Int. J. Dev. Biol. 51: 535-547 (2007)
doi: 10.1387/ijdb.072344mk

Shaping the mammalian auditory sensory organ by the planar cell polarity pathway

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ABSTRACT The human ear is capable of processing sound with a remarkable resolution over a wide range of intensity and frequency. This ability depends largely on the extraordinary feats of the hearing organ, the organ of Corti and its sensory hair cells. The organ of Corti consists of precisely patterned rows of sensory hair cells and supporting cells along the length of the snail-shaped cochlear duct. On the apical surface of each hair cell, several rows of actin-containing protrusions, known as stereocilia, form a "V"-shaped staircase. The vertices of all the "V"-shaped stereocilia point away from the center of the cochlea. The uniform orientation of stereocilia in the organ of Corti manifests a distinctive form of polarity known as planar cell polarity (PCP). Functionally, the direction of stereociliary bundle deflection controls the mechanical channels located in the stereocilia for auditory transduction. In addition, hair cells are tonotopically organized along the length of the cochlea. Thus, the uniform orientation of stereociliary bundles along the length of the cochlea is critical for effective mechanotransduction and for frequency selection. Here we summarize the morphological and molecular events that bestow the structural characteristics of the mammalian hearing organ, the growth of the snail-shaped cochlear duct and the establishment of PCP in the organ of Corti. The PCP of the sensory organs in the vestibule of the inner ear will also be described briefly.

KEY WORDS: *convergent extension, organ of Corti, hair cell, primary cilia, stereocilia*

Introduction

The mammalian inner ear contains the cochlea and the vestibule for hearing and balance (Fig. 1), respectively. The functional aspects of the cochlea and the vestibule lend themselves greatly to the mechanical properties of the tissues and the precise arrangement and polarity of sensory hair cells in their sensory organs. The cochlea has one sensory organ, known as the organ of Corti and the vestibule has five sensory organs (Fig. 1A-C). Each hair cell has several rows of apical protrusions known as stereocilia in a staircase-like arrangement with the tallest stereociliary bundles located toward one side of the hair cell. The stereocilia of hair cells within each sensory organ is coordinately oriented (Fig. 1B,D). The coordinated orientation of stereocilia in inner ear sensory organs manifests perhaps the most distinctive form of a type of tissue polarity, known as planar cell polarity (PCP), in vertebrates (Figs. 1, 2).

The entire inner ear is derived from a patch of ectodermal cells near the hindbrain known as the otic placode (Kikuchi *et al.*, 1988, Morsli *et al.*, 1998). How does such an incredibly complex structure develop from what is presumably an equivalent group of ectodermally-derived cells? The various sensory regions must be organized, orientated and innervated in an extremely well-controlled manner to allow for proper function. The overall structure and cellular organization require that tight temporal and spatial regulation be coordinated during development. The precise cellular patterning, morphological differentiation and innervation of the organ of Corti, therefore, depend on early developmental events and are determined by molecular pathways regulating terminal differentiation and morphogenesis. In this review, we briefly summarize the early development of the inner ear (for detailed discussions, see other reviews in this issue by Schimmang, Ohyama *et al.,* Wu, Schneider-Maunoury and Pujades) and focus on the morphological and molecular events that create the uniform orientation of stereocilia in the organ of Corti and the

Abbreviations used in this paper: BBS, Bardet-Biedl syndrome; CE, convergent extension; DC, Deiters' cell; Dvl, dishevelled; Fz, frizzled; Hh, hedgehog; IPhC, inner phalangeal cell; IFT, intraflagellar transport; IPC, inner pillar cell; OPC, outer pillar cell; PCP, planar cell polarity; PDGFR, platelet-derived growth factor receptor; Shh, sonic hedgehog; TGF-β, transforming growth factor-β; ZNPC, zone of non-proliferating cells

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0214-6282/2007/$30.00

Printed in Spain
www.intjdevbiol.com

lengthening of the cochlea during terminal differentiation. We integrate current data into a working model and discuss PCP regulation in the morphogenesis of the organ of Corti.

The organ of Corti

The organ of Corti is a continuous array of cells tonotopically organized on the basilar membrane along the length of the snail-shaped cochlea (Sher, 1971, Lim and Anniko, 1985). Graded variations of mechanic properties of the basilar membrane (von Bekesy, 1970), as well as electrical properties of hair cells (Hudspeth, 1989, Manley, 2000, Kruse and Julicher, 2005), enable tuning of sensory hair cells to a progression of frequencies corresponding to their location along the length of the cochlea. The tonotopical response is organized in ascending order from the apex to the base (von Bekesy, 1970). Along the length of the cochlea, there are four rows of hair cells interdigitated with several types of non-sensory cells to make up the organ of Corti. The innermost row toward the center (hereinafter referred to as "medial") region of the cochlea and the three rows toward the peripheral (hereinafter referred to as "lateral") region of the cochlea are known as the inner (IHCs) and outer hair cells (OHCs), respectively (Figs. 1C,D and 2). Several rows of "finger-like" extensions or hair bundles, known as the stereocilia (Figs. 1D, 2B-D), project from the apical surface of each hair cell and form a "V" shape, which is shallower in IHCs than in OHCs. Invariably, the vertices of all the "V"-shaped stereocilia point in the medial-to-lateral (hereinafter referred to as "mediolateral") direction (Figs. 1C,D and 2B-D). A single primary cilium, known as kinocilium, is placed at the vertices of "V"-shaped stereocilia during development. Therefore, each hair cell is intrinsically asymmetrical in terms of the arrangement of stereociliary bundles and kinocilia and all the hair cells are uniformly polarized along the mediolateral axis of the cochlear duct (Figs. 1 and 2). The uniform orientation of stereocilia of hair cells along the mediolateral axis of the cochlea manifests a distinctive form of planar cell polarity (PCP) (Lewis *et al.*, 1985, Lewis and Davies, 2002) (Figs. 1 and 2).

The nonsensory cells of the organ of Corti, commonly referred to as supporting cells, also have distinctive morphologies and organizations. Hair cells are separated from each other by supporting cells in a precise and invariable pattern. The supporting cells of the organ of Corti include the border cells, inner phalangeal cells (IPhC), the inner and outer pillar cells (IPC and OPC)

