Update

Research Focus

Dishevelled links basal body docking and orientation in ciliated epithelial cells

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Some epithelia contain cells with multiple motile cilia that beat in a concerted manner. New tools and experimental systems have facilitated molecular studies of cilium biogenesis and the coordinated planar polarization of cilia that leads to their concerted motility. A recent elegant study using embryonic frog epidermis demonstrates that Dishevelled, a key regulator of both the Wnt–β-catenin and planar cell polarity pathways, controls both the docking and planar polarization of ciliary basal bodies.

Introduction

Multiciliated epithelial cells are present in numerous tissues of a wide range of organisms, from embryonic amphibian skin to vertebrate respiratory, oviduct and ependymal epithelia [1]. These cells contain hundreds of motile cilia that beat together to propel substances over the epithelial surface. Cilium biogenesis begins with the generation of basal bodies (the organizing structures at the base of cilia) in the cytoplasm that then traffic to the apical surface, dock with and anchor to the plasma membrane and elongate a ciliary axoneme. Each cilium has an intrinsic ultra-structural and functional asymmetry. Concerted ciliary motility is achieved by the co-orientation of cilia structure and direction of beating, both within each cell and between individual ciliated cells [2] and is essential to the physiological functions of ciliated epithelia.

Most of the understanding of multicilated cells comes from extensive electron microscopic analyses that have documented the basic steps of ciliogenesis and provided ultra-structural evidence for the planar polarization of ciliary basal bodies and axonemes. The introduction of well-characterized *in vitro* and *in vivo* model systems, together with improved tools, now makes it possible to understand these processes at the molecular level.

The planar cell polarity (PCP) pathway orientates cellular structures in multiple systems [3] (Figure 1) and is, thus, a likely candidate for controlling the planar polarized orientation of motile cilia. The PCP protein Dishevelled (Dvl) was previously shown to localize to the apical surface of multiciliated epithelial cells [4]. New work from Park *et al.* [5], using embryonic frog (*Xenopus laevis*) epidermis, demonstrates that Dvl, together with Rho guanosine triphosphatase (GTPase), regulates both the docking and planar polarization of basal bodies. This work both advances the molecular understanding of motile ciliogenesis and contributes to understanding Dvl, cytoskeletal dynamics and the PCP pathway, all of which are involved in processes that are key to development and disease.

Dvl regulates actin assembly and docking during ciliogenesis

Ciliogenesis occurs through a series of highly conserved steps [6]. Basal bodies form in the cytoplasm and, subsequently, one end of the basal body is thought to associate with a vesicle. This complex then migrates apically and fusion of the vesicle with the plasma membrane anchors the basal body to the surface (Figure 2a). A massive apical meshwork of actin assembles in ciliating cells. Studies using cytochalasin D showed that this network is essential for basal body migration [7], thereby indicating its involvement in the vesicular transport step.

Previous work by Park *et al.* [4] found that the PCP effectors Inturned and Fuzzy are involved in the assembly of apical actin filaments during ciliogenesis [4]. They also found that the core PCP protein Dvl localizes in puncta to the apical surface of ciliated epithelial cells [4]. In the current work, Park *et al.* [5] tested the role of Dvl in these cells by knocking down the three frog *dvl* genes (*dvl1–3*) with morpholinos and found that depletion of individual or multiple Dvl proteins results in loss of the apical actin meshwork and trapping of basal bodies in the cytoplasm.

Rho GTPase activation is also required for assembly of the apical actin meshwork in ciliating cells and Rho inhibitors block ciliogenesis [8]. Because Dvl is a known binding partner and activator of Rho in other systems [9], Park *et al.* [5] hypothesized that basal body docking could involve localization and activation of Rho by basal body-bound Dvl. Consistent with this hypothesis, they showed that, although RhoA–green fluorescent protein accumulates at the apical surface, activated GTP-bound Rho specifically localizes to basal bodies and Dvl knockdown leads to the loss of activated Rho from basal bodies without affecting apical enrichment of bulk RhoA. Interestingly, Inturned knockdown delocalizes all RhoA from the apical surface. The authors, therefore, propose a mechanism in which Inturned localizes and Dvl controls activation of Rho GTPase during basal body docking.

