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Ciliogenesis: Polarity Proteins on the Dispatch Move

Olaf Bossinger and André Bachmann

The formation and maintenance of cilia and flagella require a selective and directed transport along the axoneme, a characteristic central bundle of microtubules. Recent evidence suggests an interesting link between the generation of cilia and the protein complexes that establish apico-basal cell polarity.

Virtually all eukaryotic cilia and flagella are remarkably similar in their organization, with a central bundle of microtubules called the axoneme in which nine outer doublet microtubules surround a central pair of single microtubules (9+2). These microtubules are enclosed by a membrane that is contiguous with the cell membrane. While the mammalian spermatozoon and the unicellular green alga *Chlamydomonas* have only one or two flagella, respectively, the unicellular protozoan *Paramecium* carries a few thousand cilia on its surface. Most mammalian cells, however, carry on their surface a non-motile primary cilium which lacks the central pair of single microtubules (9+0). Cilia contribute to locomotion, fluid movement, chemoreception and patterning of the left–right body axis. Defects in their formation and function are associated with polycystic kidney disease, retinal degeneration and the inherited left–right inversion known as *situs inversus*.

Cilia are assembled and maintained by a process called intraflagellar transport (IFT), which was first studied in the biflagellate alga *Chlamydomonas reinhardtii* but which is also now receiving increasing attention in other model organisms [1]. IFT is generally believed to involve transport of cargo needed for assembly, maintenance and function of cilia and flagella. A basic question in cell biology today addresses the mechanisms underlying this process. A recent paper in *Current Biology* by Fan *et al.* [2] reveals a novel role for proteins required for the establishment of cell polarity — originally identified in the nematode *Caenorhabditis elegans* and in the fruitfly *Drosophila* — in the formation of cilia.

Two protein complexes, Crb3–Pals1–Patj and Par3–Par6–aPKC, have previously been shown to be involved in the establishment of intercellular junctions along the lateral membrane domain [3]. These junctions have a number of functions: they connect epithelial cells and separate their membrane domains; they are involved in the regulation of paracellular transport; and they create a diffusion barrier between different biological compartments. In vertebrates, the latter functions are provided by the tight junction. Components of the tight junction are conserved in invertebrates, where they localize to the subapical region/marginal zone [4].

Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, D-40225 Düsseldorf, Germany. E-mails: olaf.bossinger@uni-duesseldorf.de; bachmana@uni-duesseldorf.de

The Crb3–Pals1–Patj complex contains the transmembrane protein Crb3, which recruits the MAGUK protein Pals1 and the multi-PDZ domain protein Patj. The Par3–Par6–aPKC complex includes the three-PDZ-domain-containing protein Par3, the single PDZ domain protein Par6, and an atypical protein kinase C. Both protein complexes are interconnected by interactions of Par6 with either Pals1 or Crb3 [5,6] (Figure 1A). The large number of protein–protein interaction domains provided by these protein scaffolds is thought to recruit additional signaling molecules and may also provide a link to the cytoskeleton in epithelial cells.

As cilia project from the apical surface of epithelial cells, Fan *et al.* [2] asked whether the Crb3–Pals1–Patj and Par3–Par6–aPKC complexes might also be involved in cilia formation. High-resolution immunofluorescence analysis showed that, in MDCK and IMCD3 renal epithelial cells, Crb3 is expressed on the apical membrane domain, as well as in a punctate staining pattern along the ciliary axoneme. Strikingly, all components of the Par3–Par6–aPKC complex colocalize with Crb3. Knock-down of Crb3 or the Par3-interacting protein 14-3-3η by small interfering (si)RNAs or drug inhibition of aPKC blocks ciliogenesis completely. These results suggest the involvement of proteins known to regulate polarity in IFT particle transport.

IFT particles are composed of at least 17 polypeptides, separable into two complexes known as A and B, which are highly conserved in all ciliated organisms. IFT particle proteins are rich in interaction motifs — such as WD40 or coiled-coil motifs [7] — suggesting that they might act as versatile docking modules for the binding of both cargo and polarity-regulating proteins. For example, the IFT52/NGD5/OSM-6 protein contains proline-rich (PxxP) motifs which have the potential to interact with SH3 domains [8]. Interestingly, immunogold labeling of IFT52 has identified the transitional fibers of the basal body (see below) as a docking site for IFT particles [9]. If these fibers indeed act as a gateway for the entrance of specific proteins to the ciliary and flagellar compartments [1], proteins regulating polarity might play an important role for the positioning of the basal body or the regulation of IFT. During IFT, large protein particles travel along the ciliary microtubules by the action of microtubule motors [10]. Anterograde (outward, to the tip) and retrograde (inward, to the cell body) IFT are powered by the heterotrimeric kinesin II or cytoplasmic dynein, respectively.