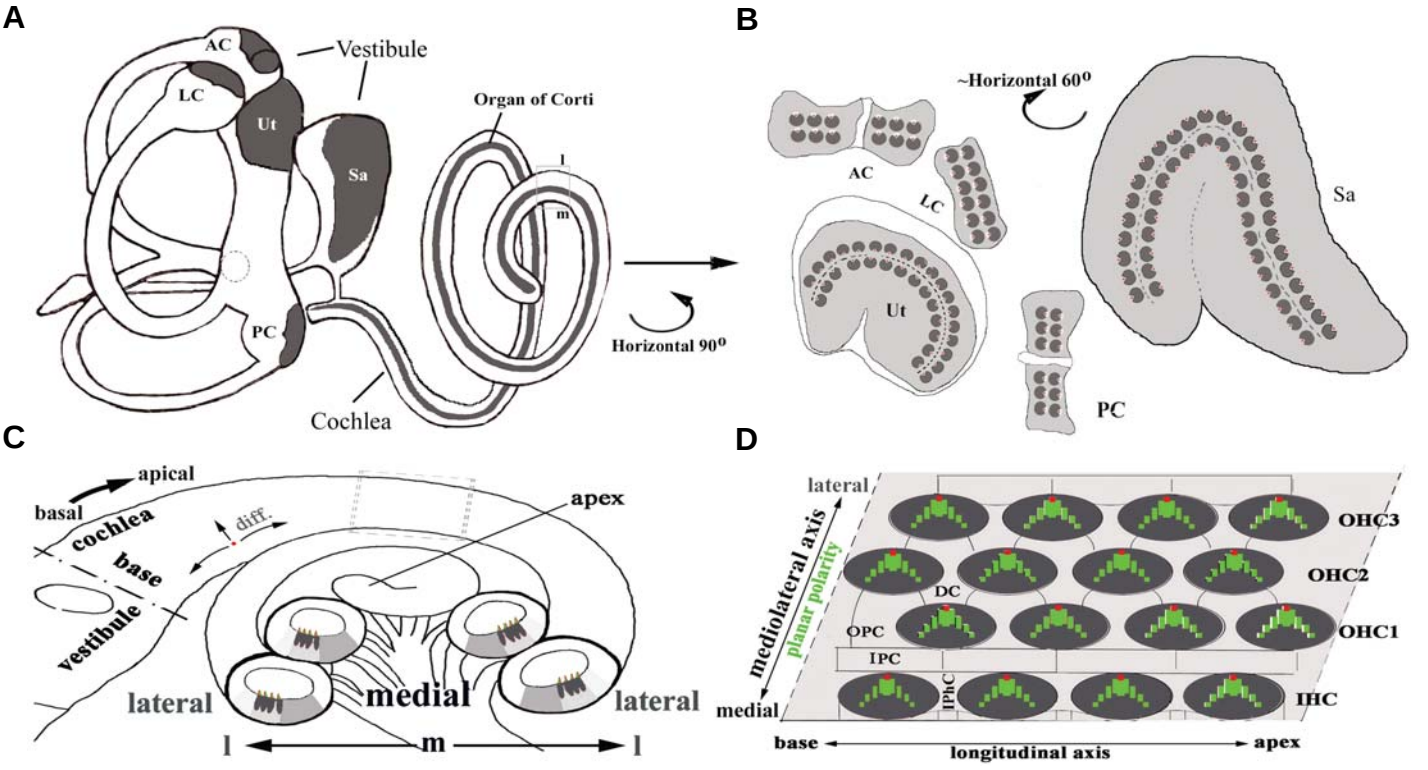

Fig. 1. Planar cell polarity (PCP) of the inner ear sensory organs. (A) *The structure of a right inner ear is illustrated. The shaded areas represent one sensory organ in the cochlea, the organ of Corti and five sensory organs in the vestibule, maculae of the saccule and utricle (Sa, Ut) and cristae of lateral (LC), anterior (AC) and posterior (PC) semicircular canals.* **(B)** *The five vestibular sensory organs show distinctive forms of PCP. Hair cells (circles) on opposite sides of the striola (dashed lines) in the saccule and utricle have reversed planar polarity, as shown by the location of the kinocilium (red dots) which is placed near the tallest stereociliary bundles. The vestibular sensory organs in (B) were rotated counterclockwise 90º horizontally as they appear in (A) and the lateral crista was further rotated clockwise ~60º horizontally to show their whole mount views.* **(C-D)** *The cochlea is a fluid-filled labyrinth. The organ of Corti is suspended along the length of the cochlea and situated on the basilar membrane (C). In a whole mount surface view, the organ of Corti shows a mosaic arrangement of sensory hair cells (D, dark grey) and supporting cells (D, light grey). The stereociliary bundles (green) are arranged in a "V"-shaped staircase on the apical surface of each hair cell and the vertices of all the "V"-shaped stereocilia are uniformly oriented away from the center of the spiraling cochlea (D), displaying a PCP along the mediolateral axis of the cochlea. Abbreviations: IHC, inner hair cells; OHC1-OHC3, the first –third rows of outer hair cells; IPC, inner pillar cell; OPC, outer pillar cell; DC, Deiters' cell.*

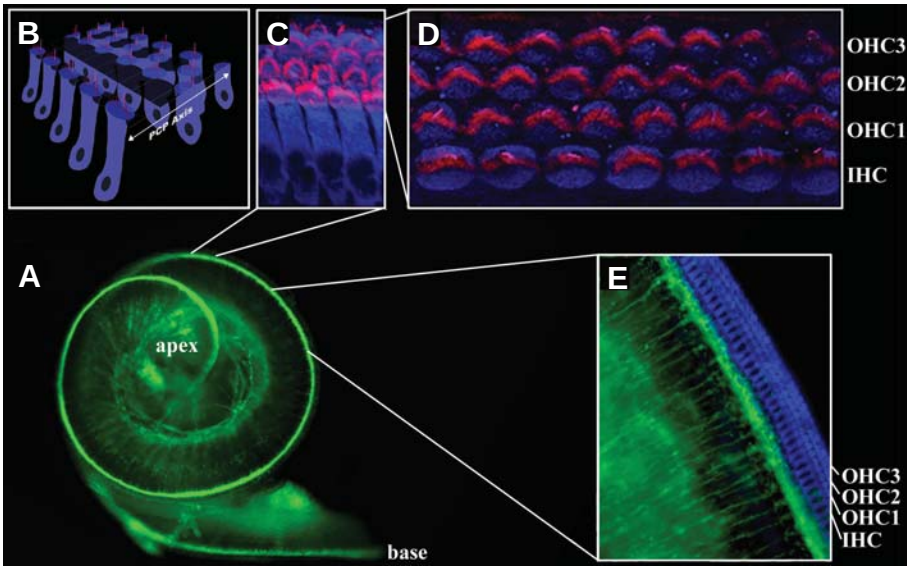

Fig. 2. Stereotyped cellular patterning, polarity and neural connection in the organ of Corti stretch the entire length of the snail-shaped mammalian cochlea. (A) *A dissected mouse cochlea with YFP expressed in the spiral ganglion neurons to visualize the general morphology of the cochlea and innervation of its sensory organ along the length of the cochlea from the base to apex. The highest YFP signal is under the row of inner hair cells (IHC).* **(B)** *Schematic diagram showing the arrangement of auditory hair cells (blue) and their stereocilia bundles (red), with kinoclium (magenta) at the vertex, along the PCP axis. Supporting cells are not shown, but also exhibit distinct polarity. An example is the Dieter's cells, each of which cup the base of an outer hair cells and form a phalangeal process that forms the separation between the more lateral row of outer hair cells at a distance along the longitudinal axis of the cochlea.* **(C)** *Hair cell polarity occurs in both the apical-basolateral axis and in a perpendicular axis within the plane of the epithelium, the PCP axis. Cell nuclei of myosinVI-labelled auditory hair cells (blue) are towards the basal regions of hair cells, whereas the phalloidin-labelled stereocilia bundles (red) are apical towards the lumen. On the apical surface, the stereocilia bundles are oriented uniformly along the mediolateral PCP axis.* **(D)** *Coordinately polarized orientation of the stereocilia bundles (red) on the apical surface of the auditory hair cells (blue) with kinocilium (magenta) seen at the vertices of the bundles. All four rows of the hair cells, with one row of inner hair cells and three rows of outer hair cells can be seen here.* **(E)** *YFP-expressing spiral ganglion neurons (green) show precise connections to auditory hair cells (blue). The spiral ganglion neurons and the sensory cells are derived from the same region within the otocyst and the development of spiral ganglion neurons influences the shape of the cochlea and its sensory organ. m, medial; l, lateral.*

and the Deiters' cells (DC) (Figs. 1D, 2B) (Slepecky, 1996, Jones and Chen, 2007) The nuclei of these supporting cells are localized basally; from their soma, the supporting cells project phalangeal ("finger-like") cellular processes toward the lumen of the cochlear duct (Fig. 2B). Their flattened ends of phalangeal processes separate hair cells from each other and form tight cellular contacts with the hair cells (Figs. 1D, 2B). Notably, the phalangeal processes of the supporting cells are highly polarized along the mediolateral axis of the cochlea. Morphologically, the pillar cells and DCs extend their cytoplasmic stalks and phalangeal processes along the mediolateral axis of the cochlea toward the periphery of the cochlea and contact the apical surface of the hair cells in the next row (Fig. 2B). Molecularly, several proteins display polarized subcellular localization along the mediolateral axis of the cochlea. The phalangeal processes of OPCs and DCs are also polarized along the longitudinal axis of the cochlea and contact hair cells at a distance (toward the apex of the cochlear duct) from the base of these supporting cells. Bundles of microtubules, intermediate filaments and microfilaments span the phalangeal processes of supporting cells to support for structural integrity of the organ of Corti (Slepecky and Ulfendahl, 1992, Henderson *et al.*, 1995, Slepecky *et al.*, 1995). The tight juxtaposition of hair cells and supporting cells at their apical surface allows the separation of the endolymphatic fluid that baths the apical domain from the perilymph in the basolateral domain for proper mechanotransduction (Slepecky, 1996). The unique morphology of supporting cells and their cellular organization with sensory hair cells allows a morphologically defined yet mechanically flexible structure to permit movement of the sensory epithelium in response to mechanical stimuli (Hudspeth, 2000).