Previous work in ciliated epithelial cells placed Rho activation downstream of Foxj1 [8], a multiciliated cell-specific transcription factor. Basal bodies fail to dock with the apical surface in mouse Foxj1 null airway epithelial cells [10] and, similarly to cells lacking Dvl, apical actin filaments are absent [8]. It will be interesting to test whether Dvl is somehow regulated by Foxj1 or if it is in a parallel pathway to Rho activation and whether Dvl phosphorylation regulates its RhoA-activating function.

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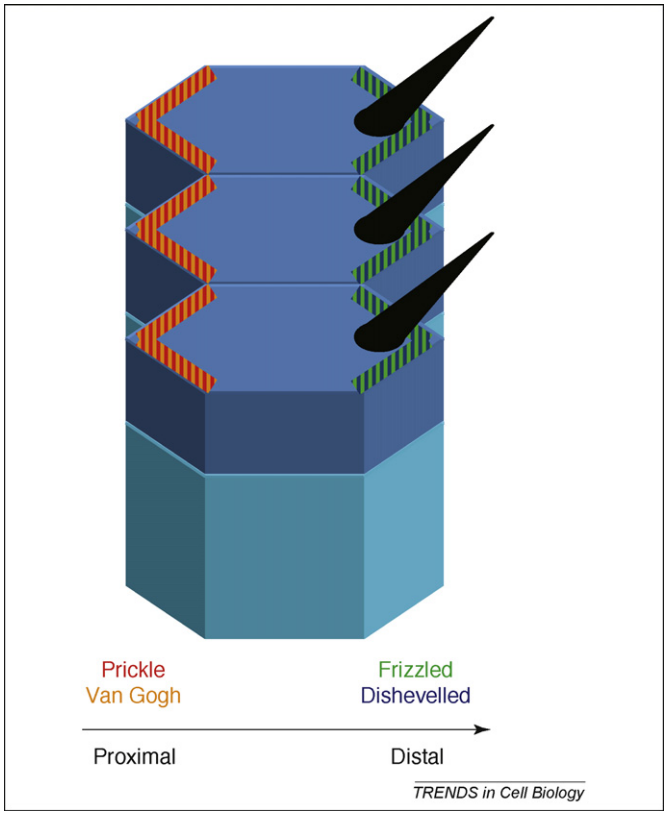

Figure 1. Schematic of the planar cell polarity pathway. This schematic presents the planar polarized fly (*Drosophila melanogaster*) wing epithelium, in which the PCP pathway positions the wing hair (black cones) to the distal side of each cell [16]. PCP proteins are distributed asymmetrically: Dishevelled and Frizzled accumulate on the distal side and Prickle and Van Gogh on the proximal side of cells. These polarized cortical domains are responsible for both aligning the wing hair and communicating polarity information between cells. Similar asymmetric distribution of PCP homologs was observed in the inner ear epithelium [3], indicating that the pathway and the mechanism are highly conserved.

Park *et al.* [5] observed that basal bodies in Dvl morphants fail to closely associate with vesicles, thereby pointing to a defect in vesicle-mediated apical transport as the cause for the docking defect. In other polarized tissues, PCP components have been shown to interact with the exocyst complex of the secretory machinery [11,12]. The authors, therefore, examined the exocyst component, Sec8, and found that it localizes to basal bodies in a Dvl-dependent manner. Sec8 is the first identified molecular component of ciliary vesicles, which thus far have only been characterized through electron microscopy. It is worth noting that similar vesicles surround centrioles in monociliated cells during primary cilium biogenesis [6], implying a conserved mechanism. The movement and fusion of basal-body-associated vesicles resembles exocytosis [6], indicating that it also relies on cytoskeleton-based motility. This could explain the importance of apical actin filaments for basal body migration, although this remains to be experimentally confirmed.

The mechanism through which vesicles attach to basal bodies also needs exploration. Dvl might bind Sec8, but it is more likely to facilitate attachment indirectly, possibly through the Rho-dependent actin polymerization needed to bring basal bodies and vesicles into proximity. One candidate for vesicle attachment is the basal body component centriolin [13]. In cells undergoing cytokinesis, centriolin anchors the exocyst complex at the mid-body, which is required for secretory-vesicle-mediated abscission of daughter cells [14]. Centriolin has been shown to physically interact with Sec8 and other exocyst components, so it would be interesting to test whether this interaction also occurs in ciliated cells.

