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Line up and listen: planar cell polarity regulation in the mammalian inner ear

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Abstract

The inner ear sensory organs possess extraordinary structural features necessary to conduct mechanosensory transduction for hearing and balance. Their structural beauty has fascinated scientists since the dawn of modern science and ensured a rigorous pursuit of the understanding of mechanotransduction. Sensory cells of the inner ear display unique structural features that underlie their mechanosensitivity and resolution, and represent perhaps the most distinctive form of a type of cellular polarity, known as planar cell polarity (PCP). Until recently, however, it was not known how the precise PCP of the inner ear sensory organs was achieved during development. Here, we review the PCP of the inner ear and recent advances in the quest for an understanding of its formation.

Keywords

planar cell polarity; cochlea; vestibule; hair cell; cilia

1. Introduction

Fields across which cells are oriented with stunning precision are a recurring theme in biology and have riveted scientists for centuries. To understand the biological mechanism that generates the regular alignment of cuticular hairs and bristles in *Drosophila melanogaster*, Gubb and Garcia-Bellido exploited the power of fruitfly genetics and identified a small set of genes that constitute conserved components of what is now termed the planar cell polarity (PCP) pathway, which governs the genesis of this reiterative pattern of uniformly oriented structures [1]. Planar cell polarity refers to the coordinated orientation of cells in the two dimensional plane of a cell sheet. It is now well known that the PCP pathway operates in both invertebrates and vertebrates, orchestrating complex tissue movements and patterning events in different types of tissues during development. During evolution, intrinsic differences in morphogenetic processes between invertebrates and vertebrates and among different types of tissues have led to variations in the ways in which the PCP pathway has been deployed. However, in many systems showing PCP features, PCP signaling functions as a crucial biological switch-board integrating long range signals with local ones to precisely orient diverse sub-cellular structures or entire cell populations along a specific axis. Among the finest model systems in which to study the

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exquisite capacities and detailed mechanics of the PCP signaling pathway in vertebrates are the sensory epithelia of the inner ear. This review will therefore focus on the role of PCP signaling in the development of the fine cellular architecture of the mammalian inner ear-the basis of its extraordinary operational capabilities for hearing and balance.

2. PCP of the inner ear

2.1. The inner ear-a marvel in miniature

The mammalian inner ear detects and processes both auditory and positional information over a considerable range with remarkable sensitivity and resolution. It contains precisely organized fields of mechanosensory hair cells, intervening supporting cells and neurons that are functionally arranged in fluid-filled chambers to produce three types of highly specialized sensory epithelia (Fig. 1A): (a) the organ of Corti that detects auditory signals; (b) the maculae of the utricle and the saccule that detect linear acceleration in the horizontal and vertical planes respectively; (c) the cristae of the semicircular canals that sense angular acceleration.

Despite the differences in the appearance of the inner ear sensory organs, they all comprise spatially organized ensembles of sensory cells each sensitive to different modalities of mechanical stimuli. Strikingly, they all share a common feature and exhibit perhaps the most distinctive forms of vertebrate planar cell polarity (PCP), which are described in greater detail in the following sections. These epithelia have now become established model systems to elucidate the molecular mechanisms responsible for the generation of planar polarity in vertebrates.

2.2. PCP in the cochlea

The sensory organ within the mammalian cochlea is the organ of Corti that runs along the entire length of the snail-shaped cochlea. Typically, the mammalian organ of Corti comprises three rows of outer hair cells (OHCs), a single row of inner hair cells (IHCs) and several types of interdigitating non-sensory supporting cells (Fig. 1B). The IHCs are located towards the center of the cochlear spiral and are described as being “medial”. In contrast, the OHCs are on the outer periphery of the cochlear spiral, away from the center, and are hence described as “lateral”. Each hair cell in the organ of Corti has on its luminal surface a set of actin-rich stereocilia to form a hair bundle. The stereocilia function by pivoting at their bases in response to sound stimuli. Stereocilia are graded in height and are arranged in multiple tightly-packed rows with the shorter rows positioned closer to the center of the apical surface. The stereocilia form a bilaterally symmetrical “V”-shaped descending staircase (Fig. 1B, C). The cochlear hair bundle also transiently contains a single primary cilium, the kinocilium, which is eccentrically positioned near the tallest stereocilia at the vertex of the “V”-shaped hair bundle and is physically linked to adjacent stereocilia. Individual hair cells are therefore intrinsically polarized. Furthermore, all the sensory hair cells show precise and identical orientations of their “V”-shaped hair bundles with the vertices aligned in the medial-to-lateral direction (planar polarity axis), producing an ordered array of sensors endowed with directional sensitivity to mechanical stimulation (Fig. 1C, lower panel).

