Understanding cargo specificity in intraflagellar transport

David S Parker and Nicholas Katsanis*

Departments of Cell Biology and Pediatrics, Center for Human Disease Modeling, Duke University, Durham, NC, USA

*Correspondence to: katsanis@cellbio.duke.edu

The EMBO Journal (2011) 30, 2518–2519. doi:10.1038/emboj.2011.196

Cilia biogenesis and maintenance depends on intraflagellar transport (IFT) that at the same time mediates shuttling of cell-type-specific cargo. Mechanistic understanding of how the general IFT transport machinery links to the movement of specific ciliary cargo remains sparse. In this issue of *The EMBO Journal*, Zhao and Malicki (2011) show that two components of the IFT machinery (Fleer and IFT88) interact with axoneme and basal body localized factors (B9d2 and Inversin) to regulate transport of specific ciliary cargo.

Primary cilia are complex microtubule-based organelles that nucleate at the basal body and project apically from most post-mitotic vertebrate cells (Gerdes *et al.*, 2009). Cilia have fundamental roles in developmental and homeostatic signalling processes, and mutations that disrupt their structure and/or function underlie a myriad of human clinical phenotypes, collectively termed the ciliopathies (Badano *et al.*, 2006).

Cilia biogenesis and function rely heavily on IFT, the bidirectional transport system that shuttles cargo along the axoneme. Two independent multi-protein complexes are required to transport proteins towards the tip of the cilium (anterograde transport, IFT complex B) and back to the cell body (retrograde transport, IFT complex A; see Figure 1A). IFT complexes A and B serve to link the microtubule bases motors (dynein and kinesin, respectively) to cargo proteins (Pedersen and Rosenbaum, 2008; Silverman and Leroux, 2009).

In addition to maintaining structural integrity of cilia, IFT also selectively transports cargo in a context- and cell-type-dependent manner. Zhao and Malicki report on mechanisms that link the general IFT transport machinery to the movement of specific ciliary cargo. Their analyses show that Fleer and IFT88 (both components of the IFT machinery) bind to B9d2 and Inversin (two proteins that localize to the axoneme and basal body). Functional perturbation of these proteins results in phenotypes that are consistent with a role in transport of a specific subset of ciliary cargo.

To identify Fleer-binding partners, Zhao and Malicki performed a yeast two-hybrid screen that identified B9d2, a gene identified previously as having a role in ciliary function (Town *et al.*, 2008; Williams *et al.*, 2008). In zebrafish, knockdown of *b9d2* alone results in defective opsin transport in photoreceptors, but no overt ciliary structural phenotypes, while concomitant knockdown of sub-effective

Figure 1 Discrete cellular functions are dependent on the IFT machinery and associated binding partners. **(A)** Ciliogenesis/maintenance defects. Loss of B9d2 in conjunction with Fleer results in ciliogenesis defects in the zebrafish neural tube and nasal pit. Cilia length is unaffected by a high dose of B9d2 or low dose of Fleer morpholino. However, concomitant knockdown of B9d2 with a low dose of Fleer (or a high dose of Fleer alone) causes a reduction of cilia length in these tissues. **(B)** Discrete cellular function defects. Loss of B9d2 inhibits the transport of non-structural ciliary cargo proteins in the zebrafish retina. Knockdown of Inv or B9d2 does not affect cilia length, but leads to a persistence of GFP opsin in the inner segment of photoreceptors. Under these conditions, peripherin-GFP transport is unaffected. Cartoon represents the binding interactions between Inv, B9d2, Fleer and the anterograde IFT machinery (IFT B). For clarity, the distal portion of the outer segment is not shown. Morpholino doses are represented by number of vertical arrows.

doses of *flee* and *b9d2* lead to profound ciliogenesis defects (Figure 1A).

B9d2 also interacts directly with Inv, a nephrocystin that does not bind to the IFT machinery. *In vitro* binding experiments show that Flee, B9d2 and Inv can form a complex, and that B9d2 is required for Flee and Inv to interact. Inv on its own does not have a role in ciliogenesis or axonemal maintenance, as loss of *inv* does not result in structural ciliary defects (Otto *et al.*, 2003; Watanabe *et al.*, 2003; Phillips *et al.*, 2004). However, *in vivo* assays in the zebrafish photoreceptor show that efficient opsin transport requires both *inv* and *nphp5* (another nephrocystin). Curiously, cilia-dependent transport of another photoreceptor outer segment transmembrane protein, Peripherin/Rds, is unaffected by knockdowns of *inv*, *nphp5* or *b9d2*, suggesting that either opsin and peripherin are transported by IFT across distinct loading paradigms, or that some, but not all, components of the photoreceptor outer segment are translocated by IFT (Figure 1B).

Previous studies have shown that Inv binds to Vangl2, a transmembrane protein that can localize to cilia and is a core regulator of the planar cell polarity (PCP) pathway (Das *et al.*, 2004; Ross *et al.*, 2005; Simons *et al.*, 2005). Combinatorial knockdown of *b9d2*, *inv* and *vangl2* showed genetic interactions to propagate PCP in the zebrafish L1 neuromast, but did not affect convergent extension in the midline.

