Chapter 10
Seven-Pass Transmembrane Cadherin CELSRs, and Fat4 and Dchs1 Cadherins: From Planar Cell Polarity to Three-Dimensional Organ Architecture

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Abstract In this chapter, two subfamilies of atypical cadherins are described: the subfamily of seven-pass transmembrane cadherins (7-TM cadherins) and Fat and Dachsous cadherins. Pioneering genetic studies in Drosophila have defined both subfamilies and dissected their roles in animal development. It is now clear that the founding members in Drosophila and their respective vertebrate homologues make critical and essential contributions to a variety of dynamic behaviors of cell populations, and that malfunctions of those atypical cadherins cause anomalies in embryonic development, resulting in postnatal organ malformation or embryonic demise. Here we discuss how the atypical cadherins control cell behaviors with the emphasis on one particular orchestration of cells along the axes of tissues, organs, or bodies, inclusively designated as planar cell polarity (PCP). Nowadays the purview of PCP ranges from the unidirectional orientation of subcellular structures, such as wing hairs of Drosophila and vertebrate motile cilia, to three-dimensional dynamics of multicellular units, such as tilting hair follicles, neural tube closure, epithelial folding in the oviduct, and collective cell migration. The PCP field is at an extraordinarily exciting juncture, bursting with questions about functions of 7-TM cadherins and Fat and Dachsous cadherins at the cellular and molecular level.

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10.1 Introduction: 7-TM Cadherins, Fat and Dachsous Cadherins, and Planar Cell Polarity

As we know, things inevitably fall apart. But in the meantime, what holds them together? In the hierarchy of the animal body, how do cells connect with each other to realize tissue integrity, and how are the myriad different cell types sorted out? Assembled cells are far from static during development; they sometimes reorient themselves along global axes of tissues, organs, or bodies, dramatically converting simple local geometries into three-dimensional organs or whole bodies. How are these dynamic processes regulated, and what types of problems do we face if the underlying machineries lose control? Behind almost all of these fundamental questions, there exists a large superfamily of cell-to-cell adhesion proteins, the cadherin superfamily.

The cadherin superfamily comprises "classic" cadherins and atypical cadherins (see the chapter by Suzuki and Hirano). It is assumed that atypical cadherins are responsible for cellular and molecular functions distinct from classic members. In this chapter, two subfamilies of atypical cadherins are described (Fig. 10.1). The first is the subfamily of seven-pass transmembrane cadherins (7-TM cadherins), where the founding member is Flamingo (Fmi)/Starry night (Stan) in Drosophila (Chae et al. 1999; Usui et al. 1999) and its vertebrate homologues are CELSRs (cadherin epidermal growth factor laminin G seven-pass G-type receptors; Hadjantonakis et al. 1997; Nakayama et al. 1998). The second subfamily comprises single-pass transmembrane proteins in Drosophila, named Fat (Ft) and Dachsous (Ds; Mahoney et al. 1991; Clark et al. 1995), and their respective vertebrate homologues (Nakajima et al. 2001; Hoeng et al. 2004; Rock et al. 2005), all of which are collectively designated as Fat and Dachsous cadherins (Thomas and Strutt 2012; Matis and Axelrod 2013; Sharma and McNeill 2013; Sadeqzadeh et al. 2014). Ft and Ds (and their vertebrate orthologues Fat4 and Dcsh1, respectively) bind in a heterophilic manner at the cell surface (Strutt and Strutt 2002; Ma et al. 2003; Matakatsu and Blair 2004; Ishiuchi et al. 2009; Tsukasaki et al. 2014).

Understanding the in vivo functions of the two subfamilies has been tightly coupled with deciphering the mechanisms of one particular orchestration of cells, namely planar cell polarity (PCP). In many organs, epithelial cells are polarized not only along the apicobasal axis (the outside–inside axis) of the two-dimensional sheet, but also along a perpendicular, second axis within the plane. The latter polarity is the original definition of planar cell polarity, and is crucial for specialized cellular functions (Fig. 10.2; Adler 2012; Lawrence and Casal 2013). Nowadays the purview of PCP has expanded to include various directional behaviors of

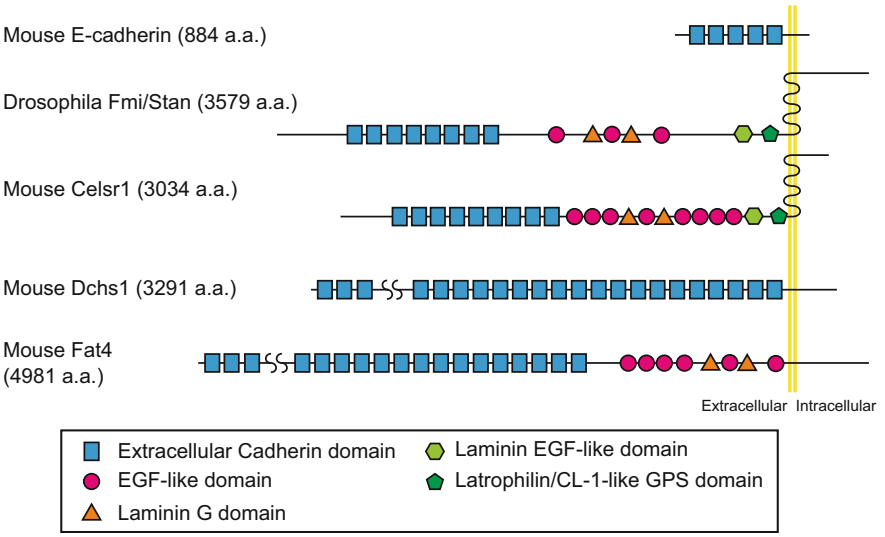

Fig. 10.1 Subfamilies of 7-TM cadherins and Fat and Dachsous cadherins. Schematic drawings of a classic cadherin and representative members of two atypical cadherin subfamilies. Mouse E-cadherin, Drosophila Flamingo (Fmi)/Starry night (Stan), and mouse Celsr1 in the 7-TM cadherin subfamily, and mouse Fat4 and Dchs1 (homologues of Drosophila Fat and Dachsous, respectively). All of them share extracellular cadherin domains (ECs) within their ectodomains in the N-termini. Other motifs in their ectodomains are also illustrated

rearranging cell populations of both epithelial and nonepithelial tissues (Vladar et al. 2009; Goodrich and Strutt 2011; Gray et al. 2011; Vichas and Zallen 2011; Yang 2012; Wallingford 2012). Below we discuss Drosophila and vertebrate model systems that have distinguished the two subfamilies in PCP, the remarkable subcellular localization of 7-TM cadherins, roles of the two subfamilies in generating polarity at multiple levels from cells to organs in various vertebrate developmental contexts, and a number of outstanding questions that are being addressed.