The elaborate patterning and intricate polarity of the hair bundle are morphogenetic readouts of a very complex developmental program that controls hair cell maturation. Terminal differentiation of the organ of Corti begins near the base of the cochlea, and extends toward the apex. Concurrently, another maturational wave advances from the medial to the lateral side of the epithelium. The formation of the highly polarized stereociliary bundles follows the differentiation gradients of hair cells. Initially, a single primary cilium is present on all epithelial cells in the center of the apical surface and is surrounded by microvilli of uniform height. Subsequently, this primary cilium, known as the kinocilium on hair cells, begins to migrate

towards the lateral side of the apical surface, and thus becomes asymmetrically positioned. This movement is concomitant with a graded enlargement of microvilli. In the cochlea, this process results in the production of descending stereociliary staircases with a defined “V”-shaped orientation. The expansion of the cuticular plate, which comprises a dense network of cross-linked actin filaments and associated proteins, and anchorage of core filaments from the stereocilia in the actin meshwork of the apical cytoplasm, proceed concurrently with the maturation of stereocilia on the luminal surface. The basal body that nucleates and lies at the base of the kinocilium is located just below the apical plasma membrane in the fonticulus, an actin-free hole in the cuticular plate. The kinocilia in the cochlear hair cells are lost prior to the onset of hearing.

The PCP of the cochlea also manifests in another form, known as convergent extension (CE). CE originally described a process in which the elongation of the body axis occurs by narrowing of mesodermal and neuroectodermal tissues along one axis and a concomitant lengthening of the body axis in a perpendicular direction during gastrulation and neurulation [2-4]. The cells in the field become highly polarized along the mediolateral axis during CE, manifesting a fundamental process of PCP. A vast body of evidence has now shown that the PCP signaling pathway governs both CE and the coordinated orientation of epithelial cells. The development of epithelial PCP in the organ of Corti occurs concomitantly with cellular rearrangements characteristic of CE. The mature organ of Corti is elongated from a shorter and thicker primordium. It is noteworthy that, although there is substantial circumstantial evidence in favor of a CE-like process mediating cochlear extension, cochlear CE has not been formally demonstrated.

2.3. PCP in the vestibular system

The vestibular sensory organs also exhibit an alternating arrangement of sensory hair cells and supporting cells with each hair cell being surrounded by a rosette of supporting cells (Fig. 1D, E, F, G). In addition, the sensory hair cells of the vestibule also show distinct intrinsic polarity, and all of the hair cells in each of the vestibular sensory organs are coordinately oriented. The orientation of vestibular hair cells is similarly manifested by the polarity of the stereociliary bundle and the location of the kinocilium, although the shape of hair bundles is different. The hair bundles in the cristae (Fig. 1D, E) show uniform alignment towards one side of the epithelia. In the macular sensory organs of the utricle (Fig. 1F, G) and saccule, the stereociliary bundles are coordinately oriented along the mediolateral axis of the epithelia. Strikingly, the hair cells on either side of a line of polarity reversal in the macular sensory organs have opposite orientations. In the utricle (Fig. 1F, G) and the saccule, hair cells are all oriented toward or away from, respectively, the corresponding line of polarity reversal.

3. The vertebrate PCP signaling pathway

The generation of planar polarity in any tissue presents two important challenges: first, to polarize individual cells in the field and second, to ensure that all the cells in the field are aligned along the correct axis and perfectly with respect to each other. Based on data accrued over the last decade from several model systems, it has become amply clear that conceptually, PCP signaling comprises at least three regulatory modules to accomplish coordinated alignment of all the cells in a given field: (1) directional cues for the cell field, (2) cellular factors that read and interpret spatial information and establish the primary PCP axis and (3) tissue-specific downstream effectors that impinge on the cytoskeletal apparatus and transform individual cells in the field into intrinsically polarized units in coordination with neighboring cells [5,6]. Emerging data in the mammalian inner ear have contributed valuable insights toward the understanding of vertebrate PCP signaling and tissue morphogenesis.

