

Role of Polarity Proteins in the Generation and Organization of Apical Surface Protrusions

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Protruding from the apical surfaces of epithelial cells are specialized structures, including cilia, microplacae, microvilli, and stereocilia. These contribute to epithelial function by cushioning the apical surface, by amplifying its surface area to facilitate nutrient absorption, and by promoting sensory transduction and barrier function. Despite these important roles, and the diseases that result when their formation is perturbed, there remain significant gaps in our understanding of the biogenesis of apical protrusions, or the pathways that promote their organization and orientation once at the apical surface. Here, I review some general aspects of these apical structures, and then discuss our current understanding of their formation and organization with respect to proteins that specify apicobasolateral polarity and planar cell polarity.

The apical surface of epithelial cells, which lines the lumen of sac- and tube-shaped organs and the inner surfaces of the body cavities, forms the interface between the extracellular milieu and underlying tissues. It has numerous functions, including absorption and secretion, immunological surveillance, sensory transduction, and barrier formation. These functions are made possible by several specializations, including the glycocalyx, which cushions and hydrates the apical membrane, the lipids of the apical plasma membrane, which limit water and solute flux, and channels and receptors, which regulate active and passive transport and sense the presence of growth factors, cytokines, and mechanical stimuli (e.g., stretch and shear stress). Finally, projecting from the apical domain of epithelial cells is one or more of the following

apical protrusions: cilia, microplacae, microvilli, or stereocilia (Fig. 1). Below, I review the form and function of these apical protrusions, I summarize the polarity proteins that specify apical identity and that organize the apical surfaces of epithelial cells, and then I describe our current understanding of how these polarity proteins promote the biogenesis and organization of these structures. An expanded review of epithelial polarity is available elsewhere (Apodaca and Gallo 2013).

APICAL MEMBRANE PROTRUSIONS

Cilia

These are projections, 2–20 μm in length, that extend from the apical surface of epithelial cells

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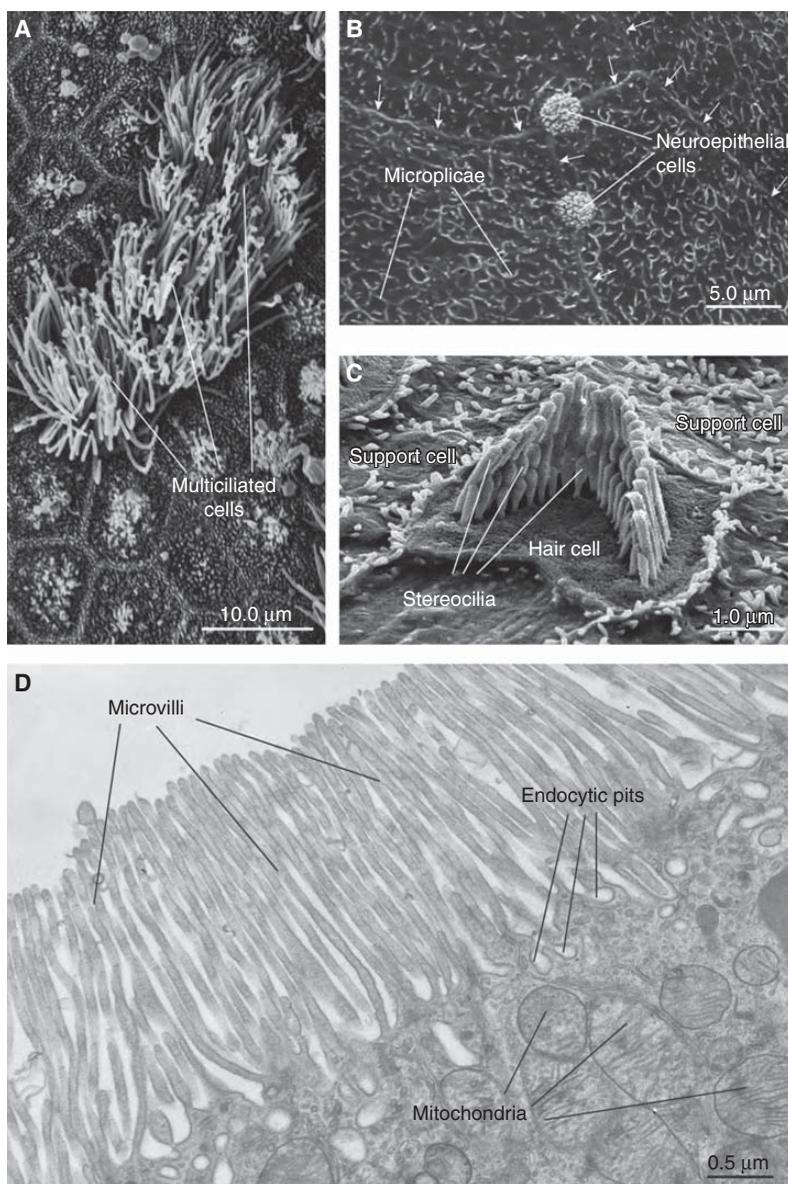


Figure 1. Survey of apical surface protrusions found in epithelial cells. (A) Scanning electron micrograph (SEM) of the apical surface of the rat trachea showing multiciliated cells. Adjacent cells are nonciliated or have rudimentary cilia. (B) SEM of the mucosal surface of the rat proximal urethra. Microplacae are observed at the apical surface of umbrella cells found in this region. Arrows mark the junctional ring of adjacent cells. The apical surfaces of neuroepithelial cells, which are covered by an apical tuft of short microvilli, are interspersed between adjacent cells. (C) SEM of the cochlea of the adult mouse showing a hair cell with associated “hair bundle,” which is comprised of stereocilia arranged in a stair-step configuration. The apical surfaces of adjacent support cells are studded with microvilli. (D) Transmission electron micrograph of microvilli at the apical surfaces of the rat proximal tubule epithelial cells. Endocytic pits and mitochondria are marked. (Electron micrograph in panel C was kindly provided by Jonathan Franks, Center for Biological Imaging, University of Pittsburgh; and micrographs in panels A, B, and D were kindly provided by Wily G. Ruiz, Kidney Imaging Core, University of Pittsburgh.) (Figure is from Apodaca and Gallo 2013; adapted, with permission, from the authors.)

(Fig. 1A) (Ishikawa and Marshall 2011). They have been the focus of intense research as of late, because defects in cilia formation and function lead to a variety of human diseases known as ciliopathies (Waters and Beales 2011). Cilia are the only apical protrusion that contain microtubules at their core, and motile cilia in particular have a peculiar 9+2 organization in which nine peripheral doublets of microtubules surround two single centrally localized ones (Fig. 2A,B). These microtubules are cross-linked by axonemal dyneins, which promote ciliary beating as a result of their intrinsic ATPase activity. Some cells such as the epithelial cells lining the airways (e.g., tracheal epithelial cells) and the ependymal cells lining the brain ventricles have multiple, motile cilia. However, the majority of cells in the body contain a single, “primary” cilium some time during their development (Ishikawa and Marshall 2011). Primary cilia play important roles in development and sensory perception (Tasouri and Tucker 2011). The latter function is thought to be mediated in part by the ability of cilia to initiate Ca^{2+} signaling in response to mechanical deflection, although this role has recently been questioned (Delling et al. 2013, 2016). The microtubules in primary cilia are arranged in a 9+0 pattern: Nine outer doublets are found at the periphery, but the two central microtubules are absent (Fig. 2A). Because they lack dynein arms, 9+0 cilia are most often immotile; however, the 9+0 cilia of nodal cells are motile, undergo clockwise rotation, and play a role in generating left–right asymmetry in the developing embryo (Hirokawa et al. 2009).

Structurally, the base of each cilium is anchored by a cytoplasmic basal body, which in the case of primary cilia is derived from the older, “mother centriole” (Figs. 2 and 3) (Kobayashi and Dynlacht 2011). Basal bodies are composed of nine triplets of microtubules but have no central microtubules. Above this structure is a transition zone that leads to the main body of the cilium called the axoneme. This zone controls the entry and exit of proteins from the cilium and is comprised of transitional fibers, Y-shaped links, and the ciliary necklace (Fig. 3). Transitional fibers link the basal body to the

ciliary membrane. Septins regulate the assembly of the nine-subunit B9 complex, which along with other proteins form the Y-shaped links that couple the microtubules to the ciliary necklace that surrounds the transition zone (Fig. 3) (Hu et al. 2010; Chih et al. 2012).

All ciliary proteins must be transported from the cell proper to the axoneme (Fig. 3). These events require ciliary targeting sequences (e.g., V-X-P-X) (Wang and Deretic 2014). The GTPase requirements for the ciliary traffic of PC1 (alias polycystin-1) are defined: Its exit from the *trans*-Golgi network (TGN) depends on ARF4A (which recognizes the ciliary targeting sequence in PC1), the Arf GTPase-activating protein ASAP1, and RAB6 (Mazelova et al. 2009a; Ward et al. 2011; Wang et al. 2012), and its subsequent transport to the cilia occurs by way of endosomes that are RAB11A, RAB3IP (alias Rabin8), RAB8A, and TRAPP2 complex positive (Knodler et al. 2010; Westlake et al. 2011). Apparently, this pathway is not shared by all cilia-localized proteins as Kim1 uses a pathway dependent on RAB5/RAB8 and Smoothed requires RAB8/RAB23 (Boehlke et al. 2010). Final fusion is likely mediated by the eight-subunit exocyst “tethering factor,” subunits of which are found in the cilium, along with SNAP25 and STX3 (alias syntaxin3) (Mazelova et al. 2009b; Zuo et al. 2009; Fogelgren et al. 2011; Feng et al. 2012). Additional components implicated in transport into the cilium include the BBSome, a family of eight conserved proteins mutated in patients with Bardet–Biedl syndrome (Nachury et al. 2007; Knodler et al. 2010), other centrosomal components (e.g., PCM1 and CEP290) (Kim et al. 2008), as well as the Ran GTPase, KPNB1 (alias β -importin), and nucleoporins (Lechtreck et al. 2009; Dishinger et al. 2010; Fan et al. 2011; Hurd et al. 2011; Kee et al. 2012; Wei et al. 2012). Once delivered to the axoneme, intraflagellar transport (IFT) particles, comprised of IFT-A and IFT-B protein complexes, move ciliary components from the basal body to the tip of the cilia by interaction with the heterotrimeric motor protein KIF3 (alias Kinesin-2) (Fig. 3). Movements in the reverse direction are mediated by IFT-dynein 1b complexes. Only one IFT protein, IFT20

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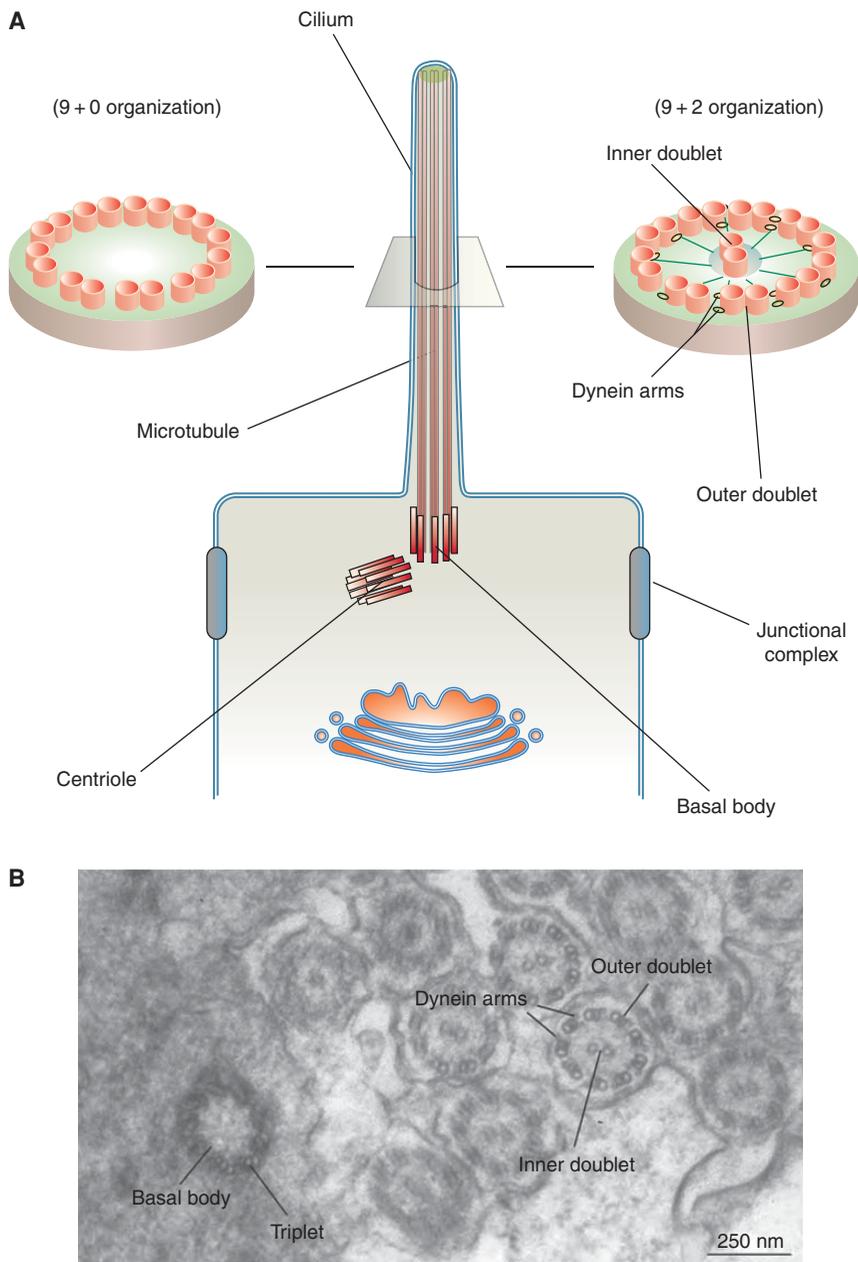