In addition to sensory hair cells and supporting cells, the spiral ganglion neurons that innervate hair cells are important for the structural integrity and function of the organ of Corti (Fig. 2) (Rubel and Fritzsch, 2002). The tonotopic organization of the auditory sensory organ is maintained in the auditory neural pathway, beginning immediately postsynaptic to hair cells (Hudspeth, 2000). The innervation of hair cells by spiral ganglion neurons is required for the structural integrity of the organ of Corti (Ma *et al.*, 2000, Rubel and Fritzsch, 2002, Matei *et al.*, 2005). During development, the formation of the sensory lineage and the neuronal lineage within the developing inner ear is tightly coupled (Ma *et al.*, 2000, Radde-Gallwitz *et al.*, 2004, Matei *et al.*, 2005, Satoh and Fekete, 2005). It is no surprise that not only the pathways directly involved in sensory differentiation are essential for the morphogenesis of the organ of Corti, but also the development of the neuronal lineage influences the shaping of the organ of Corti (Ma *et al.*, 2000, Matei *et al.*, 2005).

The specification and differentiation of the organ of Corti

The otic placode is recognizable around embryonic day 8.5 (E8.5) in mice (Kikuchi *et al.*, 1988, Morsli *et al.*, 1998, Riley and Phillips, 2003). By E10.5, the otic placode has invaginated and formed a hollow epithelium called the otocyst and at this time the regions of sensory and non-sensory epithelium begin to be determined. At these early stages of induction three-dimensional spatial cues, including Sonic hedgehog (Shh), Wnts, BMPs and FGFs (McKay *et al.*, 1996, Morsli *et al.*, 1998, Chang *et al.*, 1999, Cole *et al.*, 2000, Gerlach *et al.*, 2000, Chang *et al.*, 2002, Maroon *et al.*, 2002, Riccomagno *et al.*, 2002, Liu *et al.*, 2003, Wright and Mansour, 2003, Chang *et al.*, 2004, Ozaki *et al.*, 2004, Solomon *et al.*, 2004, Riccomagno *et al.*, 2005, Fritzsch *et al.*, 2006, Ohyama *et al.*, 2006), play important roles in setting up sub-

domains of the otocyst and controlling subsequent morphogenesis of various structures and specific cell types. Very soon after the initial specification, the newly formed otocyst begins specification and forms regions that will become the cochlea and vestibule, while the cochleovestibular neurons delaminate from the same regions in the otocyst that are designated to become the sensory epithelia. The regional specification of the otocyst is illustrated by expression of molecular markers in individual regions.

Soon after the cochlear duct has formed at the ventral region of the otocyst, sharp boundaries are formed to molecularly mark each half of the cochlear epithelium at E12.5. Otoconin90 (Zhao *et al.*, 2007) is specifically expressed in the roof while the expression of several other genes including Isl1 (Radde-Gallwitz *et al.*, 2004) and Sox2 (Kiernan *et al.*, 2005b) (personal communication, A. Kiernan) is restricted to the floor of the duct. The pathways that set up the sharp boundaries of the cochlear duct at this stage are not known. Isl1 knockout mice die by E11.5 (Pfaff *et al.*, 1996) and its role in inner ear development has yet to be determined. The analysis of molecular markers has not been reported in Sox2 mutants at E12.5 (Kiernan *et al.*, 2005b) and the role of Sox2 for early cochlea development is unknown.

Regional specifications within the floor of the cochlear epithelium continue and result in the formation of a morphologically and molecularly distinctive sensory primordium. By E13.5-E14.5, the organ of Corti is recognized as a thickened ridge in the cochlear epithelium and marked by the expression of multiple genes. In particular, the precursor cells of the organ of Corti withdraw from the cell cycle around E14.5 (Ruben, 1967) and form a zone of non-proliferating cells (ZNPC) that is marked by two cyclin-dependent kinase inhibitors, p27/Kip1 and p19/Ink4d, and Isl1 and Sox2 (Chen and Segil, 1999, Chen *et al.*, 2002, Chen *et al.*, 2003, Radde-Gallwitz *et al.*, 2004, Kiernan *et al.*, 2005b, Matei *et al.*, 2005, Lee *et al.*, 2006). p27/Kip1 and Sox2 are required for the precursor cells of the organ of Corti to withdraw timely from the cell cycle (Chen and Segil, 1999) and for the specification of the sensory primordium (Kiernan *et al.,* 2005b) in the cochlea, respectively. In addition, several genes in the Notch pathway show distinctive expression patterns in the vicinity of the sensory primordium, consistent with a role in specifying and/or restricting the sensory lineage in the cochlea (Haddon *et al.*, 1998, Lanford *et al.*, 1999, Eddison *et al.*, 2000, Kiernan *et al.*, 2005a, Brooker *et al.*, 2006, Kiernan *et al.*, 2006). Members of the BMP pathway are also expressed in the cochlear epithelium abutting the sensory primordium (Morsli *et al.,* 1998). Their exact role in the specification and differentiation of the organ of Corti in mice, however, remains controversial (Li *et al.*, 2005, Pujades *et al.*, 2006).

Following specification of the sensory primordium in the cochlea, terminal differentiation of the organ of Corti initiates near the base of the cochlea. The gradient of hair cell differentiation begins with the onset of Math1 in the near-basal region and moves in both directions, finishing in the apical portions of the cochlea around E17.5 (Sher, 1971, Chen and Segil, 1999). Simultaneously with the longitudinal gradient of differentiation, a mediolateral, or inner-to-outer hair cells, gradient of differentiation is also observed. By E18.5, nearly all the cells of the organ of Corti along the length have differentiated into the highly patterned structure of one row of IHCs and three rows of OHCs (Fig. 2). The expression of p27/Kip1 is down-regulated in differentiating hair cells and remains in supporting cells (Chen and Segil, 1999), which differentiate and form the full complement of specialized supporting cells at the same time as the hair cells achieve their terminal morphology (Fig. 3A). The Notch pathway clearly plays an essential role in determining the fate of hair cells vs. supporting cells (Haddon *et al.*, 1998, Kiernan *et al.*, 2005a, Brooker *et al.*, 2006).

Morphogenesis of the organ of Corti: convergent extension and establishment of planar cell polarity (PCP)

Convergent extension in the developing organ of Corti

Convergent extension (CE) (Keller, 2002) is one of the most important cellular movements during gastrulation, contributing to the formation of three germ layers and the establishment of an elongated body plan with a distinctive anterior-posterior body axis from a blastula. It is a process of tissue narrowing along one axis (mediolateral axis) and concomitant extension along a perpendicular axis (anterior-to-posterior or A-P axis). During gastrulation, mesoderm cells undergo CE to establish the anterior-posterior body axis and to elongate along the anterior-posterior axis (Keller, 2002). CE also plays important roles in neurulation. At the neurula stage, cells in the neural ectoderm undergo CE to both elongate along the anterior-posterior axis and to close the neural tube (Keller, 2002). During gastrulation in *Xenopus*, it was shown, using Keller's explants, that the mesoderm cells first undergo radial intercalation to form a thinner tissue and subsequently intercalate mediolaterally (Keller *et al.*, 1985). The mediolateral intercalation of cells leads to the convergence (narrowing) of the tissue along the same (mediolateral) axis and elongation along the perpendicular anterior-posterior axis. While the cellular behavior and the driving force for radial intercalation is unclear, it is known that during mediolateral intercalation, cells are polarized or elongated along mediolateral axis and project mediolaterally oriented lamellapodia (Wilson and Keller, 1991, Keller, 2002). Computational models suggest that force generated by the protrusive activity of the cells along the mediolateral axis is sufficient to drive the intercalation of cells along the same axis (Zajac *et al.*, 2003, Brodland, 2006, Brodland and Veldhuis, 2006). This hypothesis is consistent with the observation that actin-myosin cytoskeletal components are required for cellular intercalation (Zallen and Wieschaus, 2004). In mammals, morphogenetic changes indicate that similar CE cellular movement occurs during gastrulation and neurulation. However, the process of CE in mammals has yet to be documented directly.

