace otoconia, whereas angular acceleration will be detected by a displacement of the semicircular canal cristae due to the inertia of the endolymph (Fig. 3). Such a sensor will be able to perceive both self-generated motion as well as an imposed motion generated by

outside forces (reafferent/exafferent sensory signals). In order to put the perceived motion into a behavioral context, a central nervous system with a particular computational capacity is required that matches the complexity and dynamics of the animals' self-motion capability and the necessity to generate specific motor reactions to compensate for perturbations of self-induced motion.

For example, jellyfish have a limited motion repertoire with a rather restricted locomotor dynamic. These animals simply monitor their body position in space and initiate low-dynamic corrective movements to maintain their orientation in space. In contrast, vertebrates with a locomotor capacity that covers a wide dynamic range require highly dynamic gaze- and posture-stabilizing motor adjustments. The accuracy and robustness of these reflexive motor behaviors is achieved by comparing anticipated movements [via locomotor efference copies (Lambert et al. 2012)] with exafferent sensory responses generated during effective body motion in space. Compensatory eye/head/body adjustments during predictive rhythmic locomotion are thus induced by a combination of sensory signals and intrinsic locomotion-related corollary activity (Lambert et al. 2012).

All aquatic animals, suspended in the water column, will have active, body motion-induced as well as passive, water flow-induced displacements of hair cell cilia bundles along the body surface (Fig. 3), generated by monopoles, dipoles or multipoles (Lewis and Fay 2004). The combination of body and surface movements relative to the near and far field will generate complex stimulus patterns that could serve to extract information about speed and direction of a given stimulus. In order to use body surface water movements meaningfully, the central nervous system must integrate body motion-evoked inner ear sensory signals with those related to body surface hydrodynamic events. In addition, self-generated propulsive movements stimulate both inner ear and lateral line sensory organs. The respective activation of sensory reafferent signals will be compensated for by employing locomotor efference copies or corollary activity (Lambert et al. 2012; Combes et al. 2008). In particular, during self-generated body motion, anticipated sensory consequences are subtracted from active motion-related sensory inputs thereby extracting those signals that derive from unpredictable perturbations induced by exogenous sources. This requires multimodal computational analyses performed by a well-developed central nervous system, such as found in crustaceans (Budelmann 1992), cephalopods (Bleckmann et al. 1991) and vertebrates (Fritzscht and Glover 2007). In conclusion, lateral line organs in motile organisms require additional information from inertial senses about body motion in order to faithfully reconstruct and interpret the pattern of water motion at the body surface created by external sources.

These considerations indicate that graviceptive and motion-related senses are intricately linked with the functional trait of locomotor capability and should be essentially absent in fully or mostly sessile organisms as substrate contact will provide the necessary information about the largely invariant position in space. Indeed, the known distribution of gravity sensing organs fits such a distribution (Markl 1974). In contrast to vertebrates, neither the chordate nor the non-chordate deuterostome outgroup developed an ear or the molecular machinery to make anything but single sensory cells with molecular and anatomical similarities to hair cells (Joyce Tang et al. 2013; Short et al. 2012; Candiani et al. 2011; Pierce et al. 2008). Recent molecular data indicate that a group of marine organisms without coelom (acoelomata; Fig. 2), long considered to be the outgroup of triploblasts, are actually deuterostomes (Philippe et al. 2011). Many of these organisms have a single midline structure usually referred to as 'statolith' (Achatz and Martinez 2012). However, it is unknown if this structure actually serves as a graviceptive organ. Likewise, some structures inside the head ganglion of ascidians have been referred to as 'statolith' but whether this