Figure 2. Structure of cilia. (A) Cilia, which project from the apical surface of epithelial cells, have two types of organization. Motile cilia have a 9+2 organization in which a ring of nine microtubule doublets surrounds an inner microtubule doublet. Inner rays and dynein motor arms are present. Immotile cilia, such as so-called “primary cilia” have an outer ring of nine doublets, but lack the inner doublet, dynein arms, and rays. In both types of cilia, a basal body, derived from the centriole, is present and in cross section is comprised of nine bundles of microtubules arranged as triplets (see panel B). (B) Transmission electron micrograph of the zebrafish larval pronephros, which contains multiciliated cells that project cilia into the lumen of this tubular organ. A cross section through a basal body and through the axoneme highlights the structures described above. (Electron micrograph in panel B was kindly provided by Wily Ruiz, Kidney Imaging Core, University of Pittsburgh.) (Panel A from Apodaca and Gallo 2013; adapted, with permission, from the authors.)

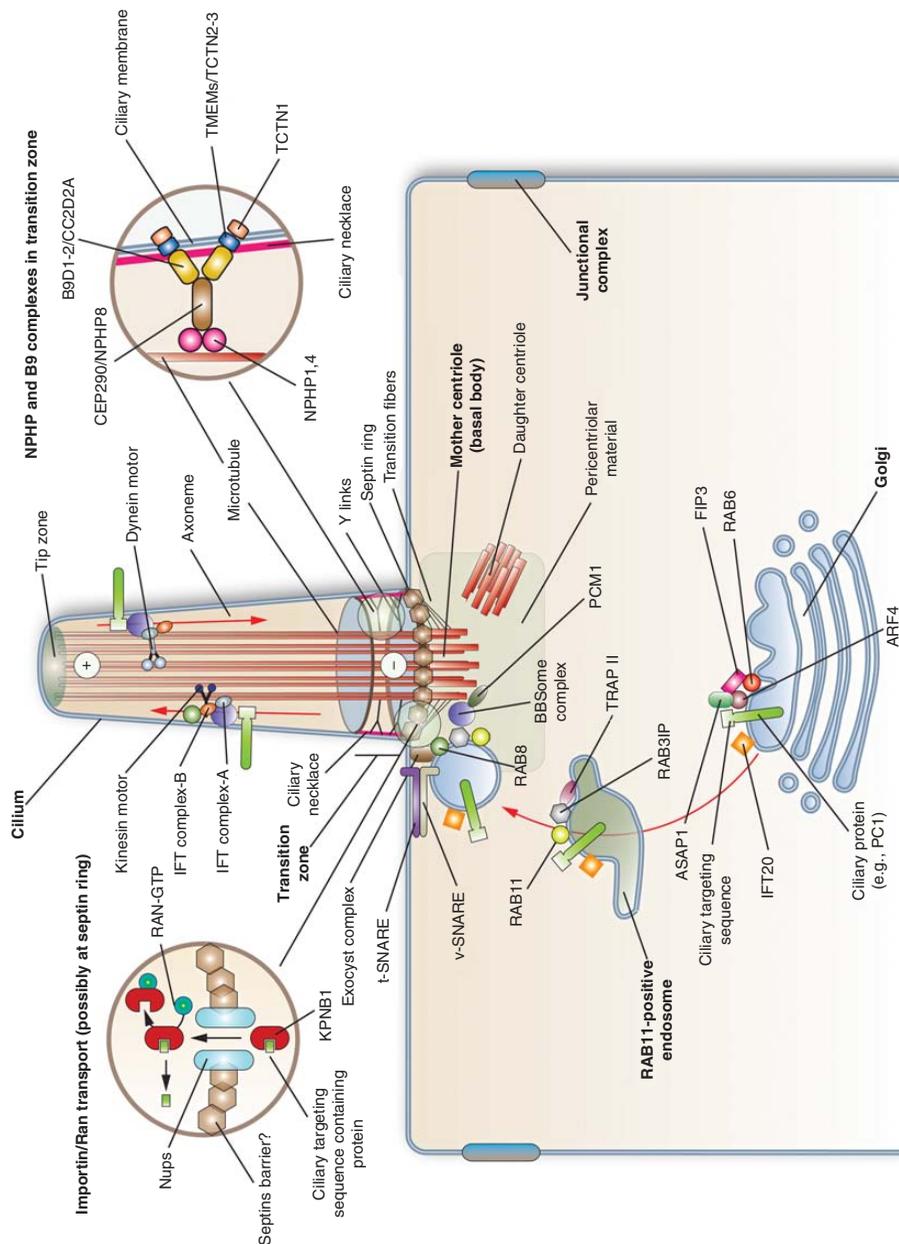


Figure 3. Mechanisms of protein transport into cilia. At the Golgi, cilia resident proteins are recognized and packaged for delivery. In the case of PC1, its ciliary targeting sequence is recognized by ARF4, and is then delivered to RAB11-positive endosomes by way of a RAB6-, ARF4-, and FIP3-dependent mechanism. (*Legend continues on following page.*)

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(a component of IFT-B), associates with RAB8A and traffics from the Golgi to the base of the cilium (Follit et al. 2006; Omori et al. 2008).

Microplicae

Whereas cilia are well-studied microtubule-based apical protrusions, much less is known about actin-rich microplicae. These are ridge-like folds of the plasma membrane and are found at the apical surfaces of stratified epithelia including those lining the cornea, alimentary tract (e.g., oral mucosa, fungiform, and circumvallate papillae of the tongue, esophagus, anal canal), reproductive tract (e.g., vagina, vulva, and the fallopian tubes of postmenopausal women), and lower urinary tract including the ureters, bladder, and upper urethra (Fig. 1B) (Andrews 1976; Collin and Collin 2006; Correr et al. 2006; Ovalle and Nahirney 2008; Khandelwal et al. 2009; Julio et al. 2010; Asikainen et al. 2012). Even nonstratified, “simple” epithelia (e.g., the intercalated cells of the kidney) can have microplicae (Evan et al. 1991). When cross-sectioned and examined by transmission electron microscopy, microplicae appear as short microvilli; however, when examined by scanning electron microscopy, they form extensive ridge-like structures that are approximately 100–200 nm in width and 200–800 nm in height (Andrews 1976). They can be straight or curved, and oftentimes show branching giving the impression of a labyrinthine organization. Whether microplicae are found at the

basolateral surface of cells remains unclear, although based solely on transmission electron microscopy this was proposed to be the case (Andrews 1976). The extracellular surface of microplicae are studded with large, O-glycosylated, transmembrane mucins (Blalock et al. 2007; Asikainen et al. 2012), whereas the cytoplasmic surface is electron dense and contains a loosely organized cytoskeleton comprised of randomly oriented actin filaments (Anderson 1977). Despite their relative abundance in the body, the function of microplicae is not well understood. One possibility is that the associated mucins help to cushion the apical surface, protecting it from abrasion and mechanical damage.

Microvilli and the Apical Brush Border

Unlike microplicae, which are ridge-like and lack an organized actin cytoskeleton, microvilli are finger-like protrusions (1–2 μm long) that contain a core of highly organized bundled actin microfilaments (Figs. 1D and 4). The latter are anchored in the subjacent terminal web. Microvilli are commonly found on epithelia that recover nutrients or water and solutes from the adjacent lumen, such as the enterocytes in the small intestine and the proximal tubule cells of the kidney (Crawley et al. 2014a). In these later two cell types, the microvilli are densely packed and uniform in length and appearance, forming a “brush border” that increases the apical surface area by 15- to 30-fold (Crawley et al. 2014a). The brush border of proximal tubule epithelial

Figure 3. (Continued) The only intraflagellar transport (IFT) protein localized to the Golgi, IFT20, may have a role in promoting protein delivery to the cilium. At RAB11-positive endosomes, RAB11 recruits the TRAPII complex and the RAB8 GEF RAB31P. The latter interacts with the basal body-localized BBSome complex (through the BBS1 subunit), and also recruits and activates RAB8. The BBSome is recruited by PCM1. RAB8 along with the exocyst complex and SNAREs, promotes the docking and fusion of the endocytic vesicle at the base of the cilium. Entry into the cilium is regulated at the transition zone by a septin ring, and the Y-links formed by the NPHP and B9 protein complexes. In addition, import into the cilium may depend on a RAN-GTP/GDP gradient (cytoplasmic RAN-GDP is not shown), which regulates KPNB1 binding to ciliary proteins and their subsequent transport across cilia-associated nucleoporins. Once in the axoneme, the ciliary proteins move along the microtubules by interactions with IFT particles and motor proteins. Anterograde traffic (directed toward the tip of the cilium) is mediated by IFT-B and KIF3 motor complex, whereas retrograde is mediated by IFT-A and the dynein-2 motor complex. The BBSome regulates the IFT complexes assembly at the basal body and its turnaround from anterograde to retrograde transport. (Figure from Apodaca and Gallo 2013; adapted, with permission, from the authors.)

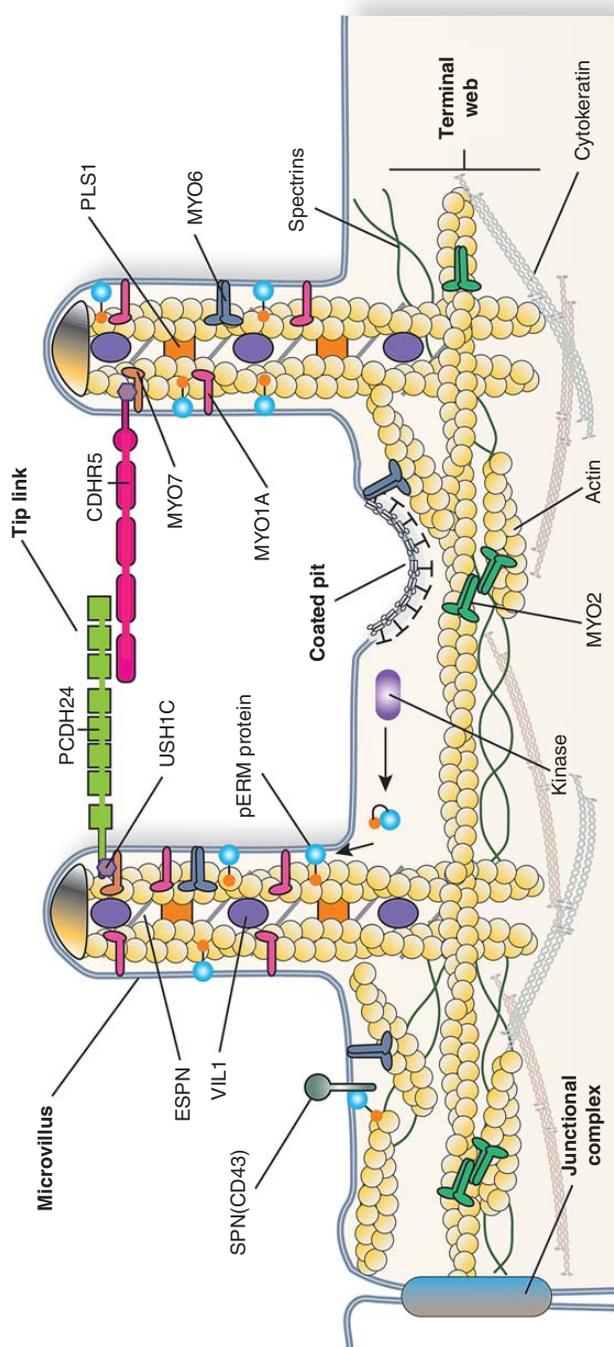


Figure 4. Protein constituents of the epithelial cell brush border. The brush border of enterocytes and kidney proximal tubule epithelial cells is comprised of a regular array of microvilli. Each microvillus contains a core of actin, which is associated with MYO1A, ESPN, PLS1, VIL1, and ERM proteins such as EZR. In their active, phosphorylated state, ERM proteins link the actin cytoskeleton to the membrane, in some cases via the membrane protein SPN (alias CD43). The microvillus actin terminates in “rootlets” that associate with actin, spectrins, MYO2, and cytokeratins to form the terminal web. Adjacent microvilli are linked to one another by tip links, which are comprised of the protocadherins PCDH24 and CDHR5. These proteins are anchored to the actin filaments by way of MYO7 and the adaptor protein USH1C. (Figure from Apodaca and Gallo 2013; adapted, with permission, from the authors.)