Are these motors candidates for the transport of the polarity regulating protein complexes in primary cilia? Fan *et al.* [2] showed that these proteins associate with microtubules and that Kif3a (kinesin II) co-immunoprecipitates with this complex. They hypothesize that

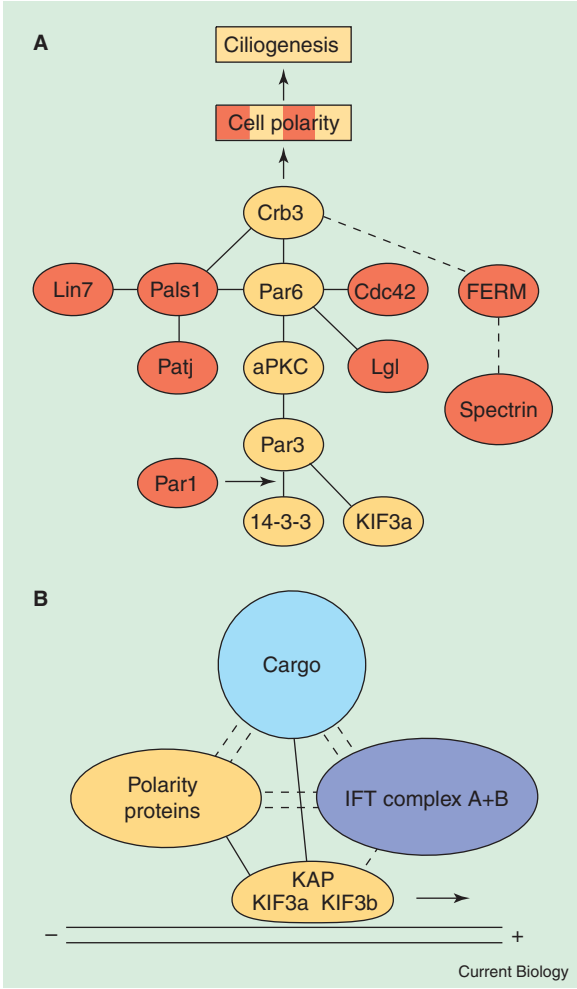

Figure 1. Interdependence of proteins regulating cell polarity and their putative function during intraflagellar transport (IFT). (A) A summary of the physical interactions (black lines) between different proteins during the establishment of cell polarity in vertebrates (dashed lines represent interactions that so far have only been described in *Drosophila*). Red and yellow colored proteins are involved in the generation of polarity; those in yellow have also been demonstrated to participate in ciliogenesis. Several proteins are involved in signaling events and/or contain protein–protein interaction domains allowing them to recruit additional proteins into the complex [4]. (B) Polarity proteins and IFT particle polypeptides may interact and provide a scaffold (dashed lines) that binds to cargo proteins. The heterotrimeric microtubule motor kinesin II (KIF3a–KIF3b–KAP) has the potential to interact with polarity proteins directly [11], bind to different cargo proteins via KAP — for example, fodrin, opsin, arrestin or APC — and to IFT complexes [7].

Crb3 connects to IFT particles and kinesin II via the Par3–Par6-aPKC complex (Figure 1B). Indeed, the four carboxy-terminal residues of Crb3, ERLI, form a PDZ binding motif and have previously been shown to interact with Par6 during the regulation of tight junction morphogenesis [6]. Furthermore, it has been shown that Par3 can interact directly with Kif3A in the establishment of neuronal polarity [11]. While the Crb3–Pals1–Patj complex participates in the establishment of epithelial polarity in mammalian cells [3], Crb3 (but not Patj) seems to localize at axonemal microtubules, suggesting evolutionary modification of this essential complex with regard to IFT (Figure 1A).

The generation of cell polarity involves the formation of large protein scaffolds for the stabilization of specific membrane domains, the local activation of signaling events, and the polarized delivery of membrane vesicles. These functions might involve the binding of protein complexes to components of the secretory machinery and the cytoskeleton [3]. During ciliogenesis, a bud-like structure containing the axoneme and the ciliary membrane projects out from the basal body. The basal body is a centriole-related structure consisting of nine triplet microtubules, which serves as a template for the assembly of the axoneme [12]. The A and B tubules of the basal body continue into the axonemal shaft, whereas the C tubule terminates within the transition zone. Here, each of the nine basal body triplet microtubules is connected to the cell membrane by transitional fibers. Hence, anchoring of basal body transitional fibers to the cortical cytoskeleton would provide a mechanism for the appropriate cellular localization of cilia and flagella.

Members of the FERM (four-point 1, ezrin, radixin, moesin) domain protein family act as linkers between membrane proteins and cortical actin filaments [13]. In pulmonary epithelial cells, ezrin was found by immunoelectron microscopy to be associated with the basal bodies, and decreased ezrin expression was shown to result in the inability of basal bodies to anchor to the apical membrane domain resulting in the loss of cilia [14]. In *Drosophila*, the intracellular domain of Crb contains a conserved FERM binding domain that is essential to recruit both the apical spectrin-based membrane cytoskeleton and the FERM domain protein *D*Moesin [15]. During ciliogenesis, immuno-labeling of Crb3 is not restricted to primary cilia, but includes the entire apical membrane domain [2]. Thus, the Crb3 complex might provide an apical cue, not only for the targeting of the basal body, but also for the Sec6/8 (exocyst) complex [16], which would be predestined to define a site for vesicle delivery and polarized membrane growth also required in cilia formation.

The mechanisms underlying ciliogenesis have recently become a major focus of research involving systematic bioinformatic screens — such as recent studies in which the proteome of the non-flagellated plant *Arabidopsis* was subtracted from those of flagella/cilia-bearing humans and *Chlamydomonas* [17,18] — and a genetic screen in zebrafish [19]. Several hundred proteins were identified as being candidates involved in ciliary/flagellar and basal body biogenesis and function. Although one would not expect these screens to identify proteins regulating polarity, a proteomic analysis of human cilia [20] has detected one of the proteins (14-3-3) discussed here. As ‘polarity proteins’ have not been identified in *Chlamydomonas* so far, their role during ciliogenesis as revealed by Fan *et al.* [2] will hopefully be complemented by further analysis in mouse, *Drosophila* and *C. elegans*.

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