structure actually functions as such and how it mechanistically works remains unclear (Markl 1974). Irrespective of the actual function of these organs, evolution of all hair cell bearing mechanosensory organs occurred obviously only after mechanosensory cells had evolved, eventually grouping together much like single celled choanoflagellates can aggregate into colonies (Fritzsche et al. 2010; Dayel et al. 2011). More work is required to understand the molecular similarity of these processes and how the formation of the otic placode was tied into the equally dramatic aggregation of a diffuse neural network into a central nervous system. Developmental biology has identified certain molecular similarities between brain and ear aggregation and invagination (Fritzsche et al. 2006; Moody et al. 2013) that transforms general epithelial into neuroepithelial properties in a stepwise fashion (Richter et al. 2002). Further work is needed to understand the fate of additional 'ear vesicles' molecularly induced outside the normal topology (Taylor and Labonne 2005) to verify if these induced ears differentiate hair cells and connect to the brain like transplanted ears (Elliott et al. 2013). Recent in vivo and in vitro work has demonstrated that hair cells induced in the dish (Koehler et al. 2013) or in the vestibular ganglion (Jahan et al. 2010) have the ability to organize vesicles into which they integrate. The molecular nature of this mechanism needs to be understood to understand how the mere aggregation of sensory cell precursors to form the otic placode could have led directly to the formation of an otocyst harboring a gravistatic sensory epithelium as proposed in the 'hair cell first' hypothesis (Duncan and Fritzsche 2012)

4. Changing a statocyst into an angular acceleration and sound pressure receiver

Historically, the vertebrate ear was considered an 'auditory organ' without understanding the second function, orientation in space, until late in the 19th century. It was assumed that the cochlea with its organ of Corti codes for frequency detection whereas the labyrinth serves as intensity coding system, using the mix of canals and otoconia bearing 'auditory maculae' in unspecified combinations (Haeckel 1877). Extirpation of the ear and careful physiologic observations combined with electric stimulation, however, showed complex disorientation pathologies including vertigo, suggesting that the semicircular canals detect head/body rotation in space with possible relations to gaze stabilization (Mach 1873; Breuer 1873). Shortly afterwards, the even more cryptic graviceptive sense was experimentally associated with the 'otoliths' leaving their somewhat misleading initial name as 'hearing-stones' as a historical trait until today. Subsequent anatomical work demonstrated the presence of a similarly structured labyrinth, referred to as 'hearing organ' (Gehörorgan) in all aquatic vertebrates (Retzius 1881), whereas only land vertebrates evolved an organ of Corti. de Burlet (de Burlet 1934) suggested that during evolution of the ear, a sensitivity for airborne sound was achieved by a transformation of the basilar papilla sensory epithelium, uniquely associated with a single perilymphatic space. Later work discovered that multiple transformations occurred already in the aqueous environment in many bony fish and at least one ancestor of land vertebrates apparently evolved a distinct endorgan associated with a perilymphatic space, precisely as predicted by de Burlet (Fritzsche et al. 2013; Fritzsche 1987).

In an ironic twist to the progressive insight of the different functions of the vertebrate ear, it turned out that ancestral vertebrates may have had only a statocyst for gravity-dependent orientation as is the case in many invertebrates, while angular and linear translational acceleration as well as sound pressure hearing evolved later. This is in contrast to initial functional ideas that considered the ear to be an organ, exclusively associated with hearing with the gravistatic sense being the last one to be associated with the ear.

Linking the cellular evolution with the obvious phylogeny of the vertebrate ear requires an understanding of the molecular steps underlying inner ear development and the sequences of the transformations that caused a diversification into the distinct ear phenotypes with unique functions known in present-day vertebrates (Retzius 1881; Lewis et al. 1985). While past ideas revolved around a ‘lateral line organ first’ idea, comparing the developmental adaptation of the otic placode with such organs, the cellular evidence presented above has been theoretically combined with the existence of sensory cells prior to placode formation into a ‘hair cell first’ hypothesis (Duncan and Fritzscht 2012). Accordingly, the otic placode is viewed as an embryonic adaptation to congregate diffuse single cell formation into a single location for complex organ establishment (Duncan and Fritzscht 2012), comparable to the aggregation of the central nervous system out of a diffuse epidermal neural network (Narimatsu et al. 2009). This evolutionary re-patterning of ontogenetic events is clearly beyond the scope of this overview. We will emphasize only two pertinent aspects of ear evolution beyond the otocyst: how to transform a two canal system into a system with three canals and how the single graviceptor anlage is repeatedly split into two to three sensors that variably reside in their own recesses. Since one such split and segregation into a separate recess and transformation into a hearing organ has recently been reviewed in detail (Fritzscht et al. 2013), we will concentrate here on aspects of the vestibular ear’s morphological evolution and the underlying molecular changes associated with gravistatic and angular acceleration sensing.