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cells contains a specialized intermicrovillar domain, less apparent in enterocytes, which contains coated pits and associated endocytic machinery (see Fig. 1D) (Larsson 1975; Biemesderfer et al. 1992).

Each microvillus contains a core of bundled and cross-linked actin filaments, which are associated with VIL1 (alias villin), PLS1 (alias plastin-1/fimbrin), and ESPN (alias espin) (Fig. 4) (Heintzelman and Mooseker 1992; Revenu et al. 2012; Crawley et al. 2014a). The actin filaments terminate as “rootlets,” which are embedded in the terminal web by MYO2 and spectrins (Fig. 4). Other components of the terminal web include TPM (alias tropomyosins), CALD1 (alias caldesmon), and cytokeratins (Takemura et al. 1988; Heintzelman and Mooseker 1992). Brush-border MYO1A forms a helically arranged series of cross bridges that connect the actin bundles of the microvillus to the plasma membrane (Tyska et al. 2005). Other associated myosins include MYO6, which links the apical surface with the underlying actin cytoskeleton (Ameen and Apodaca 2007; Hegan et al. 2015a), and MYO7B, which is associated with the electron dense distal tips of microvilli (Wolfrum et al. 1998). The latter also forms interactions between the cytoplasmic scaffolding protein USH1C (alias harmonin-a) and the transmembrane protocadherins PCDH24 (alias protocadherin-24) and CDHR5 (alias the mucin-like protocadherin) (Fig. 4) (Reiners et al. 2005, 2006; Crawley et al. 2014b). By forming *trans*-heterophilic interactions, these protocadherins generate the thread-like links that adhere adjacent microvilli in the brush border to one another in a manner analogous to the tip links observed in stereocilia (*vide infra*). An additional major component of microvilli is the EZR (alias ezrin)-RDZ (alias radixin)-MSN (alias moesin) (ERM) family of proteins (Bretscher et al. 2002). In the presence of PtdIns(4,5) P_2 , and on phosphorylation of a conserved threonine residues (T₅₆₇ in EZR), ERM proteins leave their autoinhibited state and form interactions with membrane proteins (via an amino-terminal FERM domain), regulatory molecules, and the microvillar bundles of actin (by way of a carboxy-terminal actin-binding domain)

(Bretscher et al. 2002). In addition to amplifying apical surface area, microvilli are also important for sensory perception, flow sensation, ion channel regulation, Ca²⁺ signaling, and immune system modulation (McConnell et al. 2009; Lange 2011).

Stereocilia

The final actin-based structure discussed are the stereocilia, which are long structures (10–50 μ m in length) that protrude from the apical surfaces of the pseudostratified columnar cells that line the epididymis and the vas deferens, and the apical surface of the hair cells of the middle ear (Figs. 1C and 5A) (Ovalle and Nahirney 2008; Drummond et al. 2012; Barr-Gillespie 2015). Although their function in the male reproductive tract is not well delineated, it is hypothesized that the epididymal stereocilia may act to increase surface area and thus promote fluid resorption as developing sperm move from the seminiferous tubules to the epididymis. In contrast, the stereocilia of the middle ear are required for hearing (Drummond et al. 2012; Barr-Gillespie 2015). Sound vibrations, captured by the pinnae, are transmitted by small bones within the inner ear (malleus, incus, and stapes) across the oval window, creating hydraulic waves in the fluid that fills the cochlea. The latter is a spiral canal covered by rows of one inner and three outer epithelial hair cells arranged in a mediolateral orientation, as well as interdigitating support cells (see outer hair cells in Fig. 5A). Hair cells rest on the basilar membrane, which fluctuate as the sound waves pass through the cochlear fluid (Fig. 5B). The epithelium is innervated from below by afferent and efferent nerve processes.

Protruding from the apical surfaces of the hair cells is a “hair bundle,” comprised of 100–200 stereocilia, which are organized in a staircase-like manner that forms a “V” pattern (Figs. 1C and 5A) (Drummond et al. 2012; Barr-Gillespie 2015). The stereocilia are connected to one another by fine tip links, which are comprised of CDH23 (alias cadherin-23) and PCDH15 (alias protocadherin-15) (Nayak et al. 2007). Other links are found at the base of

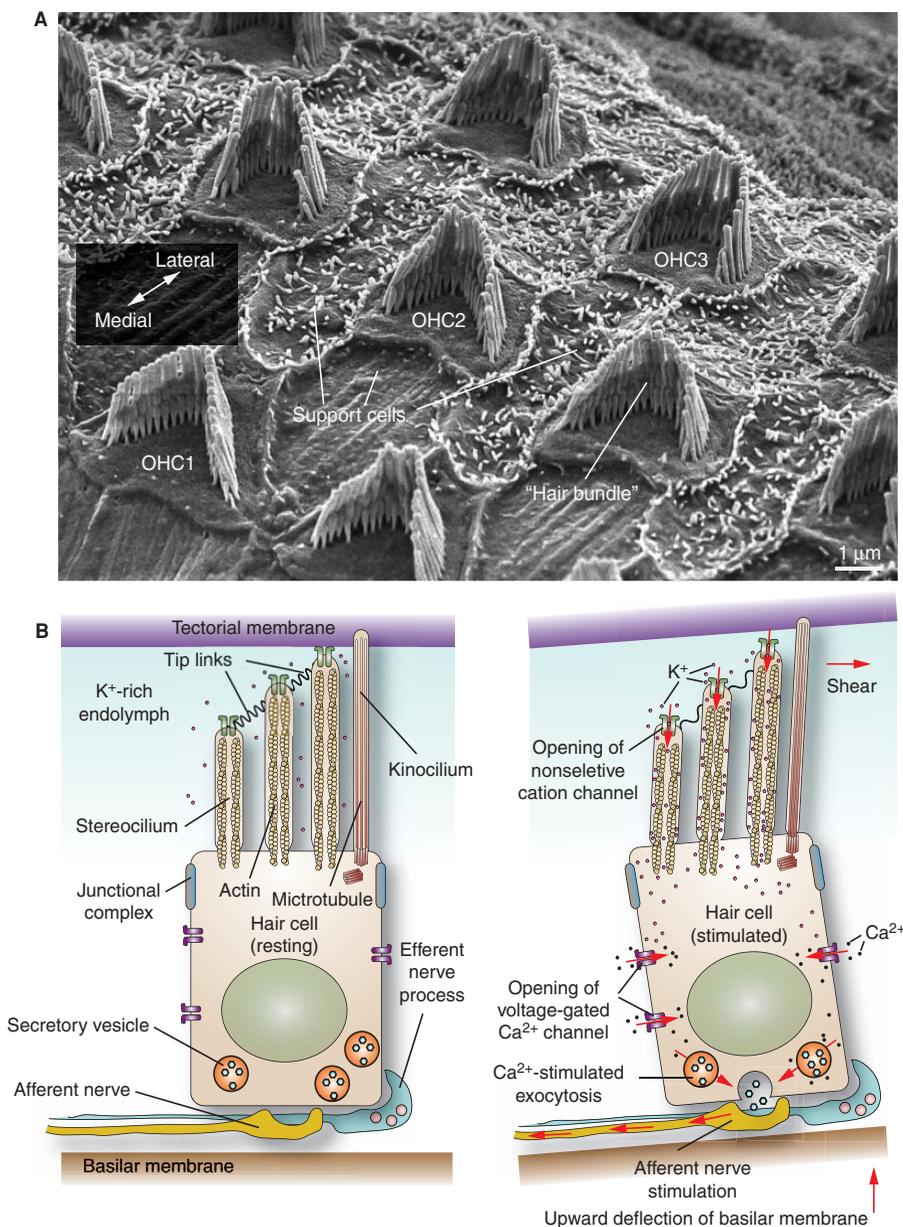


Figure 5. Organization and function of hair cells in the inner ear. (A) Scanning electron micrograph of mucosal surface of adult mouse cochlea after removal of the overlying tectorial membrane. The position of the three rows of outer hair cells (OHC1-3) with associated hair bundle is indicated. The single row of inner hair cells is not shown. Hair cells are surrounded by support cells. The orientation of the lateromedial axis is shown. (B) (Left panel) The basal surface of the hair cell rests on the basilar membrane, while its apical stereocilia, surrounded by K^+ -rich endolymph fluid, insert into the tectorial membrane. Stereocilia are attached to one another via tip links. (Right panel) Sound waves, propagated through the cochlear fluid, cause an upward deflection of the basilar membrane, generating a shear force between the tectorial membrane and the stereocilia. The bending of the stereocilia promotes the opening of a mechanosensitive, nonselective cation channel. The inward movement of K^+ ions through this channel depolarizes the cell, stimulating the opening of voltage-gated Ca^{2+} channels, which increase cytosolic Ca^{2+} . The subsequent release of neurotransmitters triggers an action potential in the afferent neuronal processes. (Panel B from Apodaca and Gallo 2013; adapted, with permission, from the authors.)

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the stereocilia (Nayak et al. 2007). Just behind the apex of the stereocilia bundle lies a single microtubule-rich kinocilium, which in cochlear hair cells disappears soon after birth (Barr-Gillespie 2015). The tips of the stereocilia are embedded within the overlying tectorial membrane. In response to sound waves, upward movements of the basilar membrane causes the stereocilia to bend, gating open a mechanically sensitive ion channel, which allows for the influx of potassium ions into the cell (Fig. 5B) (Richardson et al. 2011). The resulting depolarization opens voltage-gated calcium channels that conduct calcium inward, triggering the release of neurotransmitters that stimulate action potentials in nearby sensory nerve processes.

POLARITY PROTEINS AND THEIR FUNCTIONS

Temporally, apical protrusions develop once the epithelial cell establishes distinct apical and basolateral plasma membrane domains separated by a junctional complex. The generation of this apicobasolateral polarity is a complex process that requires membrane trafficking events, the cytoskeleton, signaling cascades, and polarity proteins, which specify and reinforce cortical membrane domain identity (Bryant and Mostov 2008; Datta et al. 2011). In addition to apicobasolateral polarization, which occurs in an orientation that is orthogonal to the tissue plane, epithelia also show polarity in an axis that runs parallel to the apical surface of the tissue. This latter type of polarity, called “planar cell polarity” (PCP), accounts for the orientation of hairs, feathers, scales, insect bristles, and cilia, as well as polarized cell division, coordinated beating of cilia, and collective cell migration (Devenport 2014, 2016; Sebbagh and Borg 2014). The latter includes convergent extension, a process in which sheets of cells converge in one axis and then extend in the other and is important during gastrulation and neurulation. In the following section, a brief overview of the proteins involved in apicobasolateral polarity and PCP is provided; however, those readers seeking additional information about these pathways are directed to other reviews in the literature

(Suzuki and Ohno 2006; Assemat et al. 2008; Schluter and Margolis 2012; Devenport 2014, 2016; Sebbagh and Borg 2014).