3.1. Establishment of the planar polarization axis in the inner ear sensory epithelia

A critical requirement for PCP is the establishment of a planar axis along which cells direct their polarity across the tissue. A set of evolutionarily conserved genes, known as core PCP genes (Figs. 2 and 3), that play a vital role in the planar polarity of all tissues with PCP features, appears to fulfill such a role. Among the “core” PCP genes are: Frizzled (Fz), Van Gogh/Strabismus (Vang), Disheveled (Dsh/Dvl), Flamingo (Fmi), Diego/Dgo, and Prickle (Pk) [5, 7]. In *Drosophila*, these core PCP proteins sort asymmetrically into specific membrane-bound signaling complexes at opposing sides of the cell to specify the axis of planar polarity. In the mammalian inner ear, studies have shown that mouse homologs of core PCP proteins, Dvl2 (Fig. 2F) and Dvl3, Vang-like 2 or looptail-associated protein (Vangl2/Ltap) (Fig. 2E), and Fz3 (Fig. 2E) and Fz6, show asymmetric membrane localizations in the organ of Corti [8–11]. The asymmetric localization of Vangl2 is observed prior to the morphological polarization of cells in the organ of Corti, and manifests a planar axis along which PCP of the organ of Corti develops subsequently [12]. These observations support a conserved role for polarized core PCP proteins in establishing the axis for PCP in vertebrates.

Loss-of-function in mouse core PCP genes causes the characteristic phenotype of stereociliary bundle misorientation (Fig. 2C) and is associated with loss of polarized membrane localization of core PCP proteins (Fig. 2G), consistent with a role for polarized core PCP proteins in establishing the axis for PCP [11, 13]. Many of the core PCP gene mutations are also associated with a shortened and widened cochlear duct (Fig. 2A) and open neural tube phenotypes, presumably resulting from defective CE during cochlear extension and neurulation, respectively [9, 14, 15]. These data strongly support the notion that PCP signaling not only governs the uniform alignment of hair bundles but also links it to the physical process underlying cochlear extension. Studies of PCP signaling in the inner ear also revealed two novel vertebrate core PCP components, Scribble 1 (Scrib1) [14] and Protein Tyrosine Kinase 7 (Ptk7) [16]. Interestingly, mutations in core PCP genes do not appear to affect the actual polarized structure of the individual stereociliary bundles (Fig. 2C). The hair bundles in core PCP mouse mutants retain the polarized “V”-shape, and the kinocilium remains at the vertex of the hair bundle. Although it is possible that gene redundancy in mammals may have masked an essential role for core PCP genes in the formation of stereociliary bundles, the stark contrast between their effect on cellular polarity coordination and on intrinsic cellular polarity suggested that core PCP genes are required for cell-cell communication to establish coordinated polarity among neighboring cells but may be dispensable for hair cell intrinsic polarity.

Consistently, emerging data from inner ear studies suggest that the polarity of the core PCP complexes does not always indicate the polarity of individual cells. In the utricle of the vestibule, a mosaic expression of a mouse homolog of *Drosophila* core PCP protein Pk, Pk2, and Fz6 revealed the localization of Pk2 and Fz6 relative to the polarity of each hair cell. Hair cells have opposite polarity across a line of polarity reversal in the utricle (Fig. 1). However, Pk2 is preferentially localized on the same (medial) side in hair cells across the entire epithelium [17]. Similarly, Fz6 localizes to the same (lateral) side in cells with opposite polarity on either side of the line of reversal [17]. These observations support the notion that core PCP protein complexes do not determine the cell's intrinsic polarity but function as a means to achieve tissue-level coordination of cell alignment.