10.2 Landmarks of Planar Cell Polarity: Drosophila Wing Hairs and Vertebrate Cilia

Roles of Fmi/Stan, Ft, and Ds in PCP have been best studied in the Drosophila wing, where individual epidermal cells localize an assembly of actin filaments at the distal cell vertices on the apical surface, and produce single wing hairs pointing distally (Figs. 10.2a, b, and 10.3a; Adler 2012; Carvajal-Gonzalez and Mlodzik 2014). If any of these genes malfunction, the mutant cells no longer respect the proximal–distal axis of the wing and fail to select the correct sites on the apical surface for hair formation (Adler 2012). For example, *fmi*/*stan* mutant cells form wing hairs at the center of the apical surface (Fig. 10.3b), supporting the hypothesis

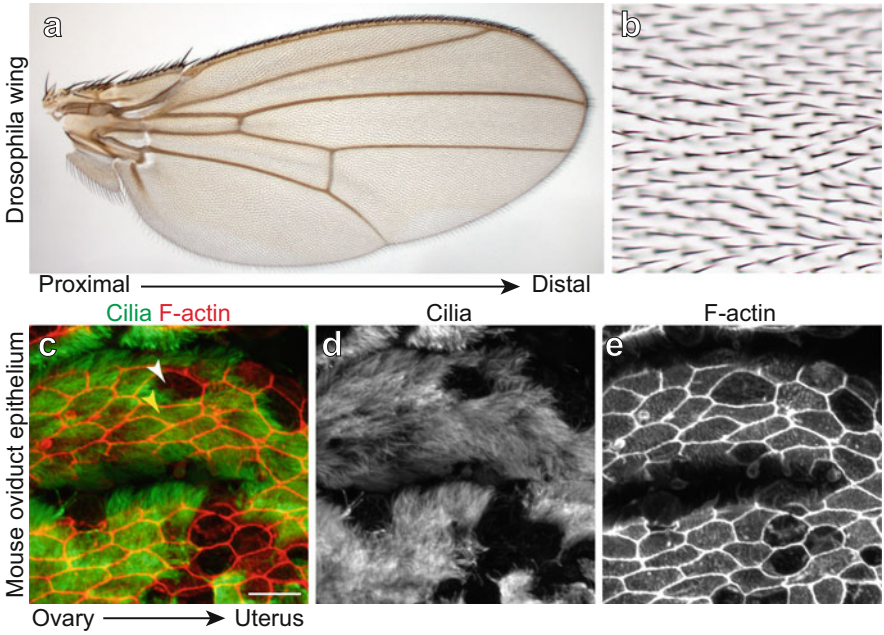

Fig. 10.2 Landmarks of planar cell polarity. (**a** and **b**) The Drosophila wing. (**a**) The whole image where the proximal–distal (P–D) axis is running from left to right. (**b**) The high-power image shows hairs pointing in a distal direction along the tissue axis. (**c–e**) A maximum intensity projection of confocal images of the oviduct epithelium, where the ovary–uterus axis is running from left to right. The oviduct is opened longitudinally and the epithelium is stained with antiacetylated α-tubulin antibodies (**d**, and *green* in **c**; cilia) and phalloidin (**e**, and *red* in **c**; F-actin). (**c**) A secretory cell and a multiciliated cell are marked by *white* and *yellow arrowheads*, respectively. The epithelium of the infundibular region (close to the ovary end of the oviduct) is mostly covered by multiciliated cells. *Scale bar*: 10 μm

that Fmi/Stan functions to limit the region where the cytoskeleton is activated to form a hair.

In vertebrate epithelia, directional alignments of apical appendages are seen in the inner ear, where actin filament-based stereocilia protrude from the apical surface of hair cells (May-Simera and Kelley 2012; see the chapter by El-Amraoui and Petit), and in many other epithelia that develop microtubule-based motile cilia (Brooks and Wallingford 2014). Cilia-forming epithelial tissues or organs include the node that gives rise to axial mesoderm (Sulik et al. 1994), the oviduct (Fig. 10.2c–e; Agduhr 1927; Boisvieuxulrich et al. 1985), the airway (Lucas and Douglas 1934), the lateral ventricle in the brain (Del Bigio 1995), and tadpole epidermis (Assheton 1896). The planar axis along which cells are polarized is referred to as the tissue, organ, or body axis, or more simply the global axis, depending on the context. In the oviduct and the airway, cilia cover the entire apical surface of each epithelial cell (Figs. 10.2c–e and 10.3c); by contrast, in the ependymal cells in the ventricle they are clustered and off-centered at a fixed corner

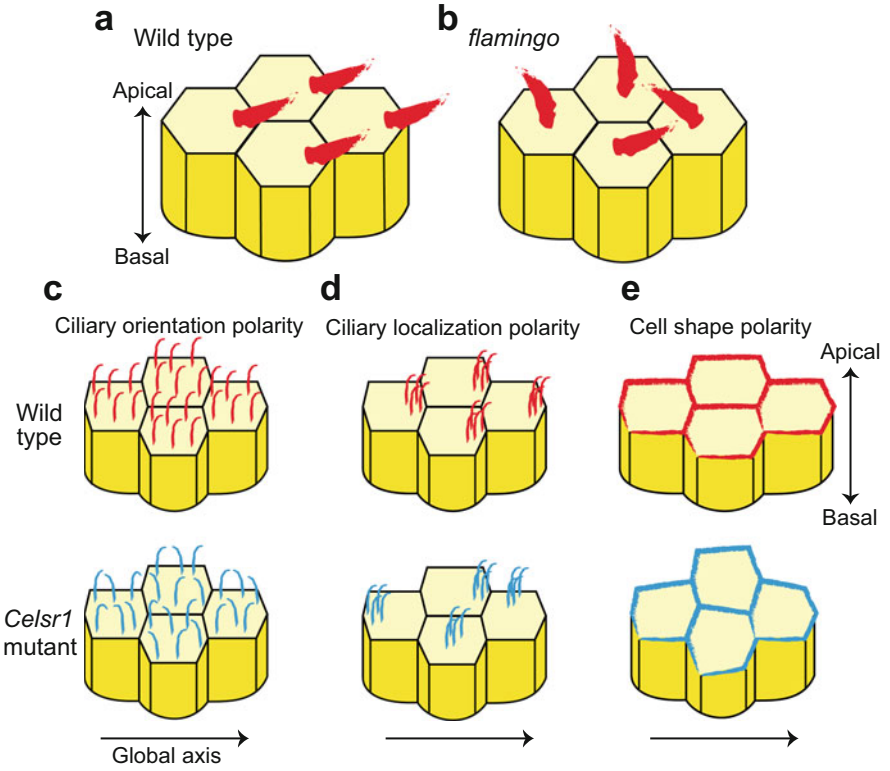

Fig. 10.3 Cellular phenotypes caused by loss of function of 7-TM cadherins. (**a** and **b**) (**a**) Diagrams of epidermal cells of the Drosophila wing. In the wild-type wing, individual epidermal cells localize an assembly of actin filaments at the distal cell vertexes on the apical surface, and produce single wing hairs pointing distally. (**b**) In contrast, *fmi*/*stan* mutant cells mislocalize actin filaments at the center of the apical surface and the generated hairs are not necessarily aligned along the proximal–distal axis. (**c**–**e**) Diagrams of multiciliated cells in the oviduct epithelium (**c** and **e**) and ependymal cells in the ventricle (**d**), comparing wild-type (*top*) and a *Celsr1* mutant (*bottom*). *Arrows* indicate the ovary–uterus axis of the oviduct (**c** and **e**) or the direction of cerebrospinal fluid (CSF) circulation in the ventricle (**d**). Phenotypic landmarks of the wild-type (*red*) and *Celsr1* mutant (*blue*) are oriented cilia (**c** and **d**) and the elongated shape of the apical cell surface (**e**). Cilia cover the entire apical surface of each cell in the oviduct (**c**), whereas in ependymal cells they are clustered and off-centered at a fixed vertex (**d**). (**e**) To highlight the elongated shape of the apical cell surface along the axis in the wild-type, cilia are not included in the drawing; similarly, in "c" the phenotype of abnormal cell shape in the *Celsr1* mutant is not drawn in order to emphasize the misoriented cilia (See descriptions of the mutant phenotypes in the text)

along the organ axis (Fig. 10.3d), much like the wing hairs in Drosophila that are generated at the distal cell vertex (Guirao et al. 2010; Hirota et al. 2010; Mirzadeh et al. 2010). Each multiciliated cell forms tens to hundreds of cilia, all of which beat in a defined direction (Fig. 10.2c, d).