Apicobasolateral Polarity

The development of apicobasolateral polarity depends on the activity of at least three highly conserved polarity complexes, originally described in *Drosophila* and *Caenorhabditis elegans* (Suzuki and Ohno 2006; Assemat et al. 2008; Bryant and Mostov 2008). These include the following (see Table 1 for summary of these and other polarity genes described in this review) (Fig. 6A): (1) The *Drosophila* Crumbs complex is comprised of Crumbs (CRB1-3 isoforms in vertebrates), Stardust (MPP5 in vertebrates; alias PALS1), and Discs lost (INADL and MUPP1 isoforms in vertebrates; alias PATJ). In vertebrates, this complex is localized to the apical side of the junctional complex and is required for tight junction formation; (2) the *Drosophila* Par complex is comprised of atypical protein kinase cells (aPKCs) (PRKCI and Z isoforms in vertebrates), Bazooka (PARD3 and 3B isoforms in vertebrates), and Par-6 (PARD6A, B, G isoforms in vertebrates), as well as the Rho family GTPase Cdc42. The Par complex, like the Crumbs complex, is localized near the junctional complex and modulates its assembly; (3) the *Drosophila* Scribble complex includes Scribble (SCRIB in vertebrates), Discs large (DLG1-5 isoforms in vertebrates), and Lethal giant larvae (LLGL1-2 isoforms in vertebrates). In vertebrates, the Scribble complex is found along the lateral membrane domain (Schluter and Margolis 2012; Tepass 2012). Although genetically and functionally linked, polarity complexes can form subcomplexes. For example, in *Drosophila* and vertebrates, Bazooka recruits an aPKC-Par-6 subcomplex to the adherens junction (the apical-most junction in flies), whereas PARD3 recruits the PRKC-PARD6 subcomplex to the tight junction of vertebrates. aPKC-Par-6 and PRKC-PARD6 are released to the apical domain as polarity establishment completes (Suzuki et al. 2002; Harris and Peifer 2005; Horikoshi et al. 2009). It is worth emphasizing that the vertebrate orthologs of these apicobasolateral

Table 1. *Drosophila* and vertebrate polarity proteins described in this review

<i>Drosophila</i> protein	Vertebrate ortholog/paralog	Alias
Apicobasolateral polarity proteins		
Crumbs	CRB1- 3	
Stardust	MPP5	PALS1
Discs lost	INADL; MUPP1	PATJ
Atypical protein kinase C	PRKCi; PRKCz	aPKC
Bazooka	PARD3, 3B	Par3
Par-6	PARD6A,B,G	
Cdc42	CDC42	
Scribble	SCRIB	
Discs large	DLG1- 5	
Lethal giant larvae	LLGL1-2	
Par-1	MARK3; MARK2	
14-3-3ε/ζ	YWHAZ; YWHAE	Par5
Yurt	EPB41L5	Band 4.1 like 5
Coracle	EPB41	Band 4.1
ATPα/β	ATP1A1-4; ATP1B1	Sodium potassium ATPase
Nrx-IV	CNTNAP1	Neurexin-4
Lkb1 kinase	STK11	Lkb1/Par4
Planar cell polarity proteins		
Flamingo	CELSR1-3	
Van Gogh	VANGL1-2	
Prickle	PRICKLE1-4	
Frizzled	FZD	
Diego	INVS; DIV	Inversin; diversin
Dishevelled	DVL1-3	
-	PTK7	Protein tyrosine kinase 7
Secretory 24AB	SEC24B	
Scribble	SCRIB	
Fat	FAT1-4	
Daschous	DCHS1-2	Protocadherin-16; protocadherin-23
Four jointed	FJX1	
Inturned	INTU	
Fuzzy	FUZ	
Fritz	WDPCP	BBS15
Flattop	CFAP126	
Inscuteable	INSC	
Partner of Inscuteable	GPSM2	LGN
Gαi	GNAI	

eral polarity proteins have a large number of isoforms and splice variants, which can be expressed in a tissue-specific manner (Assemat et al. 2008). However, their tissue-specific functions are often unknown.

One important function of polarity complexes is to bring about the formation and expansion of their associated membrane do-

main: In vertebrates and *Drosophila*, the Crumbs and Par complexes promote apical membrane formation, whereas, in *Drosophila*, the Scribble complex promotes basolateral surface expansion (Bryant and Mostov 2008). Whether SCRIB functions identically in mammalian cells remains to be established (Schluter and Margolis 2012), and as noted below SCRIB appears

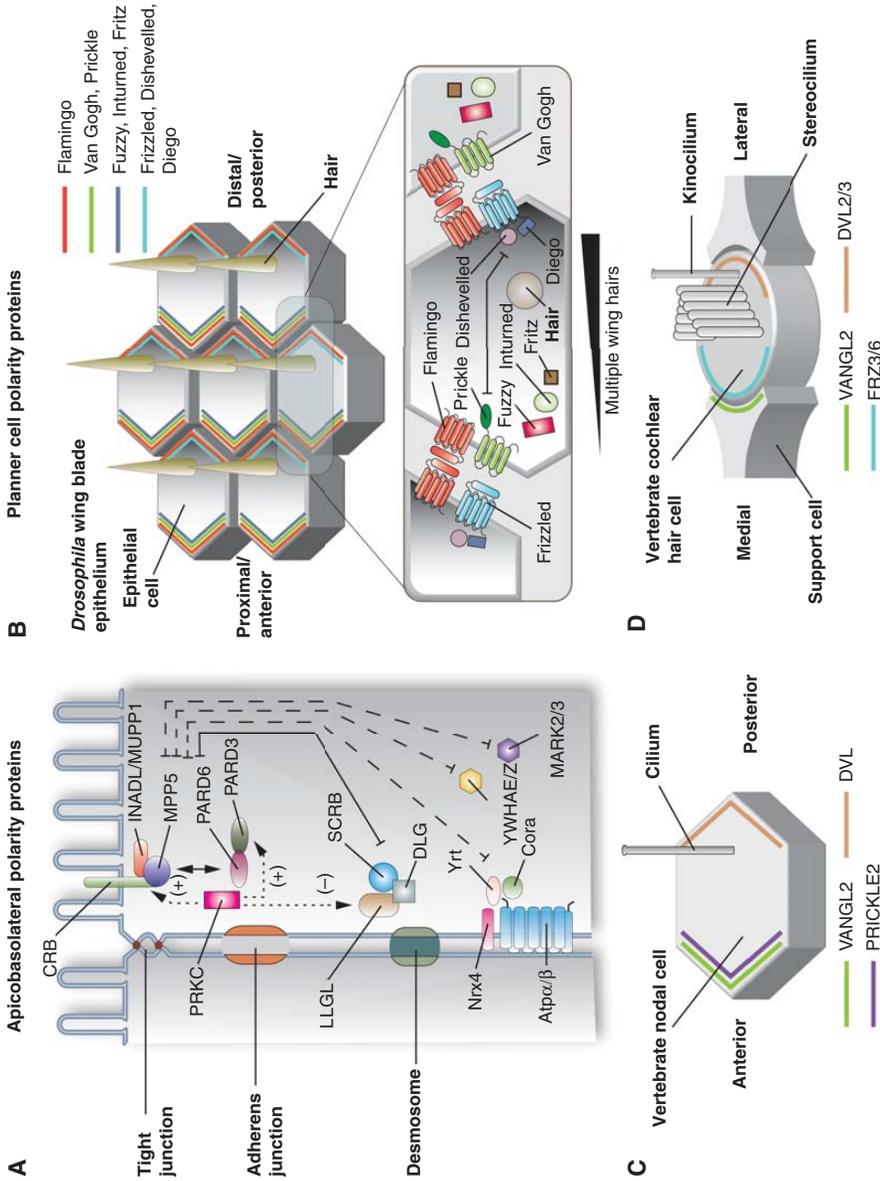


Figure 6. Polarity proteins in epithelial cells. (A) Apicobasolateral polarity proteins. The apical-most Crumbs complex includes the transmembrane proteins CRB1-3 and the cytoplasmic proteins INADL/MUPP1 and MPP5. The other apical complex is the Par complex, which is comprised of PRKC, PARD3, and PARD6. PRKC phosphorylates CRB and PARD3, promoting apical identity. (*Legend continues on following page.*)

to play an important role in PCP (Montcouquiol et al. 2003). Polarity complexes can function cooperatively or in an antagonistic fashion. An example of the former is the interaction between the Par and Crumbs complexes, which is mediated by direct binding of MPP5 or CRB3 to PARD6 in vertebrates, or Discs lost to Par-6 in *Drosophila* (Hurd et al. 2003; Nam and Choi 2003; Lemmers et al. 2004). In contrast, in *Drosophila*, binding of Par-6 (in a complex with aPKC) to Lethal giant larvae leads to its phosphorylation by aPKC, triggering the dissociation of Lethal giant larvae from the membrane (Plant et al. 2003; Laprise and Tepass 2011), reinforcing apical domain identity.

Finally, there are additional polarity proteins involved in basolateral membrane formation including vertebrate orthologs of the *C. elegans* proteins PAR-1 and PAR-5 (MARK2-3 and YWHAE/YWHAZ in vertebrates) (Benton and St Johnston 2003; Cohen et al. 2004), RAC1 and phosphoinositide-3-kinase (Chartier et al. 2011), DLG5, which binds to STX4 (alias Syn-taxin-4) and regulates basolateral trafficking of CDH1 (alias E-cadherin) (Nechiporuk et al. 2007), and the *Drosophila* Yurt/Coracle complex, which is comprised of Yurt (EPB41L5 in vertebrates), Coracle (EPB41 in vertebrates), Atp α/β (ATP1A1-4 and ATP1B1 in vertebrates; alias Na⁺, K⁺-ATPase), and Nr x -4 (CNTNAP1 in vertebrates; alias neu r exin-4) (Fig. 6) (Laprise et al. 2009). In mammalian cells, the Yurt ortholog, EPB41L5, is similarly required for lateral membrane formation (Laprise et al. 2009).

Planar Cell Polarity

Similar to apicobasolateral polarity, PCP requires a cluster of highly conserved polarity proteins, which were originally described in invertebrates (Fig. 6B). The “core complex” is required for the establishment of local polarity within and between adjacent cells, and mutation of any one of its components alters the localization of the others (Devenport 2014, 2016; Sebbagh and Borg 2014). The name of these and other PCP proteins are summarized in Table 1. Early in polarization, the core complex proteins are intermixed at the apical surface, but then become segregated to opposite sides of the apical surface (i.e., distal or proximal), with the exception of the *Drosophila* seven-transmembrane domain atypical cadherin Flamingo (CELSR1-3 in vertebrates), which is found at both locations (Fig. 6B). Segregation appears to depend on negative repulsive interactions, E3-ligase-dependent degradation of core components, positive stabilization of clusters, and directed vesicular traffic (Devenport 2014; Sebbagh and Borg 2014). In addition to Flamingo, the distal complex members in *Drosophila* include the receptor Van Gogh (vertebrate isoforms are VANGL1-2) and the cytoplasmic protein Prickle (PRICKLE1-4 in vertebrates) (Fig. 6B). The proximal complex is comprised of Flamingo, the receptor Frizzled (FZD3&6 in vertebrates), and the cytoplasmic proteins Diego (INVS and DIV in vertebrates: aliases inversin and diversin) and Dishevelled (DVL1-3 in vertebrates) (Fig. 6B). The receptors Frizzled and Van Gogh, in conjunction

Figure 6. (Continued) In contrast, PRKC-dependent phosphorylation of LLGL stimulates its degradation, and thus represses basolateral identity. Basolateral polarity is promoted by the Scribble complex, comprised of SCRIB, DLG, and LLGL. Additional basolateral polarity proteins include YWHAE/Z and MARK2/3, and the *Drosophila* Yurt (Yrt)/Coracle (Cora) complex, which includes Yrt, Cora, neu r exin4 (Nr x 4), and Na⁺/K⁺-ATPase α -subunit (NaK α). (Panel A from Apodaca and Gallo 2013; adapted, with permission, from the authors.) (B) Planar cell polarity (PCP) proteins in *Drosophila* wing blade epithelium. An actin-based “hair” extends from the apical surface of each epithelial cell. The localization of the core PCP anterior complex (Flamingo, Van Gogh, Prickle), the core PCP posterior complex (Frizzled, Dishevelled, and Diego), and PCP effector proteins (Fuzzy, Inturned, and Fritz) is shown at the apical surfaces of wing epithelial cells. A portion of the interface between adjacent cells is magnified in the *inset*, which depicts the interaction between opposing PCP protein subcomplexes and PCP effectors. The gradient of the PCP effector protein Multiple wing hairs is indicated (black indicates higher expression). (C) Localization of core PCP proteins in vertebrate nodal cells. (D) Localization of core PCP proteins in the vertebrate cochlear hair cell and associated support cells.