How are core PCP proteins sorted across the tissue to establish a polarity axis in vertebrates? Based on the observed intercellular interaction between Fz and Vang, as well as selective association of other core PCP proteins with either Fz or Vang, one of the current models posits that Fz activity is biased within a cell in response to long-range directional cues, such that it is slightly higher on one side of the cell. The model proposes that, in *Drosophila*, this initial differential Fz activity is reinforced through feedback regulation and propagated through the tissue via the intercellular interaction of Fz with Vang, resulting in a polarized distribution of

Fz-associated and Vang-associated signaling complexes of core PCP proteins on opposing faces of each cell across the tissue [18-21]. A similar mechanism may operate in vertebrates to direct coordinated subcellular polarization of core PCP proteins in all cells across the tissue. The detailed molecular interactions between vertebrate PCP proteins and the mechanisms underlying their asymmetric sorting, however, are yet to be delineated.

An additional gradient mechanism has also been proposed to operate in *Drosophila* to orient PCP axes. It has been proposed that a morphogen gradient (such as Hh or Wg) organizes a signaling module comprising two atypical cadherins, Dachous (Ds) and Fat (Ft), and a type II Golgi-localized membrane protein, Four-joined (Fj) to set up activity gradients of the Ds/Ft/Fj module across the tissue. Based on clonal gain- and loss-of-function studies of members of the Ds/Ft/Fj module in wild type or *fz*-backgrounds in the dorsal abdomen of *Drosophila*, it is proposed that the Ds/Ft/Fj module can operate independent of Fz to orient cells [6]. However, epistasis experiments in the *Drosophila* eye suggest that the Ds/Ft/Fj module acts to provide a global directional cue and functions to bias Fz activity within individual cells, feeding into the Fz-dependent signaling system [21]. The first indication that the Ds/Ft/Fj module is involved in PCP regulation in vertebrates came from a recent study that showed that the mammalian homolog of Fat, Fat4, functions in several processes regulated by PCP genes [22]. However, a more complete understanding of the exact role for Fat4 or other components of the Ds/Ft/Fj system in vertebrate PCP signaling awaits further investigation.

In summary, the vertebrate PCP signaling pathway shares striking commonality with the *Drosophila* pathway in establishing the planar axis for PCP in that the asymmetric sorting of core PCP proteins is key to the process of planar cell polarization in both invertebrates and vertebrates. The vertebrate PCP signaling pathway appears to involve additional core PCP genes and may utilize similar mechanisms involving different molecular interactions for the initiation and propagation of directional information across the tissue.

3.2. Signaling molecules in PCP regulation in the inner ear

The identity and source of spatial information that allows cells to generate the initial differential activity of core PCP proteins and to discriminate the medial from the lateral regions of the sensory epithelia are yet to be conclusively determined. Amongst the best candidates for molecules that might impart this information to the cells, presumably in the form of a gradient that initiates PCP signaling, are members of the highly conserved Wingless (Wg)/Wnt family [23,24]. Wnts bind to Fz receptors and coreceptors at the cell surface, initiating downstream activities. Because Fz has been unequivocally implicated in PCP signaling, Wnts have been extensively tested for their involvement in PCP regulation. In zebrafish, Wnt11/Silberblick and Wnt5/Pipetail activity have been shown to be required for cells to undergo correct CE movements during gastrulation [25,26]. In *Xenopus* too, Wnt5 and Wnt11 have been implicated in the regulation of CE [27,28], but again, their role, as in the zebrafish system, appears to be permissive rather than instructive.

In mice, although knockouts of several individual Wnt genes have not yielded any stark ear phenotype, one might attribute this to redundancy. Many Wnts are known to be expressed in the developing mouse cochlea [29], suggesting considerable involvement of Wnt signaling in cochlear development. In particular, Wnt5a displays a reciprocal expression pattern with a Wnt antagonist *Frzb* along the axis of planar polarization [30]. Wnt5a antagonizes *Frzb* in regulating cochlear extension and stereociliary bundle orientation in vitro. Furthermore, Wnt5a knockout mice have shortened, widened cochleae and show minor imperfections in hair bundle alignment. Furthermore, Wnt5a interacts genetically with the core PCP protein *Ltap/Vangl2* to regulate uniform orientation of stereocilia, cochlear extension and neural tube closure [30]. Another Wnt family member, Wnt7a is highly expressed at the time of stereociliary bundle orientation [29]. OHC stereociliary bundles in cochlear explants showed misorientation when

maintained in medium conditioned by Wnt7a-expressing cells. Although these data taken together substantially affirm the involvement of Wnts in PCP regulation in the inner ear, direct or conclusive evidence that implicates a Wnt gradient in directing hair bundle alignment in the organ of Corti is still lacking.