What the earliest vertebrate ears looked like is unknown. Data from fossils and extant species show two distinct morphologies, an ear with a single or two partially separated vertical canals and a largely undivided common graviceptive epithelium with otoconia in cyclostomes and three canals with at least two distinct otoconia-bearing organs in jawed vertebrates (Figs. 4,5). While it is conceivable, as outlined above, that the vertebrate ear started as a simple gravity sensor, there are no fossil data to support this notion. Limited data suggest that multiple genes had to be assembled to guide the formation of a new sensory epithelium for the horizontal canal and combine this with genes driving the morphology of the horizontal canal (Bok et al. 2007; Mazan et al. 2000; Beisel et al. 2007; Fritzscht et al. 2007). Indeed, canal cristae release signals such as Fgf10 (Pauley et al. 2003) or BMP4 (Chang et al. 2008) that drive canal development. This indicates that canal cristae evolution predates canal formation, because the former event is indispensable for the establishment of an inner ear duct system. Consistent with this idea, recent experimental data show that sensory cells can organize their surrounding cells. Selective loss of a single gene can transform neurons into hair cells within the ganglia. These ‘intraganglionic’ hair cells organize ear-like vesicles around these mechanosensory cells (Jahan et al. 2010), indicating the capacity of hair cells to organize surrounding cells into a different morphology. If inner ear hair cells have indeed the ability to transform epithelia into vesicles, for which the underlying molecular basis is unknown, this ability is not shared with lateral line organs. It remains unresolved whether lateral line hair cells of superficial neuromasts have lost this ability or never had it.

There are noticeable differences in the canal cristae organization of jawless and jawed vertebrates (Fig. 4). Hagfish have a simple ring of hair cells around the slightly enlarged ampullae of the single torus. Hair cells have long stereocilia and kinocilia that float without a cupula in the canal endolymph (Fig. 4A-C). In contrast, all other vertebrates have crista organs that are only present in localized parts of the ampullae (Fig. 4D). These cristae may be subdivided by a non-sensory structure, the cruciate eminence, into two halves in many jawed vertebrates and form several partially segregated cristae in lampreys (Lewis et al. 1985; Hagelin 1974). Since virtually no molecular or developmental data on the unique morphology of the hagfish ear exists (Fujimoto et al. 2013), it is entirely unclear how these

differences are genetically encoded and regulated and if the anatomical condition in hagfish is ancestral or derived.

What we now begin to understand at a molecular level is the complex interplay that underlies semicircular canal formation. Major factors for canal morphogenesis have been revealed, including *Fgf10* and *Bmp4* for canal growth (Chang et al. 2004). However many other, yet unknown or unidentified genes are required in addition (Beisel et al. 2005; Fritzscht et al. 2007; Chang et al. 2008). During the formation of the horizontal semicircular canal, specific genes such as *Foxg1* are necessary for sensory epithelium formation (Pauley et al. 2006), while a number of others are involved in canal growth (Chang et al. 2004; Bok et al. 2007). The number of genes that are necessary for horizontal canal formation, but are not expressed in the lamprey and hagfish inner ear, need to be determined in order to understand how gain of gene expression in the ear contributed to the morphological novelty of horizontal semicircular canal formation in jawed vertebrates and the associated evolution of eye movement control (Straka 2010). In apparent contrast to all above presented data, demonstrating that the vertebrate ear evolved in aquatic vertebrates, are suggestions that canal function will be reduced upon re-entry of terrestrial vertebrates into permanent aquatic life, such as in cetaceans (Spoor et al. 2002). The argument for this is derived from an apparent change in radius of the canals relative to their cross-sectional area (Jones and Spells 1963) compared to other mammals and is seemingly correlated with the immobility of the head. Reduced head mobility or lack of a neck is a typical feature of aquatic vertebrates such as bony fish or sharks which, however, have fairly long canals (Melvill Jones 1974). Semicircular canals with short radii and large diameters are not only found in cetaceans but are common for many amphibian species (Fritzscht and Wake 1988). Obviously, shorter canals with wider diameter improve bandwidth and velocity-sensitivity (Rabbitt 1999). However, how in detail the size configurations of a semicircular canal is causally related with particular aspects of locomotor performance in water or air of a given species remains to be determined beyond the currently existing correlation.