During terminal differentiation from E14.5-E18.5, the postmitotic organ of Corti thins from an epithelium of 4-5 cells thick to a final two cell-layered structure and extends along the longitudinal axis significantly (Chen *et al.*, 2002, McKenzie *et al.*, 2004). The extension and thinning are independent of cell proliferation and death within the developing organ of Corti, respectively, suggesting that cellular rearrangement within a shorter and thicker sensory primordium leads to the formation of a longer and thinner mature organ (Chen *et al.*, 2002). This type of cellular movement is remarkably similar to the process of CE which occurs during gastrulation and neurulation in *Xenopus*. Morphological and functional studies further suggest that radial and mediolateral intercalation of cells characteristic of CE is likely involved in extension of

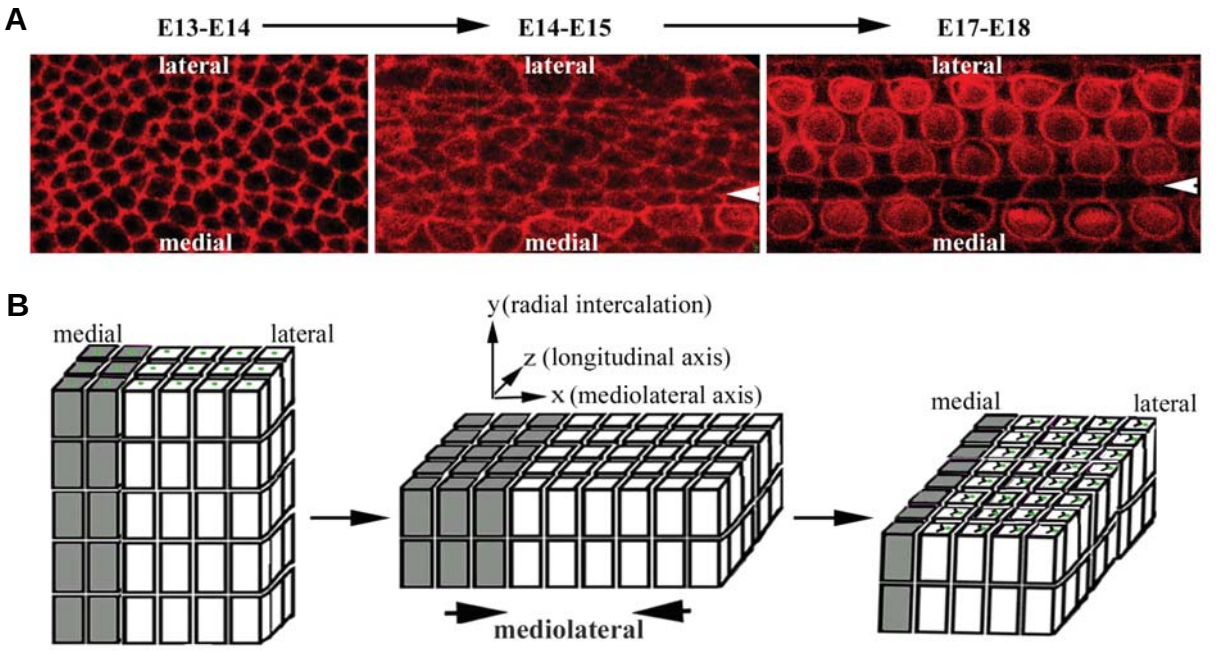

Fig. 3. Convergent extension of the organ of Corti. *Whole mount surface views of the developing organ of Corti illustrate drastic shape change and remodeling of cell-cell contact from E13-E14 to E17-E18 in mice* **(A)***, which may underlie cellular rearrangement characteristic of convergent extension during the same period* **(B)***. The Arrowheads indicate the pillar cell region that separates IHCs from OHCs. The drawing in (B) is simplified, omitting the supporting cell population. Nevertheless, the supporting cells may play essential roles in PCP and proposed convergent extension cellular intercalation. The phenotypic defects in PCP mutants indicate both radial and mediolateral intercalations are involved and PCP signaling is only required for mediolateral intercalation. However, the sequential order of radial and mediolateral intercalations of the developing organ of Corti is only hypothesized and not established experimentally.*

the cochlea and patterning of the hair cells (Fig. 3) (Montcouquiol *et al.*, 2003, McKenzie *et al.*, 2004, Wang *et al.*, 2005, Jones and Chen, 2007).

Establishment of PCP in the organ of Corti

The terminal differentiation and extension of the organ of Corti occur in a ciliated epithelium between E14.5-E18.5 in mice. Both electron microscope and immunostaining show that cells in the developing cochlea are mono-ciliated, containing a microtubule-based primary cilium (Davis *et al.*, 2006) known as the kinocilium (Figs. 1 and 2). The kinocilium is first seen centrally placed on the apex of nascent hair cells and surrounded by microvilli made up of actin filaments of uniform size (Sobkowicz *et al.*, 1995). Subsequently, these microvilli begin to enlarge and become stereocilia. The kinocilium becomes displaced to the lateral side of the cell apex and stereocilia grow in a defined "V"-shaped pattern with the vertex of the "V" closely placed near the kinocilium (Frolenkov *et al.*, 2004). The polarity of kinocilia appears to lead the polarization of stereocilia and the development of stereocilia and their polarity follow the differentiation gradient from the base to the apex along the longitudinal axis and from the inner to outer hair cells along the mediolateral axis of the cochlea. By E18.5 in mice, the polarity of stereocilia and kinocilia is established along the entire length of the cochlear duct and across the width of the organ of Corti. Once established, stereocilia continue to grow, mature and renew. In mammals, kinocilia in the cochlea regress postnatally. The transient presence and the polarity of kinocilia in the cochlea implicate a developmental role. However, the function of kinocilia in the development of the cochlea has not yet been reported.

Planar cell polarity pathway in shaping the cochlea and its sensory organ

PCP pathway

Intriguingly, as CE (Fig. 3B) was being proposed for the terminal morphogenesis of the organ of Corti (Chen *et al.*, 2002), it was revealed that CE in vertebrates is regulated by a conserved genetic pathway, the planar cell polarity (PCP) pathway (Wallingford *et al.*, 2000, Keller, 2002, Mlodzik, 2002, Wallingford *et al.*, 2002).