10.3 Phenotypes of Stereocilia and Motile Cilia Caused by Loss of Function of Atypical Cadherins

Genetic studies in mice have addressed whether homologues of Fmi/Stan, Ft, and Ds are required for correct polarization of stereocilia and motile cilia. As expected, misorientation of stereocilia is seen in *Celsr1*-mutant mice (Curtin et al. 2003). The oviduct in *Celsr1*-deficient mice also shows a PCP-specific phenotype of multiciliated cells, which is reminiscent of the polarity defect found in the Drosophila wing epidermis (Fig. 10.3b, c). Concretely, multiciliated cells of the knockout (KO) mice are differentiated with no apparent differences in the structure, length, density, and motility of cilia when compared to the wild-type oviduct; yet, the overall directionality of ciliary movements and the ultrastructure of the basal feet of beating cilia are no longer parallel to the ovary-to-uterus axis (Shi et al. 2014). Although the cilia do point in a similar direction within small local regions of the mutant cell, they are neither facing the same direction over the whole cell nor between neighboring cells. Thus Celsr1 is dispensable for ciliogenesis itself but is indispensable for the proper orientation of the cilia. As a result, the directional flow from the ovary to the uterus is not established, and the transportation of ova is demonstrably impaired in the cultured, longitudinally opened oviduct of the KO mice (Shi et al. 2014). The ependymal cells in the ventricle in *Celsr1*-deficient mice mislocalize clustered cilia, which is again reminiscent of the Drosophila wing in *fmi* mutants (Fig. 10.3b, d; Boutin et al. 2014).

Among the three Celsr paralogues in mice, their roles in the development of ciliated epithelia are not necessarily restricted to PCP. Development and planar organization of ependymal cilia are compromised in *Celsr2*-deficient mice, leading to defective circulation of the cerebrospinal fluid (CSF) and hydrocephalus. The hydrocephalus phenotype is accelerated in *Celsr2* and *Celsr3* double mutant embryos due to a more markedly impaired ciliogenesis in ependymal cells, showing contributions of Celsr2 and Celsr3 to ciliogenesis itself (Tissir et al. 2010; Tissir and Goffinet, 2013; Boutin et al. 2014).

Mammalian orthologues of Drosophila Ft and Ds are Fat4 and Dchs1, respectively, and the corresponding KO mice have also been investigated with respect to PCP defects. Loss of Fat4 or Dchs1 results in a subtle misoriented phenotype of stereocilia and various morphological phenotypes in other tissues and organs that are discussed in detail later, but abnormality of motile cilia has not been reported.

10.4 7-TM Cadherins Flamingo/Starry Night and Celsr1 Control Planar Polarity at Selective Plasma Membrane Domains

Not only genetic but also cell biological studies on Fmi/Stan have brought about breakthroughs in our understanding of the molecular mechanisms of PCP. A key finding was its subcellular localization in the Drosophila wing. Along the apicobasal axis of the epithelium in general, Fmi/Stan is localized at adherens junctions where classic E-cadherin is also located. Remarkably, when looking down at the epidermal plane, Fmi/Stan is redistributed selectively to both proximal and distal cell boundaries before the onset of hair formation, which is referred to as the polarized localization of Fmi/Stan or the "zigzag" for short (Fig. 10.4a, d; Usui et al. 1999). Similarly, on many polarizing epithelia in vertebrates, Celsr1 zigzags emerge prior to morphologically visible cell polarization such as directional cilia motility (Fig. 10.4f), providing a striking contrast to the uniform, honeycomb-like distribution of classic E-cadherin (Fig. 10.4e, g; Davies et al. 2005; Devenport and Fuchs 2008; Shi et al. 2014).

In addition to Fmi/Stan and Celsr1, several other evolutionarily conserved PCP regulators localize at the same plasma membrane domains, all of which are now categorized into the "core" group of PCP. It should be noted, however, that except for the 7-TM cadherins, the other core-group members are localized to only one end of the cell or the other along the tissue axis (Fig. 10.4b; Vladar et al. 2009; Goodrich and Strutt 2011; Devenport et al. 2011; Wallingford 2012; Vladar et al. 2012). For example, the four-pass transmembrane protein Van Gogh (Vang; also known as Strabismus; Wolff and Rubin 1998; Taylor et al. 1998) is localized at the cellular proximal end (Bastock et al. 2003), whereas the seven-pass transmembrane protein Frizzled (Fz; Wong and Adler 1993) is at the opposite end, distal in the Drosophila wing (Strutt 2001). The core-group members assemble into a complex, where the homophilic interaction of Fmi/Stan or Celsr1 across the boundary is essential for the junctional recruitment of Vang and Fz, and the resultant complex is an asymmetric one that straddles cell boundaries almost perpendicular to the global axis (Fig. 10.4c; Chen et al. 2008; Strutt and Strutt 2008; Devenport et al. 2011; Vladar et al. 2012). This asymmetric complex recruits downstream polarity effectors to restrict reorganization of the cytoskeleton spatially, leading to the array of distally pointing wing hairs in Drosophila (Adler 2012; Wang et al. 2014b; Lu et al. 2015). Less is known about how exactly Celsr1 on cell boundaries reorients cilia along the organ axis, although it has been observed that cytoskeletal reorganization coincides with and is required for the ciliary polarity formation (Vladar et al. 2012; Boutin et al. 2014). Further proteomic approaches and subsequent assays at organ and/or cell-culture levels may complete the picture downstream of Celsr1, including ciliogenesis and orientation. In fact, previous studies have identified relevant binding partners or downstream molecular events in the context of a role of Fmi/Stan in neuronal cell morphogenesis (Matsubara et al. 2011) and a control of