It is noteworthy that other signaling molecules may operate in PCP signaling in the ear as well. Hh signaling is apparently involved in orienting the denticles on the epidermis of *Drosophila* embryos [31,32]. Hh signaling appears to control cell polarity in the neuroepithelium, at least in part by means of the PCP pathway during neural keel morphogenesis in zebrafish [33]. BMPs might also contribute to PCP regulation in the cochlea, as BMPs are expressed asymmetrically along the mediolateral axis of the cochlear epithelium [34,35] and a recent study [36] revealed the regulation of graded expression and localization of Fat, Fj, and Ds by *Drosophila* BMP (Dpp). A similar action by BMP on Fat may also exist in the vertebrates. Furthermore, FGFs and their receptors show conspicuous expressions that are sometimes asymmetric along the future PCP axis of the cochlea early in development. They have been implicated in a CE process during the formation of the primitive streak in chick embryos [37], and may harbor a similar role in PCP signaling in the cochlea. The generation of genetic tools to alter the effective levels of these signaling molecules will be critical to understand better the molecular roles of these genes in PCP regulation.

3.3. Primary cilia in determination of the intrinsic polarity of hair bundles

The epithelial cells of both the auditory and vestibular sensory organs carry on their apical surface, a single microtubule-rich primary cilium (kinocilium) nucleated by an apically localized mother centriole or basal body. The generation and maintenance of primary cilia depends on intraflagellar transport (IFT) protein complexes. It has long been suspected that kinocilia somehow lead and direct the polarization of hair bundles. Supporting lines of evidence for this assertion were primarily the following: (1) the intrinsic polarity of a hair bundle is distinctly marked by the position of the kinocilium, at the vertex of the “V”-shaped stereociliary bundle; (2) the polarization of the kinocilium precedes the polarization of the stereociliary bundle during hair cell development; and (3) the transient presence of kinocilia suggests a developmental role.

The first genetic evidence that hinted of a relationship between primary cilia and PCP regulation in the development of the mammalian ear came from *bbs* mouse mutants [38]. Many of the genes implicated in the Bardet-Biedl Syndrome (BBS) have been linked to cilia and/or basal body assembly and function. *Bbs*-deficient mice showed abnormal morphology of their stereociliary bundles with the kinocilia showing an apparent loss of their close association with the stereocilia. Mice that were simultaneously mutated for the core PCP gene *Vangl2* and the *bbs* genes showed an exacerbated cochlear phenotype. More recent research further unequivocally implicated kinocilia in PCP signaling [12]. In *Ift88* ciliary mutants, the organ of Corti is shorter and wider, and cochlear hair cells were misoriented, even though the asymmetric localization of core PCP proteins was unaffected [12]. In addition, circular stereociliary bundles were present and any remaining kinocilia were often shorter and no longer tightly associated with the tallest stereocilia in *Ift88* mutants [12], indicating a perturbation in the cell-intrinsic polarity of these hair cells (Fig. 2). In contrast, in core PCP mutants, no aberrations have been reported so far in the polarized morphology of the hair bundles. Combined with the fact that core PCP proteins are partitioned normally in the *Ift88* mutant mice (Fig. 2H, I), these observations suggest that cell-cell communication is normal in the *Ift88* mutant mice, but there is a failure in responding to the signal that emerges from the asymmetrically partitioned core PCP proteins.

The hair bundle misorientation and cochlear extension phenotypes were both significantly worsened by combining the *Ift88*^{CKO} and *Vangl2*^{Lp} mutations, providing compelling evidence

in favor of a genetic interaction between the ciliogenic and PCP pathways. The molecular nature of the genetic interaction between core PCP proteins and the ciliogenic pathway, however, is not clear. Nevertheless, studies in *Xenopus* and in cultured cells [39-41] indicated a direct link between the actin- and microtubule cytoskeletal networks and ciliogenesis. Findings from cilia studies further implicate the basal body of the primary cilium as a key player in intrinsic polarity determination during PCP signaling. The pair of centrioles was found to align along the axis of planar polarity in the cochlear hair cells. In *Ift88* mutant mice, the hair bundles were consistently oriented in the general direction of the (mis-positioned) basal bodies, strongly suggesting that (i) IFT88/cilia function is essential for positioning the basal body and (ii) the position of the basal body somehow plays a cell-intrinsic decisive role in determining hair bundle orientation.