In sum, these experiments show that Dvl disruption leads to failure of apical actin meshwork assembly and failure of basal bodies to join with exocyst-containing secretory vesicles. Although it is not directly demonstrated that these events are linked, the simplest interpretation is that Dvl regulates ciliogenesis via Rho GTPase activation to establish the apical actin network necessary for vesicle binding, transport and docking of basal bodies (Figure 2b).

Dvl and Rho regulate ciliary PCP

The planar polarized orientation of basal bodies, which is necessary for coordinated ciliary motility, develops in a two-step process [15]. After docking, basal bodies are aligned approximately with the axis of polarity. This orientation is then perfected by directional fluid flow produced by concerted ciliary beating. The planar polarity phenomenon in ciliated epithelia closely resembles other polarized tissues such as the fly wing or the mammalian inner ear, in which the PCP pathway regulates cellular orientation in a remarkably conserved manner. In these systems, asymmetric membrane localization of Dvl and other core PCP proteins along the axis of polarity is essential to the PCP process [3,16] (Figure 1). However, in frog epidermal ciliated cells, Dvl localizes to basal bodies and Vangl2, another core protein of the PCP pathway, localizes to cilia in mouse kidney and airway epithelia [17], so the characteristic asymmetric PCP protein localization might not occur in ciliated epithelia.

Nonetheless, Park *et al.* [5] demonstrated that Dvl and Rho GTPase are involved in the planar polarized orientation of basal bodies (Figure 2c). Using a clever immunofluorescence-based assay, basal body orientation was observed to be randomized upon overexpression of Xdd1, a dominant-negative Dvl deletion mutant that, in this system, only slightly impairs basal body docking. Expression of dominant-negative RhoA–N19 also disrupted basal body alignment. Both interventions also lead to disorganized ciliary beating, thereby underscoring the relationship between coordinated motility and basal body alignment.

Dvl can function through both the Wnt–β-catenin and the PCP pathway, so it will be important to determine which signaling mechanism controls the docking and orientation of basal bodies. Both pathways could be involved in and responsible for distinct steps, given that Xdd1 expression only slightly affects docking. However, it is also possible that perfect docking is a prerequisite for planar polarized alignment.

Concluding remarks and future perspectives

How basal bodies become physically aligned with the axis of polarity remains unknown. Dvl and Vangl protein distributions indicate that the PCP pathway is active, but might not function by establishing asymmetric cortical domains. It will be important to determine the localization and