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with Flamingo, are thought to bridge the two complexes at sites of cell–cell contact, and in doing so propagate polarity from cell to cell.

Although conserved across species, and required for the organization of apical protrusions, our knowledge of the distribution of core PCP proteins in vertebrates is limited and is not always as expected. For example, in vertebrate node cells only VANGL2, PRICKLE2, and DVL have been localized (Fig. 6C) (Antic et al. 2010; Hashimoto et al. 2010). Likewise, in vertebrate hair cells only the distribution of VANGL2, FRZ3/6, and DVL2/3 are known (Fig. 6D). Surprisingly, hair cells FRZ3/6 and DVL2/3 are localized on opposite poles of the cell, and VANGL2 is apparently localized to support cells and the not the hair cell itself (Wang et al. 2005; Giese et al. 2012). Although the number of isoforms of core PCP proteins is expanded in vertebrates, knowledge about their location and function awaits further study.

In addition to the six members of the core module, PCP proteins in vertebrates have been expanded to include several additional ones, including PTK7, a member of the receptor tyrosine kinase family (that apparently lacks kinase activity), SEC24B, a component of the COPII vesicular coat, and SCRIB, which as described above plays an important role in establishing apicobasolateral polarity (Lu et al. 2004; Merte et al. 2010; Wansleben et al. 2010). All three of these proteins show genetic interactions with VANGL2, and COPII is required for VANGL2's exit from the endoplasmic reticulum (Wansleben et al. 2010). Moreover, biochemical studies have confirmed a direct protein–protein interaction between the SCRIB PDZ3-4 domains and the VANGL2 carboxyl terminus (Kallay et al. 2006). The requirement for additional PCP components likely reflects the need of vertebrates to meet the specialized needs of having multiple tissue types, all requiring some form of PCP to function.

The core module promotes asymmetry within and between cells, but it does not have the ability to orient its action to the plane of the tissue. This tissue-level alignment is transmitted by way of the evolutionarily conserved “global module” (Devenport 2014; Sebbagh and Borg

2014). The exact relationship of the global and core modules remains unclear, but the core module must have a mechanism to sense the output of the global module. In *Drosophila*, the global module is comprised of the Golgi-associated ecto-kinase Four-jointed (FJX1 in vertebrates), and two atypical cadherins: Fat (FAT1-4 in vertebrates) and Daschous (DCHS1-2 in vertebrates) (Matis and Axelrod 2013). In *Drosophila*, Four-jointed phosphorylates the *extracellular* domains of Daschous and Fat, which alters their affinity for one another. Four-jointed and Daschous form opposing gradients across various tissues, possibly in response to a morphogenic gradient, which leads to subtle gradients of Fat at the cellular level. Although the Fat-Daschous pathway is conserved in vertebrates (Matis and Axelrod 2013), and appears to regulate PCP in mammals (Saburi et al. 2008; Zakaria et al. 2014), it may not be regulated by the vertebrate homolog of Four-jointed, FJX1 (Sadeqzadeh et al. 2014). At present, our understanding of how individual tissues in vertebrates translate global orientation cues to planar polarity at the cellular level is very much a work in progress.

To end this subsection, I touch on the “PCP effector proteins,” which are thought to function downstream of the core PCP proteins. In general, PCP effectors regulate the cytoskeleton, which as noted above plays a major role in generating apical surface protrusions (Devenport 2014; Sebbagh and Borg 2014). In *Drosophila* wing blade epithelium, the PCP effectors Inturned (INTU in vertebrates), Fuzzy (FUZ in vertebrates), and Fritz (WDPCP in vertebrates) apparently act downstream of Van Gogh/Prickle to regulate the formation of the single actin-based hair that projects from the apical surface of each cell that comprises the wing blade epithelium (Fig. 6B) (Wong and Adler 1993; Adler et al. 2004; Collier et al. 2005; Strutt and Warrington 2008). Although their function remains somewhat mysterious, all three proteins interact with one another and they segregate to the anterior portion of the apical domain. Mutations in the genes that encode these proteins results in the presence of multiple hairs on wing epithelial cells. Thus, they appear to be negative regulators of the actin cytoskeleton. These proteins are

thought to act in part by modulating the activity of the protein called Multiple wing hairs, a formin-like actin-binding protein that is enriched on the posterior side of the cell (Fig. 6B) (Wong and Adler 1993; Collier et al. 2005). Interestingly, Multiple wing hairs also antagonize actin formation, indicating its primary role may be to modulate the extent of actin formation at the apical surface (Yan et al. 2008; Lu et al. 2015b). Although our understanding of PCP effector proteins in vertebrates is currently deficient, they are implicated in the formation of apical protrusions, a topic broached in more detail below.

Interactions between Apicobasolateral Polarity Determinants and PCP Proteins

As noted at the start of this section, apical protrusions only develop once the apical surface is established. As such, there is likely to be important cross talk between proteins that promote apicobasolateral polarity and those that specify PCP. Indeed, work in *Drosophila* shows that Frizzled's activity and stability are regulated by aPKC-dependent phosphorylation, and aPKC's activity is, in turn, antagonized by Bazooka (Djiane et al. 2005). Moreover, Dishevelled, possibly acting downstream of Frizzled8, regulates the localization and activity of Lethal giant larvae in the *Xenopus* ectoderm and the *Drosophila* follicular epithelium (Dollar et al. 2005). Because Lethal giant larvae antagonizes the action of the Par complex, and Frizzled8 overexpression leads to dissociation of Lethal giant larvae from the cortex, Frizzled/Dishevelled may promote apical domain formation. Finally, in *Drosophila*, the apicobasolateral polarity determinant Scribble binds directly to Van Gogh and acts as an effector of PCP establishment (Lee et al. 2003). Obviously, there is still much to be learned about how these two polarity-generating systems modulate each other's activity.

POLARITY PROTEINS AND THE BIOGENESIS AND ORGANIZATION OF CILIA, MICROVILLI, AND STEREOCILIA

This section examines the role of polarity proteins in the generation and organization of api-

cal membrane protrusions. The focus here is on vertebrate systems, but when relevant, invertebrate biology is also presented. What quickly becomes apparent is that our current understanding is limited, and there is a need for both mechanistic- and observation-based studies in multiple cell types to fully comprehend the links between apical projections and the broad diversity of polarity proteins and their isoforms. Such information would not only increase our understanding of how cells and tissues are formed, but also provide insights into why defects in the generation and function of apical projections leads to diseases that include blindness, deafness, sterility, and a raft of other developmental and adult afflictions (Muller et al. 2008; Sang et al. 2011; Waters and Beales 2011; Sebbagh and Borg 2014). I note that despite their presence on numerous epithelia and their fascinating structure there is almost nothing known about the biogenesis of microplcae or the role of polarity proteins in their formation or function. Thus, the following section is focused on cilia, microvilli, and stereocilia.

Cilia

This subsection begins with a brief overview of the process that builds cilia in vertebrates, and ends with a description of potential steps in which polarity proteins may intervene. Ciliogenesis is somewhat different in invertebrates and the reader is directed to the following review for additional insights into ciliogenesis in these organisms (Lee and Chung 2015).

Cilia Biogenesis

Depending on cell type, ciliogenesis occurs by one of two pathways (Fig. 7) (Sorokin 1962, 1968; Reiter et al. 2012; Chang et al. 2015): (1) In "intracellular ciliogenesis" a "ciliary vesicle" forms on the surface of the basal body and the forming axoneme starts to extend before docking with the apical plasma membrane. This pathway is important for the formation of primary cilia (Sorokin 1962); (2) In "extracellular ciliogenesis," the centriole is delivered below the apical plasma membrane, a ciliary vesicle is

formed, but extension of the axoneme occurs at the surface. This latter pathway is observed in vertebrate epithelial cells with multiple motile cilia (Sorokin 1968).

Intracellular ciliogenesis begins with maturation of the mother centriole, a process that includes the addition of a pinwheel-shaped structure, consisting of at least five proteins, that forms at the distal ends of the centriole (Kobayashi and Dynlacht 2011; Lee and Chung 2015). It is called the “distal appendage” at this stage, and will ultimately sit at the interface between the apical plasma membrane and the ciliary membrane, forming a key component of the transition fibers observed within the transition zone (see Fig. 3; not depicted in Fig. 7). Some centrioles also contain subdistal appendages and a “basal foot,” which anchors microtubules (Garcia and Reiter 2016). The distal appendages serve as a docking site for “distal appendage vesicles,” which are likely derived from RAB11A-positive recycling endosomes (Westlake et al. 2011). In a process that requires the activity of the membrane-tubulating proteins EHD1 and EHD3 (Lu et al. 2015a), distal appendage vesicles undergo a fusion process that culminates in the formation of the ciliary vesicle. Further maturation of this vesicle and the forming basal body requires the recruitment of IFT components, other components of the transition zone, and release of the centriolar protein CCP110 and its binding partner CEP97, both

of which negatively regulate centriole-to-basal body conversion (Spektor et al. 2007). In addition, the RAB11A effector and guanine nucleotide-exchange factor RAB3IP recruits RAB8 (Knodler et al. 2010), which further promotes maturation of the ciliary vesicle to a structure called the “ciliary sheath.” Thus, RAB11A and its effector cascade not only function after cilia are established (see Fig. 3), but are also key components of the pathway that builds cilia (Fig. 7). Encapsulated within this sheath region is the emerging “ciliary membrane,” generating a “cilia precursor” that contains a double membrane structure. After further modification, including axoneme elongation and building of the transition zone, the cilia precursor undergoes docking, which results in fusion of the ciliary sheath with the apical membrane (Fig. 7). The sheath gives rise to the “periciliary membrane” (Fig. 7).

Vertebrate multiciliated cells undergo extracellular ciliogenesis. In these cells, the centrioles are duplicated from within a specialized structure called the deuterostome, which is derived from the daughter centriole (Al Jord et al. 2014). The duplicated centrosomes are delivered to just below the surface, and then undergo differentiation to basal bodies as a ciliary vesicle is formed. Sheath formation is either reduced or absent. The ciliary vesicle/sheath then appears to directly fuse with the apical plasma membrane before axoneme and transition zone assembly (Fig. 7) (Sorokin 1968).

Figure 7. (*Continued*) Initially, a mother centriole with attached distal appendages accumulates RAB11A-positive distal appendage vesicles, which subsequently recruit EHD1/3, triggering a fusion event that results in the formation of a “ciliary vesicle.” Concomitantly, the centriole discharges CEP90 and CCP110, marking the conversion of the centriole to the basal body. Either before, or subsequent to ciliary vesicle formation, FUZ recruits DVL, the exocyst subunit EXOC4, and eventually RSG1 to the vesicle surface. In a similar fashion, RAB11 recruits the RAB8 exchange factor RAB3IP. In the next step, protein components from the Golgi (e.g., IFT20, ciliary membrane proteins, and PCP core proteins such as VANGL2), from recycling endosomes (e.g., RAB8), and from the cytoplasm (e.g., IFT and transition zone proteins) arrive at the ciliary vesicle and begin to assemble the axoneme and transition zone. This step is marked by the presence of the ciliary sheath, which will ultimately give rise to the periciliary membrane, and covers the inner ciliary membrane. Expansion of both membranes likely requires continued input from endosomes and Golgi. The ciliary precursor undergoes fusion in a process called “docking,” followed by further building of the axoneme and transition zone. The cilium is associated with both microtubules and actin filaments. (Smaller panel) Extracellular ciliogenesis is observed in cells with multiple cilia. Here, the centriole is delivered to a position just below the apical surface, undergoes maturation, and a ciliary vesicle/sheath is formed. The latter undergoes fusion with the apical plasma membrane, a step that is followed by assembly of the transition zone and axoneme at the cell surface.