The tantalizing idea that the basal body may somehow be involved in reading the asymmetric distribution patterns of core PCP proteins and relaying it to the cytoskeleton to organize and direct morphogenesis of the hair bundle in a cell-context dependent manner, is rapidly gaining credence. A good starting point for exploring the relationship between the kinocilium and hair bundle morphogenesis could lie in the gamut of physical and biochemical links that connect the two. Genetic studies of mouse models of Usher Syndrome (USH), the most frequent cause of deaf-blindness in humans, have led to the identification of part of the cellular machinery responsible for sculpting the stereociliary bundle in hair cells [42]. Mice that carry mutations in USH genes display very obvious defects in their stereociliary bundles that share a striking resemblance to phenotypes associated with ciliary mutants [43]. The USH mutant mice possess clusters of stereocilia, circularly-shaped stereocilia, and displacement of the kinocilium from the stereociliary bundle. Given the important role of USH proteins in the actin cytoskeleton for stereociliary bundle formation, and the role of the basal body in the microtubule cytoskeleton that physically co-exists and directly interacts with the actin cytoskeletal networks, it is tempting to speculate that the basal body serves as an organizing center to orient cellular and ciliary microtubules and provides a framework to coordinate the activities of USH proteins for the morphogenesis of the polarized hair bundle. USH proteins, in turn, may interact with polarized core PCP protein complexes and feed back the directional information to the basal body to coordinate the polarity of cells across the organ of Corti. However, molecular data in support of such a model that involves mutual regulation between USH proteins and core PCP proteins and between USH proteins and the basal body is still awaited.

3.4. Specification of PCP signaling for downstream cytoskeletal reorganization

PCP signaling is now known to share many components in common with canonical Wnt signaling, in which the binding of Wnt to Fz receptor and coreceptors results in stabilization and translocation of β -catenin to the nucleus for transcriptional regulation. But unlike the canonical Wnt signaling pathway, PCP signaling directs downstream cytoskeletal rearrangements, thus earning the epithet “non-canonical” Wnt pathway [5]. The downstream effectors identified in *Xenopus* and cultured cells include well-known regulators of the cytoskeleton, cell polarity, and protrusive activity, such as Rac, RhoA, and Cdc42, JNK/SAPK-like kinases, Daam1 [44, 45]. In addition, Inturned, Fuzzy and Dub have also been shown to act as downstream PCP effectors required for CE in *Xenopus* and zebrafish [46, 47]. However, the functions of these proteins have not been tested in the inner ear.

The sharing of common molecular components with the canonical Wnt signaling pathway poses a challenge for the PCP signaling pathway in selectively activating the subset of downstream genes specified to regulate cytoskeletal polarization. Recent studies have revealed a recurring scheme in which the canonical Wnt signaling is inhibited at multiple steps during PCP signaling to ensure the specificity of downstream activity (Fig. 3). First, distinct Wnt ligands and different receptor combinations activate PCP signaling. Secondly, certain core PCP components show

an inhibitory activity toward canonical Wnt signaling. Finally, the primary cilium and the basal body are also being recognized as a signaling platform serving as a switch that represses β -catenin-dependent canonical Wnt signaling, while promoting PCP signaling [48–51]. These mechanisms may collaborate to ensure the progression of PCP signaling in the ear.

3.5. Cell-cell adhesion and PCP signaling

During CE, cells change their relative positions to converge and extend along two perpendicular axes. Such a cellular sorting process requires dynamic remodeling of cell-cell contacts. Not surprisingly, there is growing evidence implicating a link between cell adhesion and the PCP signaling pathway that regulates CE.