The PCP pathway was first identified and characterized for its role in regulating various forms of PCP in *Drosophila* tissues (Gubb and Garcia-Bellido, 1982, Klein and Mlodzik, 2005, Strutt and Strutt, 2005). In the tissues that exhibit PCP, there is a well-defined planar polarity both in the intrinsically polarized structure of each individual cell and in the arrangement of different cells relative to each other within the group (Fig. 1). In the organ of Corti, the stereociliary bundles of each hair cell are arranged in an asymmetrical "V" shape. The asymmetrical nature of the "V"-shaped stereocilia represent the intrinsically polarized structure within each hair cell. Furthermore, all the stereocilia are uniformly oriented along the mediolateral axis of the cochlea, manifesting a precise coordination in the arrangement of the cells relative to each other within the group. The planar polarization of cells both in the intrinsic structure of each individual cell and in the arrangement of different cells relative to each other within the entire group

requires a three-tiered regulation (Tree *et al.*, 2002) through two alterative models (Fig. 4). Both models require: a global guidance cue for directional information (upstream PCP genes) and cellular factors to interpret the directional signal by adopting polarized asymmetric localization along the axis for polarity (core PCP genes) (Fig. 4). Upon the formation of polarized core PCP complexes, the third and final step of PCP signaling of planar polarization across the tissue can be achieved by cell-specific effectors downstream of core PCP genes (downstream PCP effector genes) that direct the formation of the asymmetrical structure within each cell and coordinate the polarization of all the cells across the entire tissue. Alternatively, the machinery that builds the asymmetrical structure within each cell is independent of PCP signaling. To achieve the third step in PCP signaling of planar polarization across the tissue in this second model, cellular mediators (downstream PCP mediator genes) link polarized core PCP complexes to the cellular machinery that creates the asymmetrical structure within individual cells to coordinate planar polarization in all the cells across the entire tissue (Fig. 4). In the first model, impaired PCP signaling will lead to defects in both the formation of the asymmetrical structure within individual cells as well as the coordination of all the cells across the tissue. In the second model, however, defective PCP signaling will only affect the coordination of planar polarization across the tissue but not the production of the asymmetrical structure within individual cells.

Genetic studies in *Drosophila* identified a set of core PCP genes that affect all known structures with PCP features (Tree *et al.*, 2002, Klein and Mlodzik, 2005, Strutt *et al.*, 2006). During establishment of PCP, core PCP proteins are sorted asymmetrically along the polarization axis and this polarized association of core PCP proteins is required for planar polarization across the tissue (Axelrod *et al.*, 1998, Axelrod, 2001, Tree *et al.*, 2002, Klein and Mlodzik, 2005, Strutt and Strutt, 2005). Studies in *Xenopus* and zebrafish revealed a conserved vertebrate PCP pathway that consists of a similar cassette of genes, including Frizzled (Fz) (Djiane *et al.,* 2000), Dishevelled (Dvl) (Sokol, 1996, Wallingford *et al.*, 2000), Ltap/Vangl2 (Goto and Keller, 2002, Jessen *et al.*, 2002, Park and Moon, 2002) and homologs of Diego (Schwarz-Romond *et al.*, 2002, Moeller *et al.*, 2006) and Prickle (Veeman *et al.,* 2003), that are required for CE during gastrulation and neurulation (Fig. 4). Core PCP proteins display polarized subcellular localization along the anterior-posterior axis (Jiang *et al.*, 2005, Ciruna *et al.*, 2006). Together with mediolaterally polarized sorting of Par complexes (Hyodo-Miura *et al.*, 2006, Mlodzik, 2006), the core PCP proteins likely provide instructive roles to direct mediolateral intercalation of cells during CE (Mlodzik, 2006).

The core PCP component Fz can serve as a receptor for Wnt signaling molecules (Moon, 2005). The binding of Wnt to Fz can activate a canonical Wnt pathway in which β-catenin is stabilized for transcriptional activation of downstream target genes. PCP signaling does not involve β-catenin mediated transcriptional activation and, instead, involves cytoskeletal targets (therefore, known as the noncanonical Wnt pathway). Since the polarization of cells in the entire field requires directional information and Wnts as morphogens can fulfill such a directional role, Wnts have been extensively tested for their potential involvement in PCP. However, Wnts appear to be dispensable for PCP in several *Drosophila* tissues (Ma *et al.*, 2003, Klein and Moldzik, 2005, Strutt and Strutt, 2005). A mathematic model (Amonlirdviman *et al.*, 2005), whose predictions have been tested experimentally, further argues for a morphogen-independent feedback loop regulation for polarization over a long range. In contrast, two recent studies indicate an instructive role for Wnt and Hh in orienting the denticles (actin protrusions) on the epidermis of *Drosophila* embryos (Colosimo and Tolwinski, 2006, Price *et al.*, 2006). Therefore, it is possible that epithelial planar polarization across the tissues may utilize a morphogen-independent mechanism for polarization over a long range, an instructive cue-dependent mechanism for polarization over a short range, or a combination of both. In contrast to their variable role in establishing epithelial PCP in *Drosophila*, upstream morphogens are involved in PCP signaling for vertebrate CE (Heisenberg *et al.*, 2000, Smith *et al.*, 2000, Tada and Smith, 2000, Kilian *et al.*, 2003, Ohkawara *et al.*, 2003). In *Xenopus* and zebrafish, Wnt5 and Wnt11 are required for CE (Heisenberg *et al.*, 2000, Smith *et al.*, 2000, Tada and Smith, 2000, Kilian *et al.*, 2003, Ohkawara *et al.*, 2003) and their

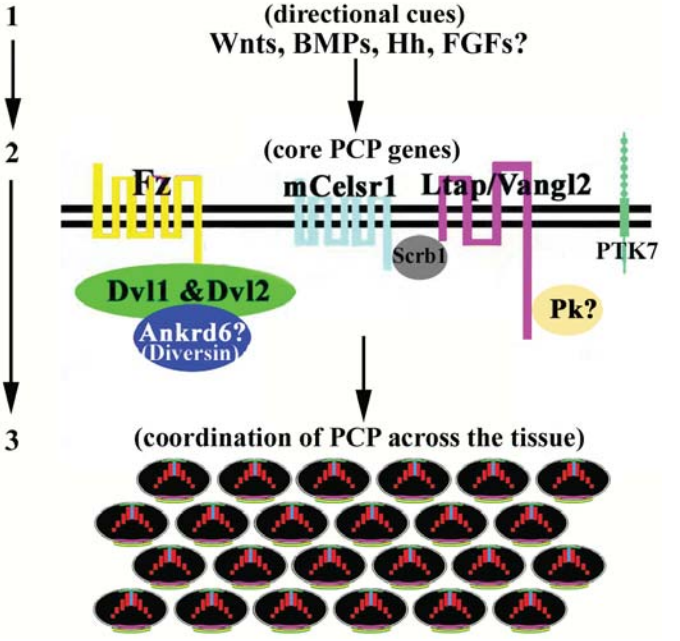

Fig. 4. A model of planar cell polarity (PCP) regulation in the cochlea. *The establishment of PCP across the organ of Corti requires a three-tiered regulation:* **(1)** *upstream factors for directional information along the mediolateral axis of the cochlea;* **(2)** *core PCP proteins form polarized complexes along the mediolateral axis of the cochlea;* **(3)** *unknown cellular mediators link polarized core PCP complexes to the machinery that builds the "V"-shaped stereocilia in individual hair cells and orient the vertices of the "V"-shaped stereocilia toward the periphery of the spiraling cochlea, establishing uniformly oriented stereocilia across the organ of Corti. The identity and the role of the upstream factors were hypothesized; and the role of Pk and Ankrd6/Diversin in inner ear was hypothesized based on their role in PCP signaling in Drosophila and in CE in zebrafish. Several core PCP proteins are observed at the boundaries between supporting cells and between hair cells and supporting cells in a polarized manner along the mediolateral axis of the cochlea. Their localization to one or both cells at the boundaries has not been determined unequivocally.*

role in CE appears to be permissive rather than instructive. In addition, graded anterior-posterior cues, such as activin and BMPs, are required for CE in *Xenopus* (Myers *et al.*, 2002, Ninomiya *et al.*, 2004). The exact mechanism underlying the involvement of graded anterior-posterior cues for CE is not clear.