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As a final note, the centriole is embedded within the actin-rich cortex underlying the apical surface, and short interconnecting actin filaments connect neighboring centrioles (Sandoz et al. 1988; Chailley et al. 1989). Thus, it should not be surprising that the actin cytoskeleton plays an important but somewhat poorly understood role in docking and ciliary movements.

Role of Apicobasolateral Polarity Proteins in Ciliogenesis

In this and the following subsection, it is presumed that many of the proteins and signaling events will be conserved, but this may depend on organism, cell type, and pathway for ciliogenesis. Both Crumbs and Par complexes are localized to cilia (Fan et al. 2004; Sfakianos et al. 2007; Zuo et al. 2011). Depletion of CRB3-CLPI, a splice variant of CRB3 that ends in the amino acid sequence C-L-P-I (single amino acid code) instead of the more common sequence E-R-L-I, leads to cilia loss, indicating that CRB3-CLPI is an important contributor to cilia formation (Fan et al. 2007). Similarly, depletion of PARD3 or inactivation of PRKC by way of inhibitors leads to defects in cilia formation and elongation (Sfakianos et al. 2007; Pruliere et al. 2011).

Crumbs and Par complexes are also important for the formation of the photoreceptor outer segment, a modified cilium that projects from the apical region of photoreceptor cells, and which contains stacks of membrane packed with RHO (alias rhodopsin) photopigment. A basal body sits at the interface between the outer and inner segments, which are bridged by a connecting cilium. In flies, a structure analogous to the outer segment is called the rhabdomere (an actin-rich structure), which forms a subdomain distinct from the rest of the apical domain. Mutations in *CRB1* lead to photoreceptor degeneration in patients with retinitis pigmentosa type 12 and Leber congenital amaurosis (den Hollander et al. 1999, 2001). Moreover, there is a strong body of genetic evidence in *Drosophila* and evidence in zebrafish that both the Crumbs and the Par complexes are important for outer segment/rhabdomere for-

mation and growth (Izaddoost et al. 2002; Pellikka et al. 2002; Nam and Choi 2003; Omori and Malicki 2006; Pocha et al. 2011; Krock and Perkins 2014). In the case of the fly, Bazooka is found at the adherence junction of the photoreceptor cell and is required for apical targeting of Crumbs and aPKC/Par-6, and loss of any of the Crumbs complex member leads to mislocalization of the Par complex proteins (Nam and Choi 2003). This loss likely reflects an interaction between Discs lost and Par-6, which is proposed to provide cross talk between these two complexes in the fly eye (Nam and Choi 2003).

How the Crumbs and Par complexes promote ciliogenesis remains an open question. A key step in ciliogenesis is the maturation of the centriole (Kobayashi and Dynlacht 2011). In this regard, PAR6G is associated with the mother centriole (Fig. 7), and it regulates the recruitment of proteins to this organelle, including DCTN1 (p150-Glued subunit of dynactin) and PAR6A, steps that are important for proper ciliogenesis (Dormoy et al. 2013). There are also some indications that one role of apicobasolateral polarity proteins is to promote vesicle traffic and protein entry into the cilium. For example, an association between CDC42-PARD6 with the exocyst subunit EXOC5 (alias Sec10) is observed at the cilium, indicating that the Par complex may recruit proteins at active sites of fusion (Fig. 7) (Zuo et al. 2011). In addition, CRB3 interacts with KPNB, an association that, as noted above, is likely to have a role in regulating the entry of proteins to the cilia (Fan et al. 2007). Furthermore, PARD3 interacts with the motor protein KIF3 (Fig. 7), and PRKC activity is required for microtubule organization during ciliogenesis (Fan et al. 2004; Sfakianos et al. 2007). Therefore, the Par complex may regulate the stability of the ciliary axoneme and also direct the polarized traffic of proteins along the ciliary microtubules by way of IFT. Obviously, much work remains to understand whether or how apicobasolateral polarity proteins regulate critical steps in ciliogenesis, including the positioning, docking, and fusion of the cilia precursor with the apical membrane, whether they regulate formation and subsequent transport

through the ciliary barrier (e.g., transition zone), and what functions they play to promote the import of proteins into the forming axoneme.

Role of PCP Proteins in Cilia Formation

In addition to the Crumbs and Par complexes, PCP proteins are also implicated in ciliary biogenesis and function. INVS and VANGL2 are localized to the ciliary axoneme, whereas DVL is asymmetrically positioned at the base of the basal body (Fig. 7) (Otto et al. 2003; Ross et al. 2005; Park et al. 2008). Moreover, several PCP proteins are associated with the centrosome, and PCP proteins are known to be important in orienting the mitotic spindle during cell division (Sebbagh and Borg 2014).

There is a growing body of evidence that implicates PCP proteins in early steps of ciliogenesis, docking in particular (Fig. 7). For example, in the multiciliated cells of the *Xenopus* mucociliary epithelium, Vangl2 is required for docking, as is Dvl (Park et al. 2008; Mitchell et al. 2009). The latter not only regulates docking of basal bodies, but also modulates polarized ciliary beating in an anterior-to-posterior direction (Park et al. 2006). In *Xenopus*, Dvl may function in at least two ways. First, it activates RhoA (Park et al. 2008), which is implicated in ciliogenesis (Pan et al. 2007), and is a well-known modulator of the actin cytoskeleton. As noted above, actin is enriched at the apical surfaces of multiciliated cells and is required for the apically directed movement and docking of basal bodies (Boisvieux-Ulrich et al. 1990; Dawe et al. 2009; Bershteyn et al. 2010). Second, in *Xenopus*, Dvl regulates the recruitment of vesicles and the exocyst subunit Exoc4 (alias Sec8) to the maturing centriole/basal body (Park et al. 2008). In vertebrates, Dvl may act at a similar step as it appears to associate with (or even recruit) Rab8-positive vesicles as ciliary sheath formation progresses (Zilber et al. 2013). Thus, DVL may regulate ciliogenesis by way of modulating membrane trafficking pathways and the actin cytoskeleton. Finally, in *Celsr2/3* knockout (KO) mice, there is a pronounced defect in ciliogenesis, including a failure to dock

basal bodies (Tissir et al. 2010). The reason for these defects is not known, but disruption of FZD3 and VANGL2 expression in these mice indicates a general disorganization of the PCP machinery.

Several PCP effectors, described above, have also been implicated in ciliogenesis (Fig. 7). In *Xenopus*, Intu is localized to basal bodies, recruits RhoA to the apical domain, and regulates the formation of the subapical cytoskeleton in multiciliated cells by way of interactions with the cilia-associated protein NPHP4 and the formin DAAM1 (Park et al. 2006, 2008; Yasunaga et al. 2015). In mice, FUZ is essential for proper ciliogenesis, apical accumulation of the microtubule-bundling protein CLAMP in the tips of cilia, and apical secretion (Gray et al. 2009). In vertebrate primary cilia, FUZ controls ciliogenesis by recruiting DVL and RAB8 to basal bodies (Zilber et al. 2013). FUZ also forms interactions with a potential regulator of membrane traffic called RSG1 (alias Rem/Rab-similar GTPase 1) (Gray et al. 2009). Although not much else is known about RSG1, if it is a bona fide regulator of membrane traffic then it would further implicate PCP pathways in trafficking and ciliogenesis. An additional PCP effector protein called CFAP126 (Flattop in *Drosophila*) is also localized to the cilium, basal body, and apical membrane, and is required for basal body docking and ciliogenesis in multiciliated cells and the kinocilium of mouse hair cells (Gegg et al. 2014).

On docking to the apical membrane, basal bodies become asymmetrically oriented, specifying the beat direction of their associated cilia. PCP proteins contribute to basal body/cilia polarity in three ways (Kishimoto and Sawamoto 2012; Devenport 2016). “Translational” polarity refers to any positional displacement of cilia/basal bodies within an individual cell, an example of which is the kinocilium in hair cells or the posterior clustering of cilia in ependymal epithelial cells or their equivalent in the *Xenopus* gastrocoel roof plate. In the latter, depletion of Prickle3 leads to defects in translational polarity as well as defects in cilia growth and basal body organization (Chu et al. 2016). In mice, translational polarity in ependymal cells is affected by the loss of MYO1D expression (Hegan et al.

2015b), an ortholog of the *Drosophila* Myo1A protein that was previously implicated in left–right visceral asymmetry (Hozumi et al. 2006; Speder et al. 2006). Although *Myo1d* KO mice do not have situs inversus (reversed organ asymmetry), they do show defects in the clustering of cilia at the posterior region of ependymal cells (Hegan et al. 2015b). Lack of *Myo1d* expression has a more pronounced effect on “rotational polarity,” which describes the alignment of basal bodies within an individual cell, and is important in those cells where cilia beat or rotate in a regular pattern. In ependymal cells, loss of MYO1D protein leads to disrupted cilia beating (Hegan et al. 2015b). Likewise, decreasing Dvl expression similarly affects rotational polarity in *Xenopus* epidermal cells and in mouse ependymal cells (Park et al. 2008; Hashimoto et al. 2010). In *Celsr1* KO mice, the multiciliated cells that line the oviduct appear to form normally, but transport along this structure is impaired as a result of defects in ciliary beating (Shi et al. 2014).

Finally, “tissue polarity” (i.e., global polarity) refers to the coordination of translational and/or rotational polarity across the tissue (Kishimoto and Sawamoto 2012; Devenport 2016). How tissue polarity comes about in vertebrates and any role for the Fat-Daschous pathway are important but unanswered questions. What is known is that *Celsr2*, *Celsr3*, and *Vangl2* are all required for establishment of polarized fluid flow across mouse ependymal cells (Mitchell et al. 2009; Tissir et al. 2010), and *Celsr1* appears to have a similar role in the oviduct (Shi et al. 2014). And, as noted above, Dvl and Rhoa appear to govern the anterior-to-posterior beating of cilia in the *Xenopus* epidermis (Park et al. 2008). Importantly, this function for Dvl appears to be distinct from its role in cilia docking, as cells expressing the *Xdd1* mutant of Dvl form motile cilia, but beat irregularly (Park et al. 2008).

One possible mechanism that may promote ciliary tissue polarity is fluid flow. When *Xenopus* epidermal tissue is explanted, the orientation of ciliary beat is governed by a previous exposure to an imposed directional flow (Mitchell et al. 2007). Interestingly, when proteins that affect cilia motility (but do not affect

ciliogenesis) are downregulated, including the ciliary proteins Spag6, Dnai1, or Tekt2a, the cilia fail to reorient when exposed to directional flow (Mitchell et al. 2007). Thus, early patterning of basal bodies in *Xenopus* likely occurs as they dock at the membrane. In contrast, polarized ciliary flow requires a positive feedback loop in which the cilia produce flow, sense this flow, and then further refine their orientation at the tissue level to optimize this flow. Somewhat like the *Xenopus* system, maturing mouse ependymal cells will also orient their beating when exposed to directional fluid flow (Guirao et al. 2010). In this system, a nonfunctional *Vangl2* mutant called “looptail” has no effect on ependymal cell ciliogenesis (and the frequency and amplitude of ciliary beating in these animals is normal); however, the alignment of cilia to flow is significantly perturbed both *ex vivo* and *in vivo*.

Besides the requirements for the actin cytoskeleton described above, ciliary beating also depends on the microtubule cytoskeleton, which connects adjacent basal bodies and ensures their spacing and rotational polarity (Sandoz et al. 1988). Interestingly, a pool of polarized microtubules emanate from the “basal foot” (Fig. 7), a nucleation center that extends from the basal body in a polarized orientation that often correlates with the axis of PCP asymmetry and cilia beat (Clare et al. 2014). The plus ends of these microtubules are oriented toward the FRZ-DVL pole of the cell (Vladar et al. 2012). When coupled with laminar flow, this could help orient cilia position and beating across the tissue.