Cadherins are key players for cell adhesion and are crucially involved in regulating vertebrate gastrulation. Furthermore, the involvement of PCP signaling pathway in CE is likely to be mediated, in part, by directly regulating cell adhesion. In zebrafish, Wnt11-mediated E-cadherin endocytosis and recycling controls cohesion of prechordal plate progenitors required for directed and coherent cell migration [52]. Wnt11 also functions locally at the plasma membrane by accumulating its receptor Frizzled 7 (Fz7) on adjacent sites of cell contact [53]. Fz7 in turn, recruits its intracellular mediator Dsh at the site of cell contact. The atypical cadherin Flamingo (Fmi) also co-localizes with Fz7 at these sites causing increased cell-cell contact persistence due to the combined local interactions of Wnt11, Fz7 and Fmi at the contact membrane [53]. This polarized sorting of core PCP proteins and modulation of cell-cell contacts is postulated to coordinate cell movements during gastrulation.

A reciprocal role for cell adhesion in PCP signaling, however, is not determined. In the fly, the wing epithelium is repacked into a quasihexagonal array from an epithelium consisting of irregularly shaped cells shortly before hair formation. Cellular junctions undergo extensive remodeling during this repacking process. Similar to the role of PCP genes in regulating cellular junctions during vertebrate CE, PCP genes regulate cadherin recycling and are required for the packing of hexagonal arrays of epithelial cells in the fly wing [54]. However, irregular packing in mutant wings disrupted for cadherin recycling or stabilization apparently does not associate with hair polarity defects [54]. By analogy, it is possible that in the differentiating cochlea, the vertebrate PCP signaling pathway regulates independently the presumptive CE and the establishment of uniform orientation. Although CE defects and uniform hair bundle orientation defects go hand-in-hand in PCP mutants, the defects in CE may be primarily due to a defect in remodeling cell junctions while the defect in coordinating hair bundle orientation across the tissue apparently results from a failure in core PCP protein-mediated cell-cell communication.

4. Conclusions

The distinct and coordinated cellular polarity in the inner ear sensory organs (Fig. 1) offers an excellent paradigm for cellular and molecular mechanisms in vertebrate PCP signaling. Together with findings from other model systems, the study of inner ear PCP revealed a potentially conserved mechanism operating in the vertebrates to regulate the formation of reiterative pattern of polarized structures in cells across a tissue field. In particular, several genes conserved from *Drosophila*, including homologs of core PCP genes Vang, Fz, Fmi, Dvl, Diego, Pk, are also required for planar polarization in the vertebrates (Fig. 3). In addition, the vertebrate PCP genes may have also retained their function in regulating cell adhesion. Such a conserved function for PCP genes could underlie their evolved essential role in the CE process in vertebrates.

The vertebrate PCP pathway, predictably, has also evolved novel mechanisms to comply with special requirements under different cellular and tissue contexts. Scrb1 and PTK7 are novel core PCP genes in vertebrates, vertebrate Wnts may indeed be bona fide PCP genes, and the

primary cilia are identified as a unique component of the vertebrate PCP signaling pathway (Fig. 3). These new members of the vertebrate PCP pathway provide insights into the mechanisms operating in the vertebrates. For instance, the primary cilia are shown to be essential for intrinsic cellular polarity in the inner ear sensory cells during PCP signaling, and the associated structure of the primary cilia, the basal body, is likely a key component of the vertebrate PCP signaling pathway that collaborates with core PCP complexes to regulate morphological polarization (Fig. 3). The requirement for Wnts in PCP-regulated cellular processes in vertebrates is met with mechanisms, including coreceptors of Wnts, dual functions of PCP genes, and the primary cilia and basal body, to suppress the β -catenin-mediated canonical Wnt signaling for specification of PCP signaling (Fig. 3).

The emerging model of vertebrate PCP signaling (Fig. 3) has evoked several key questions. The identity and action of directional cues are currently unknown; the interactions among core PCP proteins are yet to be delineated; the link between core PCP proteins and the primary cilia or the basal body is missing; the activation of downstream pathways to impact the cytoskeletal machinery for changes in cell shape and formation of polarized structures is not understood; and the mechanism underlying the role of PCP signaling in CE is not clear. The combination of advancement in research tools and further understanding of the morphogenesis of various inner ear sensory organs will undoubtedly start to address some of these issues.