Upon the formation of core PCP proteins on opposite sides of the cells along the axis for planar polarity, planar polarization across the tissue is achieved by coordinated morphological polarization. In *Drosophila*, three cytoskeleton-binding proteins, Inturned, Fuzzy and Fritz, function downstream of core PCP genes (Park *et al.*, 1996, Turner and Adler, 1998, Yun *et al.*, 1999, Adler *et al.*, 2004, Collier *et al.*, 2005). Mutations in Inturned, Fuzzy and Fritz cause the formation of multiple hairs at abnormal locations in wing cells (Adler *et al.*, 2004, Collier *et al.*, 2005), suggesting that these genes may function as downstream PCP mediators and link polarized core PCP complexes to the machinery that creates hairs in wing cells for coordinated planar polarization in all the cells across the wing. The generation of multiple hairs in these mutants also suggests that these downstream mediators directly regulate the formation of hairs by limiting the activity of the machinery that builds hairs in wing cells.

Two members of the Rho family of GTPase that are capable of modifying cytoskeletal components, RhoA and Rac, are implicated in the vertebrate PCP signaling downstream of a core PCP gene Dvl during CE in *Xenopus* (Habas *et al.*, 2001, Marlow *et al.*, 2002, Habas *et al.*, 2003, Phillips *et al.*, 2005). In addition, Inturned, Fuzzy and another cytoskeleton-binding protein Dub are required for CE in *Xenopus* and zebrafish (Oishi *et al.*, 2006, Park *et al.*, 2006). Notably, PCP signaling and Par complexes appear to be required only for polarizing lamellapodia protrusions mediolaterally (Wallingford *et al.*, 2000, Hyodo-Miura *et al.*, 2006). Disruptions in PCP signaling or Par complexes does not prevent lamellapodia protrusive activity, but rather results in random and unstable lamellapodia protrusions (Wallingford *et al.*, 2000, Hyodo-Miura *et al.*, 2006). Therefore, PCP signaling may guide only the coordinated polarization of cells across the tissue, but not the formation of the asymmetrical structure within individual cells during epithelial planar polarization nor the formation of lamellapodia during CE.

PCP pathway and morphogenesis of the organ of Corti

The establishment of PCP in the organ of Corti occurs concurrently with the extension of the cochlea involving cellular rearrangement characteristic of CE, raising the possibility that the mammalian PCP pathway may regulate both processes during terminal differentiation of the organ of Corti (Chen *et al.*, 2002).

Indeed, mice defective in core PCP genes, including Ltap/Vangl2, Dvl1/2, Fz3/6 and Celsr1, show various degrees of misorientation of stereocilia (Fig. 5) (Curtin *et al.*, 2003, Montcouquiol *et al.*, 2003, Wang *et al.*, 2005, Montcouquiol *et al.*, 2006, Wang *et al.*, 2006a, Wang *et al.*, 2006b). A widened and shortened organ of Corti is also reported in several of these PCP mutants (Fig. 5) (Montcouquiol *et al.*, 2003, Wang *et al.*, 2005, Wang *et al.*, 2006a). It was further demonstrated that the misorientation of stereocilia and the apparent CE defect are intrinsic to defective PCP signaling within the cochlea and not indirectly resulted from neural tube defects in these mutant animals (Wang *et al.,* 2005). These data support that the establishment of uniform orientation of stereocilia and CE of the cochlea are regulated and linked by the mammalian PCP pathway.

However, the process of CE and cellular behavior during terminal differentiation of the organ of Corti has not been demonstrated directly. Neither is it known whether the rearrangement of cells is an active process within the developing organ of Corti, or a passive process due to active cellular intercalation in surrounding epithelial and/or mesenchymal cells and whether the actin/myosin cytoskeletal component is required. It is worth noting that in *Math1*$^{-/-}$ animals where no hair cell differentiation and some degree of supporting cell differentiation are observed (Bermingham *et al.*, 1999, Chen *et al.*, 2002, Woods *et al.*, 2004, Matei *et al.*, 2005), the cochlea appears to have a normal length (unpublished observation). This data suggests that the differentiation of the full complement hair cells and supporting cells is not essential for the extension of the cochlea and its sensory organ. The surrounding epithelial and mesenchymal cells may be sufficient to provide the driving force for CE of the cochlea.

An intriguing question is how the mammalian PCP pathway concurrently regulates the establishment of PCP, manifested with uniform oriented stereocilia of hair cells and CE in the cochlea during terminal differentiation. The two processes occur concurrently and defects in both processes are associated in PCP mutants. However, the establishment of PCP in the organ of Corti and CE are not mutually dependent. In *Math1*$^{-/-}$ animals, no apparent PCP manifest, morphologically polarized hair cells and supporting cells, is observed in the cochlear epithelium, while the extension of the cochlea appears to be normal. The two processes may utilize overlapping signaling pathways but contain differential molecular and cellular components. Consistent with this view, several core PCP genes are expressed in the entire cochlear epithelium with higher levels at the region medial to the developing organ of Corti. Within the developing organ of Corti, Ltap/Vangl2, Dvl2 and Fz3/6 display polarized subcellular localization along the mediolateral axis of the cochlea (Wang *et al.*, 2005, Montcouquiol *et al.*, 2006, Wang *et al.*, 2006a, Wang *et al.*, 2006b). In the region medial to the organ of Corti, the subcellular localization of these core PCP proteins are also polarized, but apparently along the base-to-apex axis of the cochlea that is perpendicular to the mediolateral axis observed for PCP (data not published). In addition, the contribution of underlying mesenchymal cells of the cochlea to uniform orientation of stereocilia and cellular rearrangement within the developing organ of Corti has not been examined.

To orient stereocilia uniformly along the mediolateral axis of the cochlea, information to differentiate the medial vs. lateral direction must be presented during development. Similar to *Drosophila* PCP studies, Wnts have been investigated for their potential role in PCP signaling in the cochlea. Wnt7a is expressed in pillar cells and addition of Wnt antagonists and Wnt7a in the organ of Corti culture leads to misorientation of stereocilia *in vitro* (Dabdoub *et al.*, 2003). However, *Wnt7a*$^{-/-}$ animals do not have any apparent defects in PCP signaling (Dabdoub *et al.*, 2003), suggesting that pathways compensatory or independent of Wnts provide directional information for the organ of Corti during development. Indeed, the expression of another Wnt molecule, Wnt5a, is restricted to the region medial to the developing organ of Corti (Qian *et al.*, 2007). Genetic inactivation of Wnt5a leads to ~35% penetrance of a characteristic CE defect in the cochlea (Qian *et al.*, 2007). Furthermore, Wnt5a genetically interacts with

known PCP gene Ltap/Vangl2 in regulating the orientation of stereocilia, cochlear extension and neurulation (Qian *et al.*, 2007). Together these data indicate a role of Wnt5a in PCP signaling in mice. Intriguingly, a Wnt antagonist, secreted Frizzled-related protein 3 (Sfrp3 or Frzb), is expressed in a reciprocal pattern with Wnt5a in the developing organ of Corti (Qian *et al.*, 2007). However, it is not clear whether the reciprocal expression of Wnt5a and a Wnt antagonist is involved in generating a graded Wnt signal for an instructive role in PCP signaling.

Cell adhesion and PCP signaling in the organ of Corti

Ultimately, the affinity among constituent cells and the affinity between the cells and their extracellular matrix determine the cellular arrangement and consequently the morphology of a particular tissue or organ (Townes and Holtfreter, 1955, Nose *et al.*, 1988, Steinberg and Takeichi, 1994, McNeill, 2000, Zajac *et al.*, 2003). *In vitro*, differential cellular affinity can drive cellular aggregation and cellular rearrangement (Townes and Holtfreter, 1955, Nose *et al.*, 1988, Steinberg and Takeichi, 1994, McNeill, 2000, Zajac *et al.*, 2003, Strutt *et al.*, 2004, Saburi and McNeill, 2005). In *Drosophila*, atypical cadherins, Fat and Dachsous and protocadherin Flamingo play essential roles in initiating and propagating PCP signaling (Fanto *et al.*, 2003, Matakatsu and Blair, 2004, Simon, 2004, Strutt *et al.*, 2004). A mammalian homolog of Flamingo, Celsr1, is required for PCP signaling in the cochlea (Curtin *et al.*, 2003). However, the expression and function of atypical cadherins in PCP signaling in the cochlea has not been investigated. Furthermore, cellular packing geometry changes drastically during terminal differentiation of the organ of Corti (Fig. 3A). Cellular junctions must be remodeled during morphogenesis of the organ of Corti at the terminal differentiation stage. It is very likely that molecules mediating general cell-cell adhesion play important roles in PCP signaling and morpohogenesis of the organ of Corti. Investigation of cell adhesive activities and extracellular matrix properties in the cochlea will provide invaluable information to delineate the cellular and molecular components for PCP signaling in the cochlea.