Microvilli

The majority of studies to date have focused on exploring the roles for the major structural and regulatory proteins in formation of microvilli. In cultured cells, these proteins appear to have important roles (Takeuchi et al. 1994; Bretscher et al. 2002; Bonilha et al. 2006a; Wald et al. 2008; LaLonde et al. 2010); however, *in vivo* studies using KO mice paint a much more complicated picture. Mice with mutations in *Vil1*, *Pls1*, *Myo1a*, ERM proteins, *Slc9a3r1* (EBP-50), or a spontaneous *Espn* mutant mouse (the deaf jerker mouse) still form some microvilli (Pinson

et al. 1998; Doi et al. 1999; Morales et al. 2004; Saotome et al. 2004; Tyska et al. 2005; Bonilha et al. 2006b; Grimm-Gunter et al. 2009; Casaletto et al. 2011; Revenu et al. 2012). Although the observed phenotypes may reflect redundancy of proteins (Tyska et al. 2005), it is also likely that brush-border formation is the coordinated product of multiple regulatory and structural proteins, which cannot be appreciated by single gene knockouts. In the following section, the formation of the apical brush border is examined, followed by a discussion of those studies that implicate polarity proteins in microvilli/brush-border biogenesis.

Formation of Microvilli and the Apical Brush Border

The broad outlines of brush-border formation have been documented in kidney and intestine (Larsson 1975; Shibayama et al. 1987; Heintzelman and Mooseker 1990, 1992; Biemesderfer et al. 1992; Peterson et al. 1993; Peterson and Mooseker 1993), primarily using chicken and rodent models. Although the length of time it takes to form the brush border is species dependent, the process is similar. In brief, enterocytes, which have undergone apicobasolateral polarization, have a bulbous apical surface from which extend sparse microvillar-like structures. Over the next several days, the microvilli become denser and self-organize into clusters, which as described above are dependent on PCDH24 and CDHR5 (Crawley et al. 2014b). Higher order clustering continues until one observes consolidation of clusters into a packed brush border in which microvilli show a sixfold hexagonal symmetry. Concomitant with clustering is the formation of “rootlets,” which emerge from the base of the nascent microvilli and elongate into the forming terminal web. On completion of terminal web biogenesis, the microvilli undergo further elongation, while maintaining a highly regular and uniform length and width.

Role of Polarity Proteins in Brush-Border Formation

The events that trigger microvilli formation are not well understood and, despite the uniform

packing of microvilli in the brush border and the important role of actin in their formation, there are few if any studies that implicate PCP in the formation of microvilli or an assembled brush border. In *Drosophila*, Crumbs contains a FERM-binding site, which interacts with Moe (alias moesin), the single ERM protein in the fly (Medina et al. 2002). The complex also includes B_{heavy} isoform of Spec (alias spectrin), a component of the cortical actin cytoskeleton and terminal web. One possibility is that Crumbs recruits Moe/B_{heavy} Spec, and in doing so triggers the formation of the brush border at the apical pole of the epithelial cell (Fig. 8). An additional experimental system is the pronephros of the zebrafish embryo. When expression of the zebrafish homolog of *UPK3a* (originally called *Upk3l* but since renamed to *Upk3b*) is down-regulated in these embryos, they show edema as a result of pronephros dysfunction. One striking defect in these embryos is the complete disappearance of the brush border normally found at the luminal surfaces of the epithelial cells that line the pronephros (Mitra et al. 2012). Interestingly, these cells also lose expression of phosphorylated ERM proteins, lose expression of Prkcz, and show a redistribution of Pard3. Whether *Upk3l* interacts with ERM or polarity proteins is currently unknown, but if so it could act to promote brush-border formation by scaffolding proteins at the apical membrane domain.

The final experimental system uses an intestinal cell line that links microvilli formation to STK11 (aliases Lkb1/Par4), a master regulator of cell polarity (Morton et al. 1992; Watts et al. 2000). In the presence of the pseudokinase STRADA (STRAD) and the cofactor CAB39 (Mo25), STK11 exits the nucleus and promotes the translocation of the kinase STK26 (Mst4) from the Golgi apparatus to the apical pole of the cell where it phosphorylates T₅₆₇ in EZR (Fig. 8) (ten Klooster et al. 2009). Even in single intestinal epithelial cells, expression of STK11/STRADA stimulates the formation of an apical domain, studded with microvilli, that is separated from a basolateral domain by junctional proteins (Baas et al. 2004). More recent studies show that STK26 recruitment requires a signal-

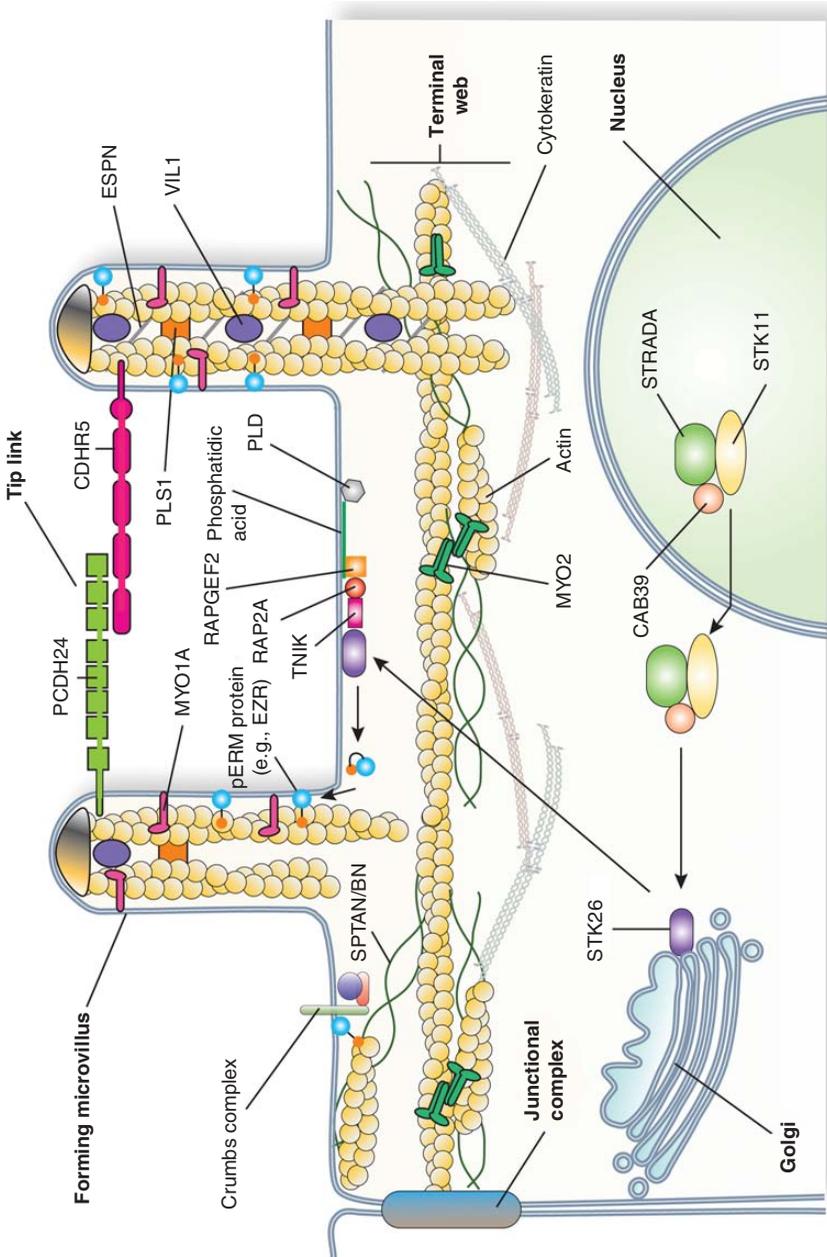


Figure 8. Biogenesis of the epithelial brush border. In *Drosophila*, Crumbs may trigger brush-border morphogenesis by interactions with Moesin, the single ERM protein in the fly, as well as the β_{heavy} isoform of spectrin (SPTAN/BN in vertebrates). An additional pathway for brush-border formation may require the activity of the STK11 kinase. In the presence of the pseudokinase STRADA and the cofactor CAB39, STK11 exits the nucleus and promotes the translocation of the kinase STK26 from the Golgi apparatus to the apical pole of the cell. (*Legend continues on following page.*)

ing cascade that initiates with PLD (alias phospholipase D) binding to apical membrane-associated PtdIns(4,5) P_2 (Fig. 8) (Gloerich et al. 2012). PLD-dependent generation of phosphatidic acid promotes recruitment of RAPGEF2 (alias PDZGEF), a GEF for RAP2A. Active, GTP-bound RAP2A then recruits the TNIK kinase, which presumably phosphorylates STK26, stimulating STK26 localization to the apical membrane. Although these above results are exciting, additional work, using genetically tractable organisms, is needed to determine whether these findings can be translated into more physiologically relevant systems.

Stereocilia

Although stereocilia are also found in the male reproductive tract, there is limited available information about their function, or the mechanisms of their formation. In contrast, the cochlear epithelium is an ideal vertebrate model to study polarity because its anatomy and development are well described, the orientation and organization of the stereocilia and kinocilium are peculiar, predictable, and quantifiable, and genetically tractable model systems are available for study including mice. Despite these features, there remain many open questions about how polarity proteins contribute to stereociliogenesis in hair cells.

Stereociliogenesis

Stereociliogenesis occurs in four stages (Fig. 9) (Nayak et al. 2007). In stage I (~E14.5 in mice), the hair cell undergoes terminal division and establishes apicobasolateral polarity. At this time, the hair cells have a pentagonal or hexagonal shape and numerous short microvilli emerge, which surround a single centrally locat-

ed kinocilium. In stage II (~E16.5), the kinocilium elongates and moves to one side of the cell. Those microvilli nearest the kinocilium undergo lengthening first, followed by those in rows further away, eventually resulting in the formation of the characteristic staircase configuration. During this stage, the kinocilium begins to initiate its inward relocalization, eventually taking up a position halfway between the outer edge of the hair cell and its center (Tarchini et al. 2013). This generates a “bare zone,” which lacks microvilli and is found between the lateral edge of the hair cell and the kinocilium (Fig. 9). In addition, the number of actin filaments increases in each stereocilium, these filaments become cross-linked, and adjacent stereocilia are linked to one another by tip links. As the cochlear epithelium matures, the hair cells become progressively ovoid in shape and are surrounded by support cells. By stage III, the stereocilia temporarily stop lengthening and instead become wider as the number of actin filaments per stereocilium increases and the filaments become more cross-linked. The base of each stereocilium begins to taper and the cuticular floor at the base of each cilium begins to form. Finally, in stage IV, the stereocilia lengthen again until they reach their final height (by E18.5 in mice).

Role of Polarity Proteins in Stereociliogenesis

The organization of stereocilia in the cochlea is one of the first to be linked to the core PCP pathway in mammals (Montcouquiol et al. 2003). Polarization of the core PCP proteins occurs early in mice, before the migration of the kinocilium and formation of the stereocilia (Vladar et al. 2012). As noted above, the expression patterns of those PCP proteins that have been localized do not fit the pattern predicted from studies in *Drosophila*. In particular, al-

Figure 8. (Continued) Here, STK26 phosphorylates T₅₆₇ in EZR, which could act to further promote brush-border formation by stabilizing the actin cytoskeleton and proteins at the apical cell surface. The translocation and activation of STK26 is dependent on PLD-induced production of phosphatidic acid, which promotes association of RAPGEF, a GEF for the small Ras-family GTPase RAP2A, with the cell surface. In turn, RAP2A recruits the TNIK kinase, which promotes translocation of STK26 to the plasma membrane, presumably as a result of TNIK-induced STK26 phosphorylation. Tip links are described in Figure 4. (Figure from Apodaca and Gallo 2013; adapted, with permission, from the authors.)