The segregation of otoconia-bearing labyrinthine endorgans will be even more complex at a molecular level, as indicated by the identification of several genes that block a segregation of the utricle, saccule and cochlea, when mutated (Fritzscht et al. 2013). As has been pointed out above, these segregations of sensory epithelia may or may not correlate with the formation of unique recesses in which the sensory epithelia will remain. For example, mutations that block the formation of an iris-like closure between the vestibular and saccular cochlear part to form the utriculo-saccular foramen (Fig. 4E,F), will result in incomplete segregation of the utricle from the saccule (Nichols et al. 2008). Before these atavistic effects can be put into an evolutionary context, the number of genes that affect this constriction as well as their interaction needs to be established. Once segregation occurred, the otoconia in either of the segregated organs will typically be placed in a different location (one horizontal and one vertical for example) to provide complementary sets of information about head position in space.

An important side aspect of otolith organ formation relates to the respective functional role of the utricle, saccule and lagena. While a vestibular (graviceptive and linear translation) function is well documented for the utricle, a comparable clear functionality is less obvious for the saccule and/or lagena (Straka and Dieringer 2004). Since the sensitivity of the hair cells in these organs cover a wide dynamic range, virtually all otolith organs have a dual function (vestibular graviceptive – substrate vibration). This also explains why some researchers consider the lagena in amphibians an auditory endorgan (Hudspeth 1989), while others demonstrated a clear vestibular function (Straka et al. 2002). The discovery of a magnetoreceptive function of the avian lagena (Wu and Dickman 2011) complies with the

notion that otolith organs can have multiple functional roles, depending on hair cell morphophysiology, biochemistry and physics of structural components and respective location on the epithelium.

Present in some but not all vertebrates is a second separation that splits the utricle from the canals (Figs. 4,5). This additional separation results in a complete isolation of the endolymph flow in the duct system and a dislocation of the canal crista that is independent from the utricular otoconia movement. Since the initial physiological advantage of such a configuration is unclear, the selection pressure on this feature remains yet to be determined. There is, however, an interesting taxonomic distribution of this trait of a unique utricular recess that lumps lungfish with ratfish and elasmobranchs and sets this apparently heterogeneous group apart from all other jawed vertebrates. Since neither the molecular basis of this extra separation nor the functional significance are clear, it can only be speculated whether lungfish obtained this feature independently or whether this is the original jawed vertebrate feature and was lost in the common ancestor of bony fish and land vertebrates. Unfortunately, neither scenario fits well with the emerging position of lungfish as the outgroup of tetrapods (Heimberg et al. 2010; Amemiya et al. 2013) and may indicate that this trait of a segregated utricle in its own recess may not be under a strong selective pressure, allowing for multiple convergent evolution through limited molecular developmental changes. It would be essential to find the molecular basis for the formation of this recess and to compare this with the condition in lungfish and elasmobranchs to see if this is a molecular parallelism or not.