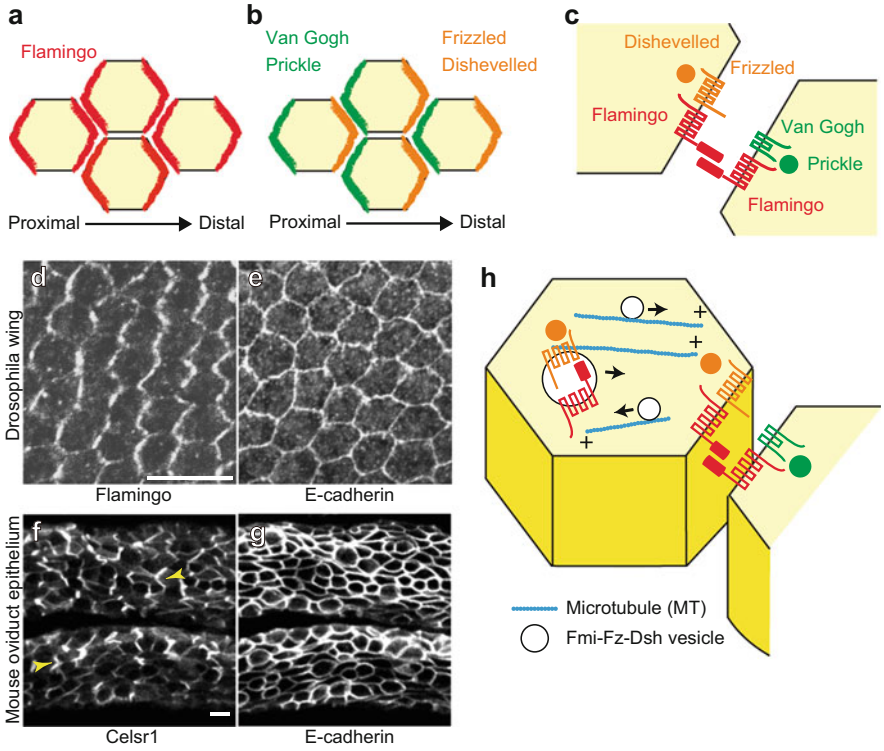

Fig. 10.4 Localization of core-group proteins to selective cell boundaries. All diagrams and confocal images are oriented as in Fig. 10.2. (**a–c**) Diagrams of apical views of wing epidermal cells show the localization of core-group proteins. (**a**) Flamingo (Fmi)/Starry night (Stan). (**b**) Transmembrane proteins, Van Gogh (Vang)/Strabismus (Stbm) and Frizzled (Fz), and undercoat proteins Prickle (Pk) and Dishvelled (Dsh). (**c**) The enlarged diagram shows the disposition of the core group at the cell junction between abutting cells along the axis. (**d–g**) Double staining of the Drosophila wing epidermis for Flamingo (**d**) and E-cadherin (**e**) and the mouse oviduct epithelium for Celsr1 (**f**) and E-cadherin (**g**). The uniform cell-boundary labeling of E-cadherin (**e** and **g**) and the zigzag pattern of Flamingo (**d**) and Celsr1 (*yellow arrowheads* in **f**). (**f** and **g**) Along the ovary–uterus axis of the oviduct, two longitudinal folds can be seen. *Scale bar*: 10 μm. (**e**) Reprinted from "Flamingo, a Seven-Pass Transmembrane Cadherin, Regulates Planar Cell Polarity under the Control of Frizzled" by Usui et al., *Cell*, Volume 98, 585–595, 1999, with permission from Elsevier. (**h**) The model for the polarized transport of Fz–Dsh and Fmi-containing vesicles along the proximal–distal (P–D)-oriented microtubules (MTs). + end-distal MTs are slightly more abundant than + end-proximal MTs, which biases the vesicles towards the distal cell cortex with the help of + end motor proteins (*arrows*). Once Fmi reaches the distal boundary together with Fz and Dsh, Fmi can engage and lock with a counterpart on the proximal membrane of the adjacent cell through its homophilic binding property. Formation of this Fmi–Fmi bridge across the cell boundary could anchor the Fz–Dsh complex at the distal cortex. This input may initiate recruitment of Vang/Stbm–Pk on the opposing proximal cortex by means of mutual exclusion between Dsh and Pk, and by means of ectodomain interactions between Fz and Vang/Stbm

subcellular localization of Celsr1 during mitosis (Shrestha et al. 2015; discussed below).

It is important to note that the polarized localization of Fmi/Stan, Celsr1, and other core-group members is thought to be a functional requirement of PCP signaling and plays an instructive role in polarity establishment (Goodrich and Strutt 2011). Fundamental unanswered questions include how such an asymmetric redistribution of the core group is achieved and why Vang and Fz are localized at opposite cell boundaries in the first place. Across the boundary, it is proposed that a positive feedback plays a critical role in the assembly of the complex; however, it is still unknown how the initial bias is generated. This question and multiple hypotheses are discussed in the end.

10.5 Celsr1 Angles Hair Follicles Along the Body Axis

Because the discoveries that polarization of apical cell appendages is under the control of the core group, the purview of PCP has expanded dramatically to include various directional behaviors of cell populations. One example alluded to earlier is convergent-extension in early embryos, more specifically, the intercalation of cells towards the midline causing elongation of the body along the anterior–posterior (A–P) axis (Gray et al. 2011; Tada and Kai 2012; Wallingford 2012; Tada and Heisenberg 2012). Below, we discuss Celsr1-dependent dynamic behaviors of cells, with the emphasis on large-scale and/or three-dimensional organogenesis in vertebrates.

In addition to unicellular apical appendages, Celsr1 regulates the polarization of multicellular units such as hair follicles, each of which comprises hundreds of proliferative basal epidermal stem cells (Fig. 10.5a; Fuchs 2007). Development of the hair follicle in mice is initiated by budding about 10 cells from the epithelium; and then invaginating nascent hair follicles become anteriorly angled and morphologically polarized through marked changes in cell shape and cytoskeletal reorganization, producing hairs that point to the posterior side (Fig. 10.5a). Individual hair follicles acquire asymmetric patterns of gene expression along the A–P axis, with P-cadherin, ZO-1, and *Shh* mRNA upregulated in anterior cells and NCAM upregulated in posterior cells. All of these events depend on Celsr1 and at least one more member of the core group Vangl2 (Devenport and Fuchs 2008). These manifestations of planar polarization within the basal layer closely resemble what happens in epithelia that give rise to Drosophila ommatidia or sensory bristles (Lu et al. 1999; Gaengel and Mlodzik 2003), which are also polarized multicellular units. Hair follicle initiation coincides with asymmetric redistribution of Celsr1 to anterior and posterior cell boundaries of basal epidermal stem cells and also in the interfollicular epidermis (whereas Vangl2 is restricted to the anterior boundary), suggesting the polarity along the body axis has spread throughout the tissue. The *Celsr1* mutant misaligns hair follicles in the entire body so that it shows a whorled