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Abbreviations

PCP	Planar cell polarity
CE	Convergent extension
AJ	Adherens junction
IHC	Inner hair cell
OHC	Outer hair cell
IPH	Inner phalangeal cell
IPC	Inner pillar cell
OPC	Outer pillar cell
DC	Deiters' cell
IFT	Intraflagellar transport

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Fig. 1. Planar cell polarity in the sensory epithelia of the inner ear

(A) The inner ear consists of 6 sensory epithelia marked by the expression of GFP: the organ of Corti of the cochlea (CO), the maculae of the saccule (SA) and utricle (UT), and the three orthogonally positioned cristae: the anterior crista (AC), posterior crista (PC) and lateral crista (LC). The white tracing outlines the fluid-filled labyrinth of the inner ear. (B-G) Schematic diagrams (B, D, F) and confocal images (C, E, G) illustrating the planar polarity of the organ of Corti (B-C), LC (D-E), and Ut (F-G). In the organ of Corti (B), the inner (IHCs) and outer hair cells (OHCs) are interdigitated with several distinct types of nonsensory supporting cells: the inner phalangeal cells (IPHs), inner pillar cells (IPCs), outer pillar cells (OPCs) and three rows of Deiters' cells (DC1-DC3s). The kinocilia (BC, blue) and stereocilia (B, purple, C,

green) constitute a “V”-shaped structure and all the “V”s are uniformly aligned, showing a distinctive PCP. In LC (E), Spectrin (red) accumulates in the cuticular plates of the hair cells but is conspicuously excluded from the fonticulus within which the basal body resides, providing a convenient marker for hair cell polarity. The hair bundles and cell membranes are visualized by staining for actin (green). (**G**) In Ut, Spectrin staining (red) permits easy visualization of the intrinsic polarity of each hair cell (white arrows) while cell membranes can be seen by staining for actin (green). The hair cells on the two sides of the line of polarity reversal (pink line) are oriented towards each other.

Fig2. Distinct cellular mechanisms underlying the cochlear phenotypes in core PCP and ciliary mutants

(A) The cochlea in a *PCP* mutant (A) or a ciliary mutant (not shown) embryo is markedly shorter and wider than that of wild-type embryo, indicating that both core PCP and ciliary genes are required for CE of the cochlea. (B-D) Compared to wild-type (B), core PCP (C) and ciliary (D) mutants share hair bundle misorientation defects (asterisks). In ciliary mutants (D), circular hair bundles were also present (white arrowheads), indicating the loss of the intrinsic polarity of hair cells that is unique to ciliary mutants. (E-I) Polarized distribution of core PCP proteins in wild-type (E, F) is lost in PCP mutant (G), but retained in ciliary mutants, even when hair bundles have rotated away from their normal orientation (H, I). (I) is a close-up of the cell outlined by a dashed square in the panel (H). M: medial; L: lateral

Fig. 3. Model showing the formation of planar cell polarity in the cochlea

PCP establishment in the cochlea involves at least 3 main morphogenetic stages: **(A)** Kinocilia initially protrude from the center of the apical surface of the newly post-mitotic cells in the sensory primordium of the cochlea. **(B)** Polarization of core PCP proteins, including Vangl2, Fz3 and Fz6, and Dvl2 and Dvl3, and eccentric localization of primary cilia and centriole alignment along the PCP axis. The interaction between Fz and Vang is hypothesized. Other core PCP proteins, such as Pk and Dvl, may preferentially interact with Fz or Vang to reinforce polarized sorting of Vang and Fz within the same cell and to recruit downstream factors for localized cytoskeletal remodeling. **(C)** Formation of uniformly oriented hair bundles in the organ of Corti. Both core PCP genes and ciliary genes are clearly required for morphological polarity of the organ of Corti. Series of red arrows in **(B)** indicate potential effectors downstream of core PCP proteins that target at actin and microtubule cytoskeletons to achieve morphological polarity. Asterisk indicates genes that have been shown to repress canonical Wnt signaling. They include Wnt coreceptor Ror, Diversin/Ankrd6, and ciliary and basal body genes. M: medial (center of the cochlear spiral), L: lateral (periphery of the cochlear spiral).