Equally enigmatic is the formation or lack thereof of a lagenar recess. In most basal bony fish, lungfish and ratfish, a lagena exists together with the saccular sensory epithelium in a single, saccular recess. In contrast, in derived elasmobranchs, derived bony fish and *Latimeria* as well as all tetrapods, the lagena is in its own recess. The most simplistic explanation is the gain of a separate lagenar recess even though it is still unclear if its formation is molecularly identical in all vertebrates. Mutational analyses in mammals have provided evidence that the lagenar recess (in this vertebrate taxon usually referred to as the cochlea) can form even when no sensory epithelium forms (Fritzscht et al. 2013; Duncan and Fritzscht 2013). This apparent autonomy of the lagenar recess/cochlear duct is not yet understood at the molecular level but could provide the necessary evidence to understand the morphological evolution driving the formation of a lagenar recess at least three times independently among jawed vertebrates (Figs. 4,5). Finally, otoconia vary widely in their composition (Carlstrom and Engstrom 1955; Steyger and Wiederhold 1995) and micro-organization (single crystal, multiple crystals). While several genes can influence massively the assembly of otoconia or even their general formation, there is no data indicating the functional significance of different crystal forms or the benefit of single compared to multiple otoconia. Indeed, the only functional evidence associated with multiple otoconia is the age-dependent degeneration of the matrix that results in single otoconia floating off and towards the cupulae in the canal organs, causing severe balance disorders (Batuecas-Caletrio et al. 2013).

Conclusion

This review provides a logical framework that will allow integrating future data on the evolution of molecular developmental programs underlying the stepwise transformation of mechanosensory cells from single-celled ancestors to present-day vertebrates into a functional assessment framework. The scenario we developed here allows appreciating how modified mechanosensory cells optimally integrate into the respective anatomical structures of complex organs in the latter group to transduce a wide dynamic range of mechanical

stimuli into appropriate cellular responses. Importantly, deuterostomes in general and vertebrates in particular show less deviation from the condition present in single celled ancestors, compared to arthropods and mollusks (Fig. 2), consistent with the emerging view that deuterostome animals show many primitive features whereas protostome animals show more derived traits.

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Figure 1. Hair cell morphology in single-celled organisms and the vertebrate inner ear
 SEM image of a choanoflagellate with the central kinocilium, the collar of microvilli surrounding the kinocilium, and a process to the opposite side of the cell to attach to the substrate (A). The vertebrate vestibular hair cell has graded stereocilia asymmetrically organized relative to the kinocilium (B). Stereocilia are connected by tip links (green) to open ion channels. Instead of a process emerging from the opposite pole, hair cells receive afferent and efferent fiber innervations. The insert shows a frog hair cell with a bulbed kinocilium on one side of the stereocilia. Image in A taken with permission from Mark J Dayel (2012) *Choanoflagellates and animal multicellularity*. url: <http://www.dayel.com/choanoflagellates>. Images in B and insert are from public websites (kamares.ucsd.edu/~ffilimon/107B/107b.html; <http://hms.harvard.edu/news/firm-molecular-handshake-needed-hearing-balance-11-8-12>).

Figure 2. Evolution and development of mechanosensory cells

Kinocilia (red) and microvilli/stereocilia (green) of known or suspected mechanosensory cells in various eukaryotic unicellular (1) and multicellular (3) organisms are shown. Left hand panel: The single celled ancestor of all multicellular animals, the choanoflagellates (1), has a single, kinocilium surrounded by microvilli utilizing an actin core (A). In some diploblasts, the central kinocilium is surrounded by an asymmetric assembly of microvilli, potentially providing directional sensitivity (B). Among deuterostomes, urochordates have various presumed mechanosensory cells that have a kinocilium with asymmetrically arranged microvilli (C). Vertebrates are unique in that a highly polarized, organ-pipe assemblies of actin-rich stereocilia are attached via tip links to each other (D). E,F Illustration of the very different organization of protostome mechanosensors, as a single kinocilium that is pulled along its length (F) or as multiple kinocilia, connected by tip links (E). Right hand panel: Mammalian hair cells develop their stereocilia in a process that starts with a central kinocilium surrounded by few microvilli. As the number of microvilli increases, the kinocilium moves into an off-center position and eventually toward one end of the developing hair cell. Microvilli in front of the moving kinocilium become reduced and eventually all disappear. In contrast microvilli trailing the kinocilium grow in length, thickness and actin content (green) to turn into stereocilia. As the kinocilium reaches its eccentric position, the distinction between kinocilium and microvilli has established the polarity with the longest stereocilia being next to the kinocilium. Development diverges through unknown molecular mechanism to generate the four different hair cell types found in the mammalian sensory epithelia. Type I and inner hair cells develop thick stereocilia and the characteristic bundles, which are C-shaped for inner hair cells. Other vestibular hair cells develop as type II and outer hair cells with thinner stereocilia. In addition, the organization of the stereocilia of the outer hair cells forms a characteristic M shape with the kinocilium in the inflection of the M. Later in development small microvilli all but disappear and the kinocilium is resorbed in inner and outer hair cells of the organ of Corti. Modified after (Duncan and Fritsch 2012; Philippe et al. 2011; Schwander et al. 2010).