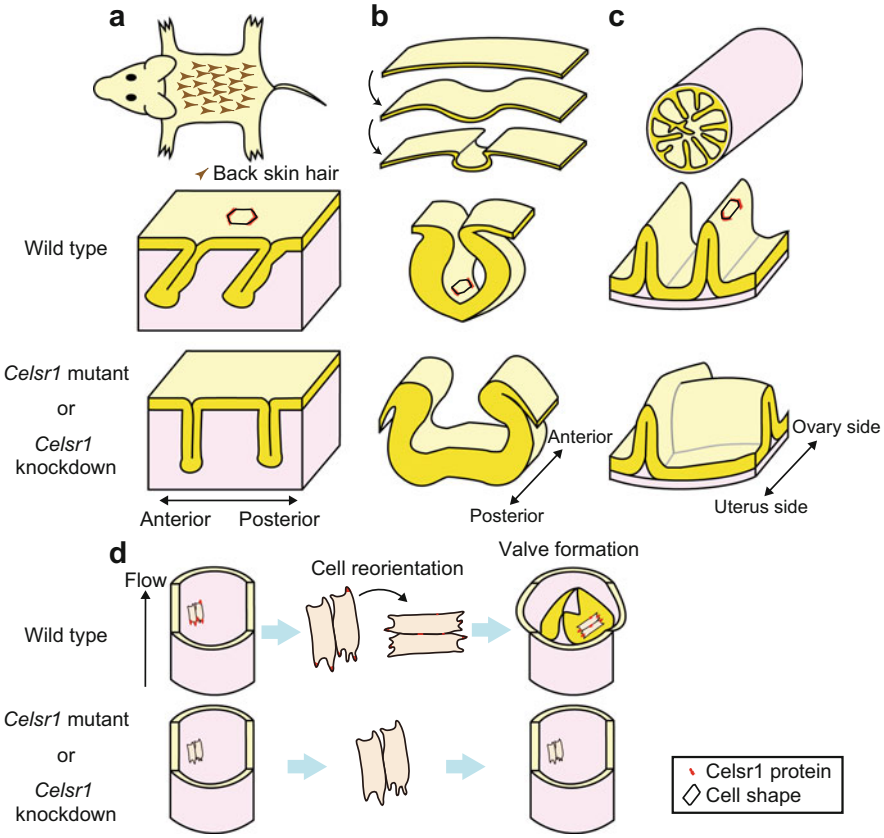

Fig. 10.5 7-TM cadherin Celsr1-dependent three-dimensional organogenesis in vertebrates. Diagrams showing anterior angling of hair follicles in mouse epidermis (**a**), neural tube closure in chick and mice (**b**), longitudinal epithelial folding in the mouse oviduct (**c**), and formation of a lymph valve in mice (**d**). Except for illustrations of overall organs in "a"–"c", wild-type structures are schematically drawn at the top and those in *Celsr1* mutant or knockdown animals are at the *bottom*. Apical cell shape of epithelial cells (*black polygons*) and localized Celsr1 protein (*red*) are illustrated in individual tissues. (**c**) The oviduct consists of a simple columnar epithelium and a thin stromal layer surrounded by smooth muscle layers. See more details in the text

hair pattern and also loss of the asymmetry of gene expression within individual follicles (Devenport and Fuchs 2008; Ravni et al. 2009).

In addition to the above demonstration of the role of Celsr1 in global asymmetric morphogenesis of hair follicles, studies of basal epidermal stem cells are answering an important question at the molecular level: how PCP is maintained in highly proliferative tissues (Devenport et al. 2011; Shrestha et al. 2015). During mitosis when the cells are rounded up, asymmetrically distributed Celsr1 risks mislocalization or unequal inheritance, which could lead to profound perturbations of the long-range propagation of polarity. How is the polarized localization

regained after cell division? Elegantly, Celsr1 proteins are internalized into endosomes during mitosis; and following mitosis, they are recycled back to the cell surface, where polarized localization is re-established with the help of the polarity of adjacent interphase cells (Devenport et al. 2011). This mechanism explains how extensive polarity disruption is minimized within tissues that must maintain function during rapid growth and/or turnover. A further proteomic approach shows that a key mitotic kinase, Polo-like kinase 1, phosphorylates conserved serine/threonine residues in the carboxyl tail of Celsr1, which promotes Celsr1 endocytosis during mitosis (Shrestha et al. 2015).

10.6 Celsr1 Orchestrates Neural Tube Formation by Way of Spatiotemporal Control of Actomyosin Contraction

The neural tube is a primordium of the brain and the spinal cord. Converting a wide flat neuroepithelial sheet—the neural plate—into the neural tube is a challenging feat (Fig. 10.5b). This is evident from clinical data, where common human birth defects are those affecting neural tube formation, including failure of neural tube closure, and from molecular genetic studies, where a variety of gene products, including Celsr1 and other PCP regulators, has been shown to be required for this tube formation in animal models and humans (Curtin et al. 2003; Doudney and Stanier 2005; Robinson et al. 2012; Allache et al. 2012; Juriloff and Harris 2012; Wallingford et al. 2013; Yamaguchi and Miura 2013). When apical cortical actin constricts locally within the epithelium, an indent forms in the sheet (St Johnston and Sanson 2011). However, if cells do not converge towards the midline, the sheet is incompletely rolled up along the A–P axis, resulting in a failure to close and the consequential inability to generate a tube. (Fig. 10.5b). How Celsr1 orchestrates other proteins and coordinates the two morphogenetic processes, apical constriction and midline conversion, has been best studied at the molecular level in chick embryos (Nishimura et al. 2012; Nishimura 2014).

Again, at the nexus of the known molecular machinery, there exists polarized localization of Celsr1. In this developmental context, Ceslr1 is enriched at anterior and posterior cell boundaries that are orthogonal to the A–P body axis in the bending neuroepithelium, and it cooperates with Dishevelled (one of the PCP core group) and other proteins to upregulate Rho kinase activity, causing shrinking of those boundaries through localized cytoskeletal reorganization (Fig. 10.5b). This planar-polarized acotomyosin contraction promotes midline convergence of neuroepithelium cells, leading to the bending of the neural plate along the A–P body axis. Knockdown of *Celsr1* and any of the other component genes of the machinery causes neural tube closure defects where the bending of the neural plate is insufficient (Fig. 10.5b). The role of Celsr1 in spatiotemporal regulation of cytoskeletal components such as phosphorylated myosin light chain and F-actin

was also reported in otic placode invagination during inner ear morphogenesis (Sai et al. 2014).

10.7 Celsr1 Links Shaping of Individual Cells with Folding of Epithelial Sheets in the Oviduct

With respect to three-dimensional organ architecture, the oviduct is much more than a simple tube. In mice, the epithelium forms about 20 straight folds running in parallel with the ovary-to-uterus axis (Figs. 10.4g and 10.5c; Agduhr 1927). These folds are thought to increase the epithelial surface area and ensure the oocyte transport from the ovary to uterus, guided by coordinated ciliary beatings. In addition to this large-scale polarized structure, the apical surface of individual epithelial cells is elongated along the organ axis (Fig. 10.3e), and Celsr1 is localized at the shortened cellular junctions that are perpendicular to the organ axis (Figs. 10.4f, and 10.5c). Thus the wild-type oviduct generates polarity at multiple levels of single cell shape, directional ciliary beating on the plane, and three-dimensional tissue morphology; and all of these polarities are disrupted by loss of Celsr1 function (Shi et al. 2014). Compared to the wild-type, Celsr1-deficient cells are less elongated and less oriented along the organ axis (Fig. 10.3e), and the morphology of epithelial folds is disorganized, with the subsequent generation of ectopic and abnormal branches that run in randomized directions (Fig. 10.5c).