Taken together, a hierarchical picture is beginning to emerge that is concomitant with discrete cellular functions that are dependent on the IFT machinery and associated binding partners. In the present model, Flee and IFT88 interact directly with B9d2. In turn, B9d2 binds to Inv, suggesting that B9d2 acts as a bridge between the IFT machinery and Inv. This idea is supported by the localization of Inv to cilia being dependent on B9d2 and Flee, and *in vitro*

binding data showing that B9d2 is required for Inv and Flee to interact.

The question is what is the physiological relevance of these interactions? From the present study, one emergent possibility is that such complexes might confer context-dependent specificity for IFT-mediated cargo transport. Under this model, one might imagine that, depending on the spatiotemporal requirements of a particular cell, selective partnering of core IFT components with proteins such as B9D2 might facilitate the movement of specific cargo, which might also explain the observed genetic interactions in regulating some aspects of PCP.

This study also raises a number of interesting questions. First, it will be important to understand the topology of such complexes, as well as the site(s) of their assembly. Both Inv and B9D2 reside primarily at the basal body. As such, it is plausible that early assembly steps of IFT88-Flee-Inv-B9D2 and other similar complexes might occur prior to axonemal loading, teaching us about the cellular sites that confer transport specificity. Second, it will be important to understand the spatiotemporal constitution of such complexes, as well as the cues that initiate their assembly. Although the data in the present study point to critical photoreceptor transport functions, we do not yet know whether this is true in other cell types and under what premise. Whatever the answer, however, these data contribute to the emergent picture of the role of IFT-associated proteins conferring cargo specificity. Elucidation of such partners will be critical for understanding the diversity (and selectivity) of ciliary functions across different cell types and developmental/homoeostatic time points.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Badano JL, Mitsuma N, Beales PL, Katsanis N (2006) The ciliopathies: an emerging class of human genetic disorders. *Annu Rev Genomics Hum Genet* **7**: 125–148
- Das G, Jenny A, Klein TJ, Eaton S, Mlodzik M (2004) Diego interacts with Prickle and Strabismus/Van Gogh to localize planar cell polarity complexes. *Development* **131**: 4467–4476
- Gerdes JM, Davis EE, Katsanis N (2009) The vertebrate primary cilium in development, homeostasis, and disease. *Cell* **137**: 32–45
- Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, Foreman JW, Goodship JA, Strachan T, Kispert A, Wolf MT, Gagnadoux MF, Nivet H, Antignac C, Walz G, Drummond IA *et al.* (2003) Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat Genet* **34**: 413–420
- Pedersen LB, Rosenbaum JL (2008) Intraflagellar transport (IFT) role in ciliary assembly, resorption and signalling. *Curr Top Dev Biol* **85**: 23–61
- Phillips CL, Miller KJ, Filson AJ, Nurnberger J, Clendenon JL, Cook GW, Dunn KW, Overbeek PA, Gattone II VH, Bacallao RL (2004) Renal cysts of *inv/inv* mice resemble early infantile nephronophthisis. *J Am Soc Nephrol* **15**: 1744–1755
- Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, Leitch CC, Chapple JP, Munro PM, Fisher S, Tan PL, Phillips HM, Leroux MR, Henderson DJ, Murdoch JN, Copp AJ, Eliot MM, Lupski JR, Kemp DT, Dollfus H *et al.* (2005) Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet* **37**: 1135–1140
- Silverman MA, Leroux MR (2009) Intraflagellar transport and the generation of dynamic, structurally and functionally diverse cilia. *Trends Cell Biol* **19**: 306–316
- Simons M, Gloy J, Ganner A, Bullerkotte A, Bashkurov M, Kronig C, Schermer B, Benzinger T, Cabello OA, Jenny A, Mlodzik M, Polak B, Driever W, Obara T, Walz G (2005) Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. *Nat Genet* **37**: 537–543
- Town T, Breunig JJ, Sarkisian MR, Spilianakis C, Ayoub AE, Liu X, Ferrandino AF, Gallagher AR, Li MO, Rakic P, Flavell RA (2008) The stumpy gene is required for mammalian ciliogenesis. *Proc Natl Acad Sci USA* **105**: 2853–2858
- Watanabe D, Saijoh Y, Nonaka S, Sasaki G, Ikawa Y, Yokoyama T, Hamada H (2003) The left-right determinant inversin is a component of node monocilia and other 9 + 0 cilia. *Development* **130**: 1725–1734
- Williams CL, Winkelbauer ME, Schafer JC, Michaud EJ, Yoder BK (2008) Functional redundancy of the B9 proteins and nephrocystins in *Caenorhabditis elegans* ciliogenesis. *Mol Biol Cell* **19**: 2154–2168
- Zhao C, Malicki J (2011) Nephrocystins and MKS proteins interact with IFT particle and facilitate transport of selected ciliary cargos. *EMBO J* **30**: 2532–2544