Figure 3. Consequences of particle motion in the near field (top) and far field (bottom) aquatic environment

Ball oscillation causes water to flow as indicated. Objects of comparable density such as a buoyant fish will oscillate in the near field (blue); this induces shearing forces between moving hair cells and lagging otoconia of the utricle (U) in the ear, thereby generating vibrational stimuli. In the far field, the same oscillation will result in pressure waves that cause compression and rarefaction of a gas-filled swim bladder (red); this alternating compression and expansion results in near field particle motion that can be channeled with specialized perilymphatic spaces through the inner ear. The resulting hair cell deflections are thus triggered by far field sound pressure, and detected mostly with a specialization of the saccule (S). Modified after (Fritzsche 1999; Ladich and Popper 2004).

Figure 4. Labyrinthine endorgan morphology in vertebrates

Hagfish have only a simple torus with two circular canal cristae in the ampullae (A-C). The hair cells are organized as a continuous band (C), have extremely long kinocilia and stereocilia (B) but no cupula (C). In contrast, the canal cristae in jawed vertebrates such as mice (D) show a separation of two sensory cell-bearing areas by a non-sensory cell-bearing area (D). Four basic types of jawed vertebrate ears can be identified, depending on the absence or presence of a lagena in its own recess and the presence or absence of a complete separation of the utricle from the semicircular canals (E, E', F, F'). Only elasmobranchs and lungfish have a separate utricular recess connected to the anterior canal by its own opening indicated by the red arrow. In contrast, all other vertebrates have only one constriction, the utriculo-saccular foramen, that connects the inferior to the superior part of the ear (indicated by a green arrow). Note that the absence of a lagenar recess is typical for many basic vertebrates (E, F), whereas many derived vertebrates of each major lineage have evolved, apparently independently, a lagenar recess (E', F'). Thus, a lagena as a sensory epithelium may have evolved with jawed vertebrates but its position in its own recess may have evolved several times, leading only in *Latimeria* and tetrapods to the formation of the basilar papilla (BP), but not in other vertebrates that also have evolved a lagena. Abbreviations: AC, anterior crista; BP, basilar papilla; e, endolymphatic duct; L, lagena; PC, posterior crista; S, saccule; U, utricle; USF, utriculo-saccular foramen.

Morphological ear evolution depicted as morphotype changes

Figure 5. Evolution of inner ear endorgans

Vertebrate ears are grouped according to systematic affinity but also taking morphologic differences in ear morphology into account to arrange them with least additional assumptions as to how anatomical traits evolved. Ears in cyclostomes are very different indicating a long segregation of the lamprey and hagfish line. Elasmobranchs, ratfish and lungfish form a single group characterized by the unique feature of the nearly complete separation of the utricle from the semicircular canals. All bony fish, *Latimeria* and tetrapods lack this trait but it remains unclear if it is a secondary loss or if it is derived again in elasmobranchs and lungfish. Assuming that the indicated groups are natural and not assemblies generated by convergence, it appears that formation of a lagenar recess occurred at least three times among vertebrates. However, only in the line leading to tetrapods (*Latimeria*), a separate epithelium was formed, the basal papilla, that evolved into the mammalian organ of Corti. Abbreviations: AC, anterior canal crista, BP, basilar papilla; CM, common macula; E, endolymphatic duct; HC, horizontal canal crista; L, lagena; PC, posterior canal crista; S, saccul, U, utricle; USF, utriculo-saccular foramen. Modified after (Beisel et al. 2005).