To address the cause-and-effect relationship between the cellular shape and the fold morphology, mosaic analyses were performed in mice, in which mutant clones of various size were generated in the wild-type background, and it was examined how the two features were affected. The results show that the cell shape is intrinsically regulated through Celsr1 and not by extrinsic cues; on the basis of this finding, it is hypothesized that the cell shape is primarily regulated by Celsr1 and as a consequence the epithelial folds are aligned. It requires future studies to unravel how Celsr1 drives polarization of cell shape, whether the cell elongation along the organ axis produces any anisotropic force, and whether such a force, if produced, leads to the parallel alignment of epithelial folds (Heisenberg and Bellaïche 2013; Sugimura and Ishihara 2013). The impact of this study is not limited to organogenesis of the oviduct, because folding of cellular sheets is observed throughout the animal body, including the sulcus in the brain (Striedter et al. 2015). It may be intriguing to re-examine directional relationships between cell shape and those folds.

Tissue-level polarity inside tubular organs is also seen in veins and lymphatic vessels, where one-way flow of blood and lymph is ensured by polarized multicellular structures, luminal valves (Fig. 10.5d). Morphogenesis of the lymphatic valve is initiated by Celsr1-dependent reorientation of endothelial cells perpendicular to the longitudinal organ axis, followed by migration of the cells into the luminal side to form a primordium of the valve (Shi et al. 2013; Tatin

et al. 2013). Celsr1 and Vangl2 are localized to membrane protrusions and also recruited to cell–cell contacts during the reorientation of the endothelial cells. What the signal is for the reorientation, how Celsr1 drives this cell orientation, and what the link between localized Celsr1 and the cell migration is should be elucidated in the future.

This chapter has focused on just a handful of developmental processes where model organisms have provided some detailed knowledge of the molecular mechanisms, however, 7-TM cadherins play important roles in other developmental contexts in Drosophila and vertebrates that are not discussed in this chapter, including neuronal wiring (Takeichi 2007; Shima et al. 2007; Hakeda-Suzuki et al. 2011; Matsubara et al. 2011; Hirano and Takeichi 2012; Schwabe et al. 2013; Tissir and Goffinet 2013; Wang et al. 2014a), pancreatic β cell differentiation (Cortijo et al. 2012; Wang et al. 2014a), and maintenance of hematopoietic stem cells (Sugimura et al. 2012). We now turn our attention to Fat and Dachsous cadherins.

10.8 Functional Relationships Between 7-TM Cadherins and Fat and Dachsous Cadherins

The most outstanding feature of PCP would be its high fidelity to global axes, which is represented not only by the unidirectional beating of all cilia within individual cells, but also by the coordination between cells entirely along the tracheal or oviduct axis. One theoretical framework proposes that two distinct mechanisms can contribute to this long-range propagation of polarity (Abley et al. 2013): one is coupling between adjacent cells (designated as "cell–cell coupling"), generating local alignment, and the other is a concentration gradient of a signaling molecule across the tissue (designated as "tissue gradients"). Experimental data suggest that cell–cell coupling operates through the plasma membrane-spanning complexes comprising 7-TM cadherins and other core group members, whereas Dachsous that binds to Fat is implicated in the mechanism of tissue gradients as suggested by its graded expression along different axes in various tissues in Drosophila (Fig. 10.6a; Yang et al. 2002; Ma et al. 2003).

The functional relationship between the core group and the Ft–Ds system in PCP has been a target of intense investigations, and apparently there are numerous mechanistic variations among different tissues or even in the same tissue depending on the readouts (Goodrich and Strutt 2011; Lawrence and Casal 2013; Matis and Axelrod 2013; Carvajal-Gonzalez and Mlodzik 2014; Olofsson et al. 2014; Ayukawa et al. 2014; Merkel et al. 2014). In some tissues, the two systems work independently, whereas they interact in other tissues, as discussed at the end of this chapter. Much less is known about the relationship in vertebrate development, and one example in the context of collective cell migration is discussed below, where both CELSRs and the Fat4–Dchs1 system participate, but their roles are segregated.

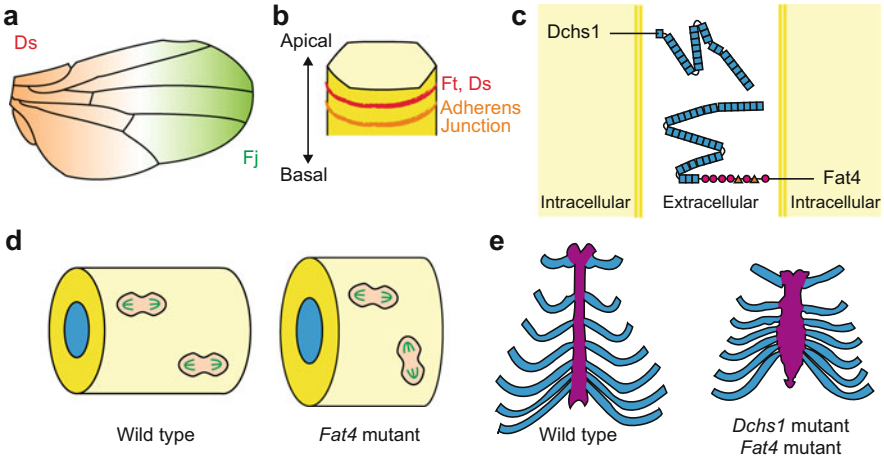

Fig. 10.6 Fat and Dachsous cadherins: expression at the tissue level, subcellular localization, protein conformations, and mutant phenotypes. (**a**) Dachsous (Ds, *orange*) and Four-jointed (Fj, *green*) are expressed in counter-gradients in the Drosophila wing. Fat (Ft) is uniformly expressed and not included in this diagram. (**b**) Ft and Ds are positioned more apically than the adherens junction where Fmi and E-cadherin are localized. (**c**) The model of conformations of Fat4 and Dchs1 ectodomains at the intercellular junction. (**d**) Fat4 is required for oriented cell division (OCD) in the kidney, and misorientation of cell division angles in the kidney in the mutant mice renders collecting ducts broader and shorter. (**e**) Illustrations of skeletal preparations of sternums (*purple*) of the wild-type and *Fat4* or *Dchs1* mutants. Sternums are wider and shorter in the mutants

10.9 Fat and Dachsous Cadherins: Control of the Heterophilic Binding by Ectodomain Phosphorylation and Protein Conformation at the Cell Interface

In Drosophila, Fat and Dachsous form a trio with Golgi kinase Four-jointed (Fj) and contribute to tissue patterning across the axis. Intriguingly, Fj phosphorylates the ectodomains of Ds and Ft to modulate their heterophilic bindings (Ishikawa et al. 2008; Sopko et al. 2009; Feng and Irvine 2009; Brittle et al. 2010; Simon et al. 2010). As discussed in the theoretical framework of PCP, Ds is thought to belong to the mechanism of "tissue gradients"; so is Fj, which is expressed in a counter-gradient fashion to Ds in multiple tissues (Fig. 10.6a; Villano and Katz 1995; Zeidler et al. 2000; Yang et al. 2002; Ma et al. 2003). It is this complementary expression pattern that controls Ft–Ds binding at cell boundaries differentially across the wing (Thomas and Strutt 2012; Mani et al. 2013; Jolly et al. 2014; Hale et al. 2015). Complementary expression patterns of *Dchs1* and the *Fj* homologue *Fjx1* and those of *Dchs1* and *Fat4* are found in mice (Rock et al. 2005) and their relevance to neuronal migration is described (Zakaria et al. 2014; and see below).