PCP signaling and ciliogenesis in morphogenesis of the organ of Corti

Cilia consist of a microtubule-based axoneme tethered to the cell at the basal body and typically protrude from the apical surface of cells (Davis *et al.*, 2006). The basal body consists of a pair of centrioles and is the organization center for both cytoskeletal microtubules and ciliary microtubules. The axoneme in the ciliary portion consists of microtubule doublets either with (9+2) or without (9+0) a centrally located pair (Davis *et al.*, 2006). Protein synthesis does not occur in cilia. Cilia are assembled and maintained by intraflagellar transport (IFT), in which multimeric protein complexes called IFT particles are moved bidirectionally along the axomeme by the coordination of IFT motors, the anterograde (toward the plus ends of microtubules or the tip the cilium) molecular motor kinesin and the retrograde (toward the minus ends of microtubules) motor protein dynein along microtubules. The loss of IFT genes leads to ciliary defects.

The polarization of stereocilia in the hair cell is led by the polarity of the kinocilium, a primary cilium (Figs. 1 and 2). Kinocilia regress postnatally in the mammalian cochlea (Sobkowicz *et al.*, 1995, Leibovici *et al.*, 2005), implicating a developmental role. Recent studies revealed tantalizing links between cilia and PCP signaling (Ross *et al.*, 2005, Park *et al.*, 2006, Jones and Chen, 2007) and raised the possibility that PCP signaling is required for ciliogenesis and the possibility that cilia are involved in PCP signaling. These findings provided the first experimental indication that kinocilia may indeed have a developmental role in the cochlea.

Upon close examination, however, there are outstanding questions regarding the links between PCP signaling and ciliogenesis. Gene knockdown of PCP downstream effectors, Inturned and Fuzzy, in zebrafish causes a ciliogenesis defect (Park *et al.*, 2006), suggesting that PCP signaling may be required for ciliogenesis. However, the PCP genes that are required for ciliogenesis are limited to these downstream cytoskeletal effectors. Kinocilia and other primary cilia appear to be normal in core PCP mutants in studies reported by several laboratories, including ours (Jones and Chen, 2007). These data argue against a requirement for PCP signaling in ciliogenesis, but rather a dual role of cytoskeletal effectors, such as Inturned and Fuzzy, for both ciliogenesis and PCP.

Receptors and mediators for hedgehog (Hh) and platelet-derived growth factor receptor (PDGFR) signaling pathways have been localized to cilia for transducing both pathways (Corbit *et al.*, 2005, Haycraft *et al.*, 2005, Liu *et al.*, 2005, Schneider *et al.*, 2005). This newly defined role of cilia as a specialized subcellular compartment for localizing and concentrating membrane bound signal receptors and complexes to relay signals from the cilium to the cell interior led to speculations that cilia may have a similar function and transduce Wnt signals (Ross *et al.*, 2005, Simons *et al.*, 2005). However, the first study implicating a role of cilia in PCP signaling has raised some issues(Ross *et al.*, 2005). A PCP component, Ltap/Vangl2, was localized to the basal body and cilia in ciliated human respiratory epithelial cells from nasal brushing (Ross *et al.*, 2005). Several Bardet-Biedl Syndrome (BBS) genes encode basal body proteins and mutations in these BBS genes cause ciliary defects and minor defects in stereociliary patterning (Ross *et al.*, 2005). However, the stereociliary defect in BBS mutants is manifested with a low percentage of misshaped stereocilia, in contrast to misoriented stereocilia observed in PCP mutants. Ltap/Vangl2 or any other PCP proteins, such as Fz (Wnt receptors), have yet to be localized to the basal body or cilia in a tissue that exhibits PCP like the organ of Corti. Although the exact role of cilia or ciliary genes in PCP signaling and the morphogenesis of the organ of Corti remains obscure, the possibility for the involvement of cilia in PCP signaling is exciting and will be pursued vigorously by the general biological field.

Coalescing multiple signaling pathways in shaping the organ of Corti during terminal differentiation

Shortened and widened cochlear ducts and misorientation of stereociliary bundles are not limited to mouse mutants defective in PCP genes. In particular, genes for the Notch signaling are expressed in the developing cochlea during terminal differentiation and mutations in some of these genes result in certain characteristic PCP defects in the cochlea and its sensory organ (Kiernan *et al.*, 2005a, Brooker *et al.*, 2006). The role of the Notch pathway in sensory competency and in determining the fate of hair cells vs. supporting cells has been established (Daudet and

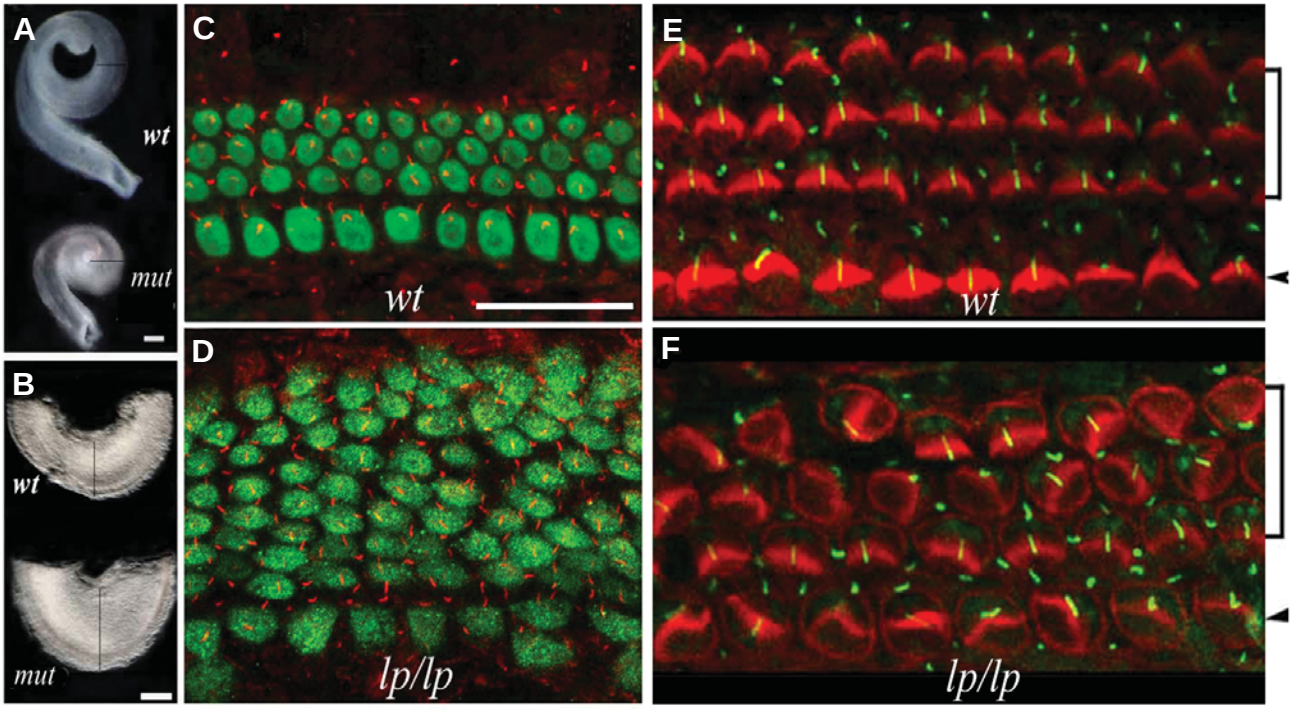

Fig. 5. Typical PCP phenotypes in the cochlea. *Defective PCP signaling leads to formation of a shorter* **(A)** *and wider* **(B)** *cochlea in mutant (mut) mice, in comparison to wild-type (wt) animals. The widening of the cochlea and its sensory organ is most prominent toward the apical region (compare C and D) by staining for a hair cell marker* **(C, D)**. *In addition, wild-type cochleae show a distinctive PCP, manifested with uniformly oriented stereocilia* **(E)** *while PCP mutant cochleae display misorientation of stereocilia* **(F)**. *A single primary cilium, the kinocilium (C-F), is seen on the apical surface of each hair cell. lp: the loop tail alleles, a loss-of-function allele for core PCP gene Vangl2 (Ltap). Brackets and arrowheads (E, F) indicate the outer and inner hair cells, respectively. This image is modified from Wang* et al. *(2005), Nature Genetics. Authors' Copyright.*