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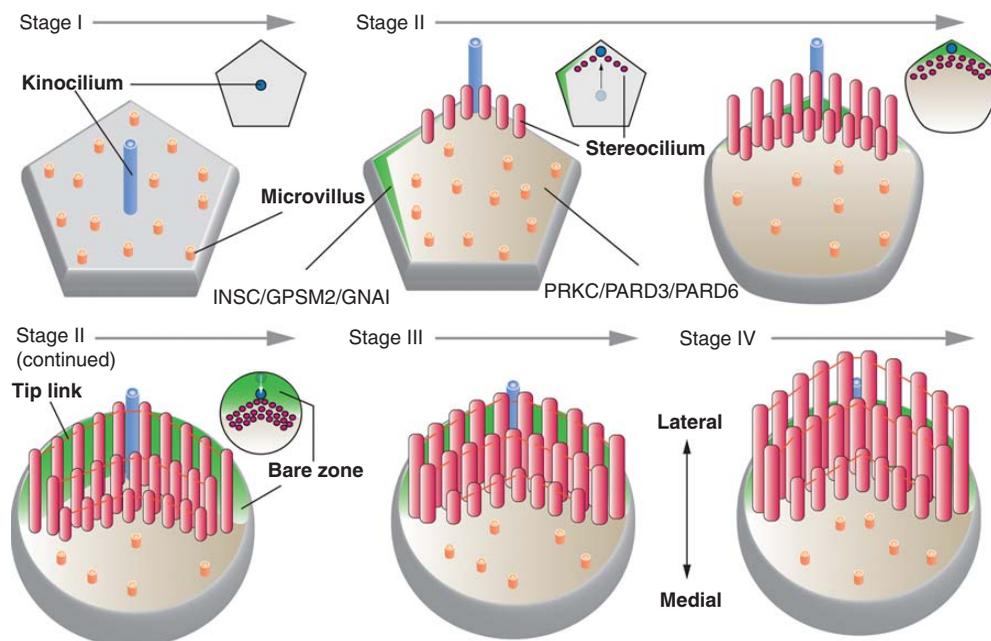


Figure 9. Stages of stereociliogenesis. Stage I: After the hair cell precursor establishes its apicobasolateral axis of polarity, a single kinocilium extends from the center of the cell. At this stage, the hair cell has a pentagonal or hexagonal shape. Stage II: The kinocilium undergoes a centrifugal displacement, positioning it at the periphery of the cell next to the tip of a polarized surface domain marked by INSC/GPSM2/GNAI and opposite of a domain enriched in PRKC/PARD3/PARD6. Stereocilia are seen to emerge in this stage, forming a “V”-shaped, staircase-like pattern as additional rows of cilia form. The vertices of each row point toward the kinocilium. Subsequently, the kinocilium undergoes a second migration, placing it midway between the cell center and cell periphery. The INSC/GPSM2/GNAI domain is now positioned in the lateral portion of the apical surface, establishing a “bare zone,” which derives its name from the lack of microvilli in this region. Stereocilia track the kinocilium and end up positioned at the border of the bare zone and the PRKC/PARD3/PARD6 domain. By the end of stage II, the stereocilia are connected by tip links and the hair cell apical surface has achieved an ovoid-shaped morphology. Stage III: The stereocilia stop growing in height and instead grow wider as additional actin filaments are packed into each stereocilium and cross-linked. Stage IV: The stereocilia continue to lengthen until they reach their final height. The small images at the upper right of each stage show an en face view of the position of the kinocilium and stereocilia at the indicated stage of development.

though VANGL2 is on a membrane domain across from FRZ3/6, it appears to be localized to adjacent support cells and not the hair cell itself (Fig. 6D) (Giese et al. 2012). This would fit with recent analysis showing that mutations in *Nectin3* disrupt supporting hair cell junctions and in doing so alters the polarity of stereocilia bundles (Fukuda et al. 2014). Largely because of the lack of isoform-specific antibodies, the localization of the other PCP isoforms in the vertebrate cochlea remains to be described.

Regardless of their localization patterns, mutants in the core PCP genes *Vangl2*, *Frz3/6*,

Celsr1, *Dvl1/2*, and *Dvl3*, as well as *Scrib* all lead to misorientation of the hair bundle (Curtin et al. 2003; Montcouquiol et al. 2003; Wang et al. 2005, 2006; Etheridge et al. 2008). However, the hair bundles still retain their stereotypical V-shaped organization. Moreover, the kinocilium is still formed and is found near the cell periphery, but its position is no longer uniformly in the lateral region of the apical surface. Thus, in hair cells, core PCP proteins do not appear to be required for formation of the kinocilium or the stereocilia bundles per se, but instead they are required to orient the kinoci-

lium and the vertices of their associated hair bundles along the mediolateral axis. If not core PCP proteins, then what allows hair cells to form V-shaped hair bundles, which remain polarized with respect to the kinocilium?

At the cellular level, the PCP of stereocilia bundles appears to depend on the centrifugal migration of the kinocilium and associated basal body from the cell center to the cell periphery, followed by its secondary relocalization to a position between the cell center and lateral end of the hair cell (Fig. 9) (Tarchini et al. 2013). Data in support of this hypothesis include the following. First, after hair bundle development is complete the kinocilium degenerates (in mice this occurs ~8 d after birth). Second, when kinocilium formation and/or function is perturbed by mutations in the genes encoding the IFT transport proteins KIF3A and IFT88, or Bardet–Biedl syndrome genes, stereocilia bundles are misoriented (in some cases appearing in straight rows instead of V-shaped ones), some stereocilia bundles appear flattened, and the basal bodies are often randomly placed in the lateral half of the cell (Ross et al. 2005; Jones et al. 2008; Sipe and Lu 2011). Third, in those hair cells with mutations in *Ift88* that lack cilia but maintain a centralized basal body (~10% of mutant hair bundles), circular bundles of stereocilia are formed (Jones et al. 2008). Finally, a pathway has recently been described that may regulate the migration of the kinocilium and the PCP of stereocilia at the cellular level. This pathway is described further below.

Strikingly, the localization of core PCP proteins is not affected in *Kif3a* and *Ift88* mutants, or mutations in several other genes that regulate ciliogenesis, possibly indicating that directed kinocilium migration may be occurring by way of a PCP pathway parallel to the core PCP signaling pathway (or that acts downstream of it) (Jones et al. 2008; May-Simera et al. 2015). However, it is worth noting that mutations in *Ift20* and *Bbs8* do alter the localization of VANGL2 (and BBS8 co-immunoprecipitates with VANGL2), which may indicate a more complex relationship between kinocilia formation and core PCP proteins (May-Simera et al. 2015). The nature of the putative, alternate PCP

pathway has recently been described and is a repurposing of one originally described to regulate the orientation of the mitotic spindle during asymmetric cell divisions. In *Drosophila* and *C. elegans*, mitotic spindle orientation requires the Par complex (which is localized to the posterior side of the cell) and an anterior module comprised of the adaptor protein Inscuteable (INSC in vertebrates), the Partner of Inscuteable (GPSM2 in vertebrates), and the heterotrimeric G protein G α i (GNAI in vertebrates). Acting together, these proteins couple mitotic spindle orientation to cortical polarity and ensure asymmetric inheritance of fate determinants between sister cells (Gonczy 2008). In a manner analogous to proteins in invertebrates, INSC and GPSM2 regulate planar divisions in vertebrate systems (Lancaster and Knoblich 2012).

During hair cell development in mice, the INSC/GPSM2/GNAI module are initially localized at the medial side of the hair cell, and by E15.5 are positioned at the microvilli-free “bare zone” that lies between the kinocilium and the distal/lateral surface of the hair cell (see green-colored zones in Fig. 9) (Tarchini et al. 2013). Mutants in *Gpsm2* disrupt the organization of the hair cell bundles, and the kinocilium position becomes dispersed within the lateral half of the hair cell apical surface (Ezan et al. 2013; Tarchini et al. 2013). A similar phenotype is observed when GNAI is inhibited with pertussis toxin or in mice with mutations in *Gnai3* (Ezan et al. 2013). The *Gpsm2* mutant does not affect the initial lateral migration of the kinocilium, but instead appears to affect its shift closer to the cell center (Tarchini et al. 2013). As a result, the kinocilium assumes a position in the lateral domain that appears random. The localization of DVL2 and FZ6 is not obviously affected by mutations in *Insc* or *Gpsm2*, nor is VANGL2 localization affected by inhibition of GNAI (Ezan et al. 2013; Tarchini et al. 2013). Thus, the INSC/GPSM2/GNAI module appears to act in a manner distinct from the core PCP machinery. At present, there is no information about the receptor(s) acting upstream of GNAI, or the proteins that act downstream of INSC and GPSM2.

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Finally, insights into the function of Crumbs/Par/Strubble complexes in stereociliogenesis have lagged those of PCP proteins considerably. In *Drosophila*, asymmetric cell division depends on the partitioning of Inscuteable/Partner of Inscuteable/G α i in a membrane domain distinct from the Par complex (Gonczy 2008). In turn, these two modules exert differential forces on the mitotic spindle, orienting it before cell division. The INSC/GPSM2/GNAI and PRKC/PARD6/CDC42 modules may act in an analogous manner in hair cells (Fig. 9). In support of this hypothesis are the following data. Whereas INSC/GPSM2/GNAI are localized to the bare zone, PRKC/PARD6/CDC42 are enriched in the opposite region of apical surface (PARD3 has been variously localized to either zone) (Ezan et al. 2013; Tarchini et al. 2013). The INSC/GPSM2/GNAI domain may limit the PRKC zone, maintaining the asymmetry at the apical surface of the hair cell (Tarchini et al. 2013). Furthermore, mutations in *Cdc42* leads to misorientation of the stereocilia bundles similar to the reported effects for the kinocilia mutants described above (Kirjavainen et al. 2015). Finally, in *Cdc42* mutants, those microtubules surrounding and emanating from the kinocilium basal body are disorganized and malformed (Kirjavainen et al. 2015). Although the underlying mechanism of how INSC/GPSM2/GNAI and the Par complex work, it is possible that these two modules may be exerting opposing pulling forces on basal body nucleated microtubules, helping to direct the movement and final positioning of the kinocilium, and by extension the stereociliary bundle. Interestingly, and as noted above, these effects happen independently of kinocilium assembly or initial migration to the lateral edge. What is responsible for this initial migration is unknown. Nor is it clear how the core PCP module interfaces with the cell autonomous INSC/GPSM2/GNAI module to generate stereocilia PCP.

FINAL WORDS

Apical projections, which are found on the luminal surface of all vertebrate epithelial cells, play critical roles in epithelial cell functions.

Moreover, defects in their formation/function leads to numerous diseases, including blindness, deafness, infertility, microvillar inclusion disease, and other developmental disorders including spina bifida and craniorachischisis (Waters and Beales 2011; van der Velde et al. 2013; Sebbagh and Borg 2014). Yet, we have a limited understanding of the early steps in their biogenesis and organization. In the case of microplacae, which are found throughout our body, we know nothing about their biogenesis or function. And although studied for decades, there is limited understanding of the early steps in the biogenesis of the brush border. Even in the case of cilia and stereocilia, in which we understand significantly more about early steps in their formation, there remains much to be learned.

Because apical protrusions are specialized regions of the apical surface, and because polarity proteins function to generate asymmetric membrane domains, it is not surprising that polarity proteins are required for their biogenesis and organization. However, there are still unanswered questions. For example, why are there so many isoforms of polarity proteins in vertebrates (see Table 1)? Although likely to contribute to tissue-specific organization and function of apical projections, currently there are limited insights into the expression patterns and functional differences between the multitude of polarity protein isoforms that have been described. Furthermore, what accounts for differences in polarity protein requirements between vertebrate tissues? For example, in multiciliated cells, cilia formation depends on core PCP proteins for docking, but these same proteins do not play an obvious role in the generation of the kinocilium. Apparently, there is an unidentified mechanism that controls kinocilium docking in the inner ear, the nature of which has not been described.

Because apical protrusions develop after apicobasolateral polarity is established, there must be a mechanism to signal the completion of the apical surface domain, before initiation of PCP. But the nature of this signal is unknown. Even after the apical surface is established, both ciliogenesis and stereociliogenesis require api-

cobasolateral and PCP proteins. But how these polarity complexes communicate and the hierarchy of this information exchange is not well understood. Based on the partitioning of the INSC module and Par complex observed in hair cells (Ezan et al. 2013; Tarchini et al. 2013), one paradigm is that the PCP proteins and apicobasolateral proteins define distinct membrane domains that ensure partitioning of effectors. Which leads to the next question: What are the effectors in vertebrates that act downstream of the polarity proteins to modulate the biogenesis and organization of apical projections? Considering that all apical projections have cytoskeletal elements at their core, regulators of the cytoskeleton are obvious candidates and ones previously implicated in PCP signaling events (Devenport 2014; Sebbagh and Borg 2014). This fits with the finding that CRB3 interacts with KIF3, and roles for INTU and FUZ in ciliogenesis (Fan et al. 2004; Gray et al. 2009; Zilber et al. 2013). However, a complete understanding will require further study using different animal and tissue models. Finally, how is polarity at the cellular level governed at the tissue level? As noted above, the Fat-Daschous plays an important role in *Drosophila*, but the roles of these proteins in vertebrates is less clear, and in the case of hair cells there remains limited understanding of what the core PCP proteins are doing to orient hair cell bundles.

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