Along the apicobasal cell axis, Ft and Ds are localized in a cell–cell contact area positioned more apically than the adherens junction (AJ) in the Drosophila wing primordium (Fig. 10.6b; Ma et al. 2003); and Fat4 and Dchs1 show a similar localization in neural progenitor cells of the mouse cortex (Ishiuchi et al. 2009). The heterophilic binding between Fat4 and Dchs1 is shown by expressing the full-length molecules in cultured cells (Ishiuchi et al. 2009) and by surface plasmon resonance analysis using their entire ectodomains (Tsukasaki et al. 2014). At the organ level, the heterophilic binding is imaged in developing kidney, which consists of three cell layers: the epithelial ureteric bud, which forms the collecting ducts; the nephron progenitors (also known as the cap or condensing mesenchyme); and the stromal mesenchyme. For normal kidney development, it is necessary to balance self-renewal and a mesenchymal-to-epithelial transition of the progenitors. This control is achieved by signaling between Dchs1 in the progenitors and Fat4 in the stromal cells, and at least Dchs1 proteins are concentrated at the interface of these two layers (Bagherie-Lachidan et al. 2015; Mao et al. 2015).

Electron-microscopic observations verify how the large ectodomains of Fat4 and Dchs1, about 4500 and 3000 amino acids in length, respectively (Fig. 10.1) are fitted into limited intercellular spaces (Tsukasaki et al. 2014). Fat4 and Dchs1 ectodomains assume kinked conformations, in contrast to the linear configuration of the E-cadherin ectodomain (Fig. 10.6c). It was found that certain linker regions of Fat4 and Dchs1, which connect ECs (extracellular cadherin domains), lack the Ca^{2+}-binding motifs that are conserved in the E-cadherin ectodomain and are important for the linear conformation of E-cadherin (Tsukasaki et al. 2014). Due to these differences in amino acid sequences, the ectodomains of Fat4 and Dchs1 bend, thereby enabling them to fit in the confined intercellular spaces. Expansion of this structural approach may provide an explanation for how phosphorylation of the Ds and Ft ectodomains by Fj modulates their heterophilic binding.

10.10 Loss of Fat4 or Dchs1 Function Causes Various Morphogenetic Phenotypes

Fat4- or *Dchs1*-deficient mice show morphological defects in various organs, including size reductions of internal organs (intestine, lung, and kidney; Saburi et al. 2008; Mao et al. 2011). Which of these phenotypes, apart from the subtle misalignment of stereocilia of hair cells in cochlea (Saburi et al. 2008; Mao et al. 2011), can be explained by our current knowledge of PCP? The core group has been implicated in the regulation of oriented cell division (OCD) in various organs or embryos in Drosophila and vertebrates (Lu et al. 1999; Gong et al. 2004; Devenport 2014). The requirement of Fat4 for OCD was shown by misorientation of cell division angles in the kidney in KO mice, which renders collecting ducts broader and shorter, leading to cystic kidney disease (Fig. 10.6d; Saburi et al. 2008). In both the *Dchs1* mutant and the *Fat4* mutant mice, such a “compressed”

morphological defect is also seen in the sternum (Fig. 10.6e; Mao et al. 2011), therefore elongation of this organ also might depend on Fat4–Dchs1 mediated OCD and/or cell intercalation.

Although Fat4 shows the highest sequence similarity to Drosophila Fat, genes of three other homologues are present in the mammalian genome, and the respective Fat proteins act both synergistically and antagonistically to affect morphogenesis of multiple organs including neural tube closure (Saburi et al. 2012; Badouel et al. 2015). Similarly, a partial redundancy between Dchs1 and its paralogue Dchs2 was shown (Bagherie-Lachidan et al. 2015). Molecular machineries downstream of Fat cadherins have been studied by hunting for binding proteins, and Fat1 and Fat4 bind to different sets of actin regulating and junctional proteins (Tanoue and Takeichi 2004; Ishiuchi et al. 2009; Sadeqzadeh et al. 2014; Badouel et al. 2015). Future studies will address the question of whether each Fat cadherin executes organogenesis in different contexts with a common set of binding partners (such as classic cadherins with catenins) or context-specific binding partners, or a combination of both. Drosophila Ds intracellular domain interacts with an unconventional myosin Dachs (D), and this binding is important for shaping the dorsal thorax epithelium (Bosveld et al. 2012). Although intracellular binders to vertebrate Dchs cadherins have not been reported, zebrafish Dchs1b regulates the actin and microtubule cytoskeleton, possibly independent of Fat in the unanticipated context of the single-celled embryo (Li-Villarreal et al. 2015).

10.11 "Intersection" of 7-TM Cadherins and the Fat4–Dchs1 System in the Guidance of Cell Migration

In Drosophila pupal development, one context of collective epidermal migration is dependent on Ds (Bischoff 2012), and the directional information is provided by an imbalance of the Ds level between the migrating cells (unpublished data of M.A. and T.U.). In vertebrate embryonic brains, the Fat4–Dchs1 system and 7-TM cadherins are shown to control migration of facial branchiomotor (FBM) neurons in the neuroepithelium (Fig. 10.7), but the roles of the two subfamilies are segregated along orthogonal axes: the A–P (anterior–posterior) axis and the mediolateral axis. FBM neurons arise within one compartment, rhombomere 4 (r4), of the hindbrain, undergo posterior migration towards r6 and then turn laterally in r6 (Fig. 10.7c; Tissir and Goffinet 2013). 7-TM cadherins control the former posterior migration, whereas the Fat4–Dchs1 system controls the latter. How each of the 7-TM cadherins contributes to the posterior migration is slightly different between Celsr1–Celsr3 and in different species (zebrafish and mice). In zebrafish, Celsr2 and Fz3a (one of the core group) act in the surrounding neuroepithelium to prevent the integration of the neurons into the neuroepithelium, thus restricting them to the correct A–P path (Wada et al. 2006; Wada and Okamoto 2009). A similar role of Celsr2 outside the neurons was reported in mice

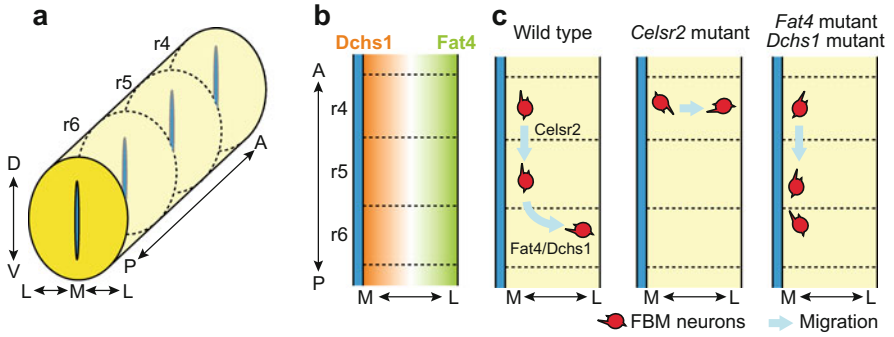

Fig. 10.7 Distinct roles of Celsr2 and Fat4 and Dchs1 in migration of facial branchiomotor neurons in the hindbrain. Diagrams of the hindbrain (**a**), of Fat4 and Dchs1 expression (**b**), and migration patterns of facial branchiomotor (FBM) neurons (**c**). The ventricular zones are highlighted in blue. (**a**) A portion of the hindbrain between rhombomere 4 (r4) and r6. The anterior–posterior (A–P) axis, the mediolateral (M–L) axis, and the dorsal–ventral (D–V) axis are indicated. (**b** and **c**) Dorsal views of lateral halves of longitudinal sections of the hindbrain. (**b**) Expression of Dchs1 is highest medially, whereas Fat4 expression is highest laterally. (**c**) Migration patterns of FBM neurons in the hindbrain neuroepithelium in the wild-type (WT) and the mutants are indicated. FBM neurons arise within one compartment, rhombomere 4 (r4), undergo posterior migrations towards r6 and then turn laterally in r5 and r6. The posterior migration depends on Celsr2, whereas the lateral migration depends on Fat4 and Dchs1

(Qu et al. 2010; Tissir and Goffinet 2013). However, the directional cue (anterior vs. posterior) is unknown.