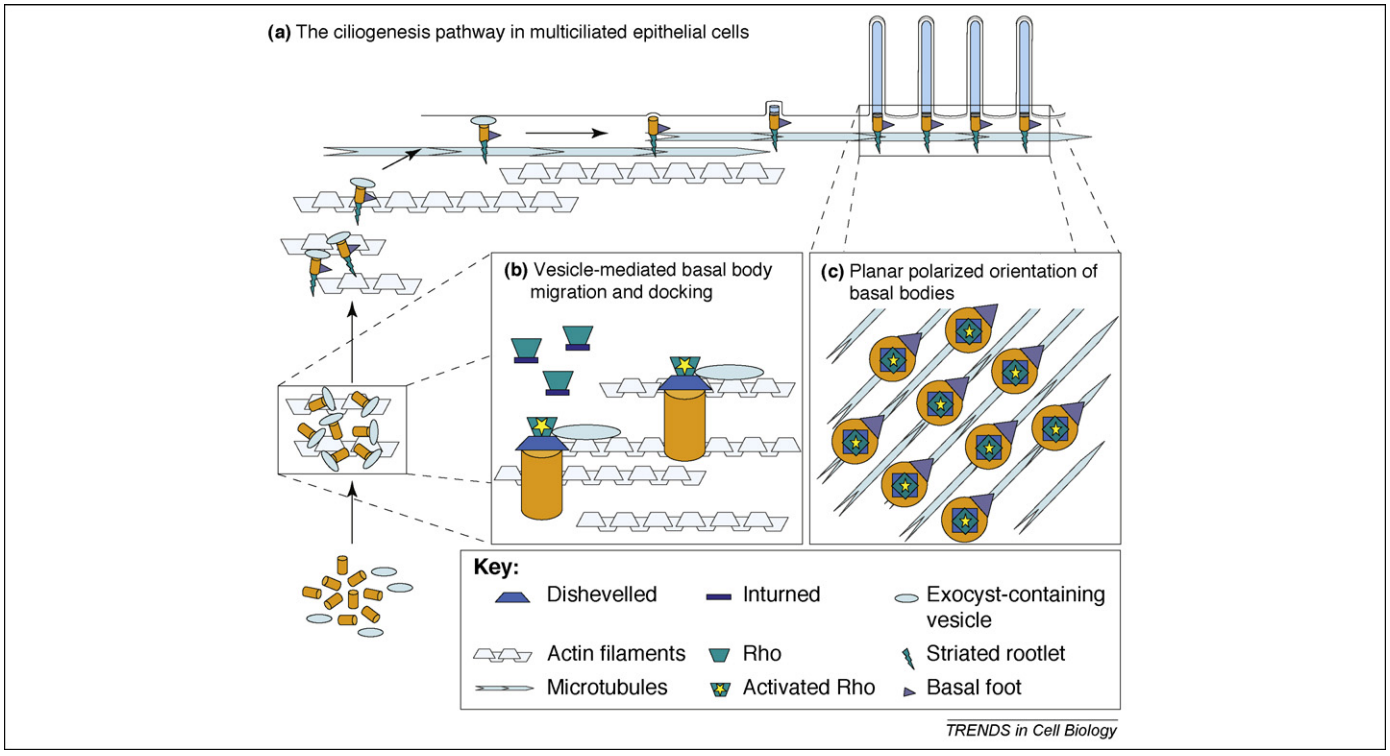

Figure 2. Dishevelled (Dvl) controls basal body docking and planar polarization during ciliogenesis. **(a)** Ciliogenesis begins with the generation of basal bodies (orange cylinders) in the cytoplasm, which then associate with vesicles (light blue ovals). The basal body–vesicle complex migrates apically, possibly using actin cables (light blue textured lines), which are abundant in the ciliating cytoplasm. Basal bodies dock with the cell surface through vesicular fusion. Basal feet (purple triangles) and striated rootlets (thin green triangles) assemble onto basal bodies before docking with the surface. The ciliary axoneme (long blue cylinders), which is ensheathed by the plasma membrane, elongates from the basal body after docking. Alignment of basal feet in the direction of ciliary motility shows the planar polarized orientation of cilia, which could result from interaction with a hypothetical, planar polarized microtubule network (blue textured lines). **(b)** Inturned (blue rectangles) brings Rho GTPase (teal trapezoids) to the basal body; then, Dvl (dark blue trapezoids) activates Rho, which is thought to lead to the assembly of actin filaments. Exocyst-containing vesicles could be brought to and join with basal bodies by means of this localized actin meshwork. **(c)** Dvl and activated Rho are necessary for the planar polarized orientation of basal bodies, although the mechanism of polarized orientation remains to be uncovered.

function of other core PCP components in multiciliated cells to begin to understand how the PCP pathway contributes to ciliary polarity. In addition to discovering factors that orientate cilia in individual ciliated cells, the nature of the global polarity cue that aligns ciliated cells with the correct tissue axis needs to be identified. In the fly, a global polarity signal is thought to exist upstream of the core PCP components [16] and this might similarly operate in vertebrate polarized epithelia [18]. Wnt proteins have also been suggested as possible candidates for the global polarity signal [19], but both need to be tested in ciliated epithelia.

The polarized basal body appendages, the basal foot and striated rootlet, which are known to interact extensively with the apical cytoskeleton [6], also deserve attention. In fly wing cells, apical microtubules organize parallel to the axis of polarity and contribute to PCP [20]. Although a planar polarized cytoskeleton has not been observed in ciliated epithelial cells, such a structure might control polarity through interaction with basal body appendages.

Finally, it will be important to determine the contributions of individual Dvl isoforms for both docking and polarity.

Given the importance of ciliated epithelia in human development and disease [21], there is a substantial need for understanding this process in greater molecular detail. Park *et al.* [5] have brought us closer to this goal by identifying Dvl as a regulator of both the docking and polarized orientation of basal bodies. Although it is implied that these are sequential but separate steps in ciliogenesis, it is also possible that these two events are not so distinct. By surgically reversing a segment of undifferentiated oviduct, it was demonstrated that polarity is determined before differentiation [22]. Furthermore, polarized appendages are already present on nascent basal bodies [6]. Interaction between these appendages and the hypothetical planar polarized apical cytoskeleton during migration could be an early event that could produce the rough alignment of basal bodies seen immediately after docking [15]. Thus, basal body docking and polarization could be closely linked and could both be consequences of a single activity of Dvl.

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