Lewis, 2005, Kiernan *et al.*, 2005a, Brooker *et al.*, 2006, Kiernan *et al.*, 2006). In the mice carrying mutations in two of the Notch ligands Jag2 and Dll1, however, misorientation of stereocilia is also apparent (Kiernan *et al.*, 2005a). Shortened cochlear ducts with increased rows of hair cells were observed toward the apical region of the cochlea in mice mutated for Dll1 (Kiernan *et al.*, 2005a, Brooker *et al.*, 2006). It has been hypothesized that misorientation of stereociliary bundles in Jag2 and Dll1 mutants may be an indirect result of defects in patterning hair cell and supporting cell mosaic (Kiernan *et al.*, 2005a). It has also been hypothesized that shortened and widened cochlear ducts in Dll1 mutants may be related to premature differentiation that shortens the period of growth and prevents the sensory primordium from elongating and narrowing normally (Brooker *et al.*, 2006). While the molecular mechanisms underlying PCP-like defects in Notch pathway mutants are not determined, these data indicated a tight coupling of cellular differentiation and patterning with PCP signaling. It is possible that the specification and differentiation of hair cells and/or supporting cells produces specific surface or membrane components that are necessary for cell-cell communication during PCP signaling for establishing uniform orientation of stereocilia across the organ of Corti, and/or for mediolaterally polarized cellular protrusive activity to drive CE of the cochlea.

The development of spiral ganglion neurons also appears to influence the morphogenesis of the organ of Corti. In studies looking at the role of neurogenin1 in making afferent connections to inner hair cells, it has been found that not only do afferent connections have strong influence on the proper connections and autonomic innervations, but it also seems to have direct role in the development of the sensory epithelium (Ma *et al.*, 2000). Although hair cells seem to differentiate normally in the absence of afferent innervation in Ngn1 null animals, other aspect of the phenotype, in striking resemblance of PCP defects (Ma *et al.*, 2000), strongly implicates a role for neuronal influence on organ of Corti morphogenesis. The organ of Corti is shortened and expanded along the mediolateral axis in the apical region (Ma *et al.*, 2000). Misorientation of stereocilia is also observed in the apical region of the cochlea (Ma *et al.*, 2000).

In addition to the Notch pathway and neural influence, Hh, transforming growth factor-β (TGF-β) and FGF signaling may also contribute to PCP processes in the cochlea. In *Drosophila* embryos, Hh and Wnt molecules function together to orient the denticles, actin-based cell projections, on segmentally repeated subsets of ventral epidermal cells (Colosimo and Tolwinski, 2006, Price *et al.*, 2006). The role of Hh in terminal differentiation of the organ of Corti is being actively investigated by several laboratories (Riccomagno *et al.*, 2002, Riccomagno *et al.*, 2005) and will help illustrate the complex upstream signaling molecules in PCP signaling. During gastrulation in *Xenopus*, anterior-posterior cues (along the extension axis), such as activin, a member of the TGF-β family, are required for convergent extension (Ninomiya *et al.*, 2004). Interestingly, BMP4, one member of the TGF-β family, is expressed in an asymmetric manner in the cochlear epithelium along the longitudinal (extension axis) and mediolateral axes of the cochlea (Morsli *et al.*, 1998). Multiple FGF ligands and receptors/mediators are also expressed in the cochlea during

terminal differentiation (Pirvola *et al.*, 2000, Mueller *et al.*, 2002, Shim *et al.*, 2005, Fritzsch *et al.*, 2006). The role of Hh, BMP and FGF signaling in early patterning and differentiation has been well characterized. However, whether they also directly regulate PCP processes, the uniform orientation of stereocilia and the presumptive CE, in the cochlea remains uncharted.

A working model of PCP regulation in the cochlea and perspectives

The organ of Corti manifests perhaps the most distinctive form of planar cell polarity in vertebrates. It has emerged as a model system to illustrate the underlying mechanisms of the PCP pathway that is essential for development and function of multicellular organisms.

Based on current understanding of PCP signaling and mutations that affect uniform orientation of stereocilia and extension of the cochlea, we propose a working model that coalesces multiple signaling pathways for PCP regulation in the cochlea (Fig. 4). Upon upstream directional cues which may consist of both morphogens and proteins that mediate cell-cell adhesions, the core PCP complexes are sorted asymmetrically along the mediolateral axis of the cochlea. Cell-cell communications reinforce the sorting of core PCP proteins and the alignment of neighboring cells. Notably, all the known mice defective in core PCP genes maintain "V"-shaped stereocilia. Although it is possible that there is residual PCP signaling in all these known PCP mutants sufficient for the formation of "V"-shaped stereocilia, these observations point to a mechanism in which the formation of "V"-shaped stereocilia is independent of PCP signaling. Therefore, we propose that cellular mediators link polarized core PCP complexes to the machinery that directs the formation of "V"-shaped stereocilia in individual hair cells and align uniformly the vertices of the "V"-shaped stereocilia of all the hair cells along the mediolateral axis of the cochlea, displaying PCP across the entire organ of Corti.

Many questions remain in PCP signaling in the cochlea. The nature of upstream directional cues remains elusive. Wnt, Hh, BMPs, FGFs, Fat-Dachsous interaction, as well as neural influence may set up the initial polarity of the core PCP complexes. Similar to PCP regulation in *Drosophila*, core PCP complexes are also polarized along the axis for PCP in the mammalian cochlea. However, the detailed molecular interaction among core PCP proteins may deviate from that of the well-characterized *Drosophila* core PCP proteins. The identity of the putative cellular mediators that link polarized core PCP complexes to the machinery for the formation of "V"-shaped stereocilia and how these putative cellular mediators communicate with core PCP proteins and components of the stereocilia are not yet unknown. The apparent involvement of cilia and/or basal body in PCP signaling opened new directions for seeking the mechanisms underlying cochlea morphogenesis. It is tempting to hypothesize that cilia may function as a specialized apparatus for directional cues for PCP processes in the cochlea and that basal body as a microtubule organization center may function in sorting of core PCP complexes, and/or linking polarized core PCP complexes to stereocilia to coordinate their uniform orientation across the organ of Corti. The adhesive properties of the cells in the cochlea will also be critical for the understanding of the cellular and molecular components driving cellular rearrangements that shape the extended cochlear and its sensory organ. Research toward these directions is exciting and will not only advance our understanding of the underlying mechanism for the morphogenesis of the cochlea but also address fundamental issues in biology.

Acknowledgements

We thank Sharayne Mark and Kristen Radde-Gallwitz for their contribution to inner ear paint-fill, immunostaining and in situ *hybridization images; Dalian Ding for helpful comments on inner ear structure. This work is supported by grants from the US National Institute of Health (to P.C.) and a predoctoral training grant from US National Institute of Health (to M.K.).*

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Published Online: 27th August 2007