In *Fat4* or *Dchs1* KO mice, the neurons fail to migrate laterally and stay medially positioned (Fig. 10.7c; Zakaria et al. 2014). A cell-level analysis showed that the neurons become polarized along their migratory path in normal development, on the basis of the localization of the Golgi apparatus relative to the nucleus and elongated cell shape, and that this polarization requires Fat4 and Dchs1. Curiously, at a specific stage of the neuronal migration, Dchs1 and Fat4 are expressed in opposing gradients along the mediolateral axis in the neuroepithelium, in such a way that the neurons migrate away from the medial region of high Dchs1 and towards the lateral region of high Fat4 (Fig. 10.7b). These expression patterns are reminiscent of the proximal–distal gradient of Ds in the Drosophila wing (Ma et al. 2003; Matakatsu and Blair 2004). Dchs1 and Fat4 are each required both within the migrating neurons and the neuroepithelial cells through which they migrate. Based on these results, along with the results of mosaic analyses, it is thought that the polarity of the neuron is established through the interpretation of the long-range gradients of Dchs1 and Fat4 across the epithelium and also by local communication between the neurons within the migration stream (Zakaria et al. 2014).

Cellular misalignment and defective migration are also observed in valvular interstitial cells of *Dchs1* mutant mice where cardiac valve formation is abnormal, and mutations in human *Dchs1* cause mitral valve prolapse, a common cardiac valve disease (Durst et al. 2015). Possibly, in multiple contexts of organogenesis,

the Fat4–Dchs1 system plays a critical role in cell migration by providing directional cues.

10.12 Remaining Outstanding Questions Include What Makes 7-TM Cadherin Polarized

As discussed above, essentially all studies on 7-TM cadherins start with those proteins that already occupy selective cell boundaries along the tissue axis (Figs. 10.4d–g and 10.5a–c). In the microscopic sense, the assembly of the 7-TM cadherin-containing complex across one cell boundary could be made by a positive feedback mechanism; however, such a cell-by-cell polarity does not transform into the zigzag pattern throughout the tissue without an initial bias along the tissue axis. One possible cell-biological mechanism generating such a bias is polarized transport to particular cell boundaries (Fig. 10.4h). This hypothesis has been addressed experimentally by quantitative in vivo imaging and conventional as well as immunoelectron microscopy, using the Drosophila wing and its primordium (wing imaginal disc; Shimada et al. 2006; Harumoto et al. 2010; Matis et al. 2014; Olofsson et al. 2014). A current composite picture is as follows: (1) the Ft–Ds–Fj system interprets the information of the proximal–distal axis (see the explanation of "tissue gradients" above). (2) This system creates a subtle asymmetry of microtubules in the apical region of the cell, orienting along the tissue axis (planar MTs); that is, + end-distal MTs become slightly more abundant than + end-proximal MTs. (3) This asymmetrical MT organization allows + end motor proteins to transport vesicles containing 7-TM cadherins and other core-group members (Fz and Dsh) better to the distal cell boundary (Fig. 10.4h; see continued explanations in Fig. 10.4h legend). (4) This mechanism may contribute to establishing an initial bias in the proximal region where the imbalance of the Ds level is large (Matakatsu and Blair 2004; Harumoto et al. 2010), not necessarily throughout the wing. Open questions related to this hypothesis include:

- The vesicular transport has been imaged in pupal wings; however, the asymmetry of the core group emerges earlier, in growing imaginal discs in larvae (Classen et al. 2005; Aigouy et al. 2010; Sagner et al. 2012). Imaging vesicular transport in the growing disc is technically challenging (explained below).
- Although epithelia generally develop apicobasal MTs, planar MTs may not always exist in all tissues that are acquiring PCP (Devenport 2014). How does the asymmetry of 7-TM cadherins arise in such tissues?
- In tissues where the core group and the Ft–Ds system do not crosstalk, how is the asymmetrical MT organization generated? In such tissues, is the polarity generated by mechanisms other than the polarized transport?

Other approaches to investigating the polarity-generating mechanism include studies of how the localized domain is aligned between cells. The Drosophila

orthologue of Wnt1, Wingless (Wg), has been long considered as an attractive candidate of the tissue-level polarity cue. One of the reasons for this is that Wg is one of the well-studied morphogens that are secreted from organizing centers in Drosophila, and it regulates the expression of *ds* and *fj* in opposite ways in the wing disc (*ds* high in the hinge and *fj* high in the wing blade; Cho and Irvine 2004; Zecca and Struhl 2010). One recent model proposes that gradients of Wg and Wnt4a are the long-range cues, which directly modulate Fz–PCP signaling (Wu et al. 2013). In contrast, an alternative model proposes that the Wg gradient is unlikely to act as a cue itself (Sagner et al. 2012). Data behind this model highlight that gradients of Wg and other morphogens do not play an instructive role in PCP. Instead, each organizing center reorients cells when the disc is small, and this "fixed" polarity is maintained as the tissue grows, and hence no longer depends on long-range biasing cues such as gradients throughout subsequent tissue growth (Aigouy et al. 2010; Sagner et al. 2012). Thus, the exact mechanisms by which the morphogens (or the organizing centers) regulate Fmi/Stan asymmetry early during tissue growth remain to be elucidated.

To address these remaining questions or to devise new models, it is preferable to perform in vivo time-lapse imaging of the growing tissue where the polarized pattern is emerging *de novo*. However, this approach often runs up against technical challenges due to folded smaller tissues that are located deep inside the body of younger animals, such as the Drosophila imaginal discs in immature larvae. One solution is the development of easy-access ex vivo tissue/organ or whole embryo cultures, such as the skin culture that provides a clue about a time window of the hypothetical directional cue and Xenopus embryos (Devenport and Fuchs 2008; Butler and Wallingford 2015). Another more drastic solution is the innovation of reconstitution systems, in which tissues are reconstituted from dissociated single cells, and they grow and acquire at least a local polarity. With this system in hand, we may be able to search for conditions to reproduce long-range polarity through genetic, chemical, and/or physical manipulations. This approach is promising, as illustrated by the airway epithelia that are reconstituted in primary cultures (Vladar et al. 2012; Vladar et al. 2015), and it is being applied for many other tissues/organs. With these new techniques, a detailed mechanistic understanding of how polarity is established may soon be within reach.

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