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Flipping a Phosphate Switch on Kinesin-II to Turn IFT Around

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Cilia and flagella are assembled and maintained by the motor-driven, bidirectional traffic of large protein complexes in a process termed intraflagellar transport (IFT). In this issue of *Developmental Cell*, Liang et al. (2014) report that IFT is regulated in part by the phosphorylation status of the kinesin-II subunit FLA8/KIF3B.

Intraflagellar transport, or IFT, plays an essential role in the assembly and maintenance of almost all cilia and flagella. IFT consists of the anterograde (from ciliary base to tip) and retrograde (from ciliary tip to base) movement of large motor/cargo complexes, termed IFT particles or trains, that deliver and remove material to and from the ciliary tip, respectively (Rosenbaum and Witman, 2002). The motor that drives the anterograde movement is kinesin-II, a heterotrimeric complex consisting of FLA10/KIF3A, FLA8/KIF3B, and FLA3/KAP subunits, whereas retrograde movement is mediated by cytoplasmic dynein 2. IFT cargo transport increases dramatically during ciliary assembly (Wren et al., 2013), indicating that cargo loading and unloading is regulated and can change in response to ciliary assembly status. However, the mechanisms governing this dynamic behavior are essentially unknown. In this issue of *Developmental Cell*, Liang et al. (2014) provide evidence for phosphorylation-mediated regulatory steps in kinesin-II entry into flagella and IFT-cargo binding and release.

Previous studies in *Chlamydomonas* and other organisms have suggested a potential role for kinases and phosphorylation in the regulation of IFT and motor-cargo interactions. For example, many of the long flagella (*lf*) mutations in *Chlamydomonas*, which result in defects in flagellar assembly, flagellar length regulation, and IFT, have been mapped to kinases in the MAP kinase and cyclin-dependent kinase families (Berman et al., 2003; Hilton et al., 2013; Tam et al., 2013). In addition, localized intraflagellar Ca^{2+} fluxes have been implicated in the control of IFT (Collingridge et al., 2013).

In mammalian neuronal cells, CaMKII phosphorylation of the kinesin family member Kif17 was shown to cause release of the Kif17 cargo Mint1 (Guillaud et al., 2008), leading Liang et al. to ask if a calcium-dependent kinase could similarly regulate IFT kinesin-II. Working with *Chlamydomonas*, which offers a unique combination of genetic, cell biological, and biochemical approaches, the authors identified the *Chlamydomonas* ortholog of CaMKII, CrCDPK1, and demonstrated that it localizes to the flagellar base and proximal half of the flagellum in steady-state (i.e., nonassembling) flagella. RNAi-mediated knockdown of CrCDPK1 resulted in a short flagella phenotype, decreased flagellar regeneration kinetics, and an accumulation of IFT components at the flagellar tip, indicating that CrCDPK1 is involved in normal flagellar assembly and the transition from anterograde to retrograde IFT. The authors then hypothesized that the critical substrate for CrCDPK1 could be a subunit of the anterograde IFT motor kinesin-II. Using mass spectrometry, they confirmed that the FLA8/KIF3B subunit of kinesin II is indeed phosphorylated on a conserved serine (S663). Then, using both in vivo and in vitro approaches, the authors demonstrated that CrCDPK1 phosphorylates S663 on FLA8. To determine the consequences of FLA8 phosphorylation, the authors generated phosphomimetic or phosphodefective FLA8 mutants and found that phosphorylation must be regulated for proper flagellar assembly. Importantly, they found through coimmunoprecipitation assays between kinesin-II and its cargo, IFT complex B, that kinesin-II interacts with IFT complex B only when FLA8 is unphosphorylated. Furthermore, the S663D phosphomimetic FLA8 mutant failed to enter the flagella.

The authors propose a model in which phosphorylation of FLA8 prevents the entry of kinesin-II into flagella and also promotes the dissociation of kinesin-II from IFT complex B at the flagellar tip. Conversely, IFT cargo binding to nonphosphorylated FLA8 results in kinesin-II activation and entry into flagella. The localizations of both CrCDPK1 and phosphorylated FLA8 (pFLA8; detected with a phosphospecific FLA8 antibody) are consistent with the researchers' model: during flagellar assembly, CrCDPK1 and pFLA8 were partially redistributed from the flagellar base to the tip. This could promote the entry of kinesin-II into the elongating flagella and increase turnover of kinesin-II at the flagellar tip, both of which are enhanced during flagellar assembly. Once at the tip, FLA8 is phosphorylated by CrCDPK1, resulting in kinesin-II dissociation from the IFT complex.

The data of Liang et al. (2014) and the resulting model raise a number of questions (Figure 1). What is the phosphatase that dephosphorylates FLA8 to allow it to enter the flagella? Where precisely at the flagellar base does dephosphorylation and motor-cargo binding occur? IFT proteins are enriched on the transition fibers at the distal end of the basal body (Deane et al., 2001); are the transition fibers the site where IFT complexes and kinesin-II come together? Additionally, it is unclear how CrCDPK1 localization and activity are regulated. The authors report that the C2 domain of

CrCDPK1, a predicted lipid-binding domain in the N terminus of the protein, is required for CrCDPK1 enrichment at the flagellar tip and proximal half of the flagellum, suggesting that concentration of CrCDPK1 at these regions requires an association with the flagellar membrane. Furthermore, CrCDPK1 redistributes during flagellar assembly; this indicates that CrCDPK1 localization is dynamic and that CrCDPK1 itself could potentially be transported to the flagellar tip, in an inactive form, by kinesin-II-driven anterograde IFT. In this scenario, kinesin-II would carry its own "deactivator" to the flagellar tip, where CrCDPK1 would then be activated, phosphorylate FLA8, and promote kinesin-II dissociation from the IFT particle.

Finally, if kinesin-II dissociates from the IFT particle at the flagellar tip, how is kinesin-II recycled back to the flagellar base? It is possible that at least some of the kinesin-II motor could diffuse back to the flagellar base. Consistent with this, direct visualization of kinesin-II by total internal reflection fluorescence microscopy of *Chlamydomonas* cells expressing KAP-GFP revealed multiple anterograde IFT tracks but very few retrograde IFT tracks (Engel et al., 2009). The study by Liang et al. (2014) sets the stage for further investigation into the intriguing and largely unexplored mechanisms that control IFT and ciliary assembly.

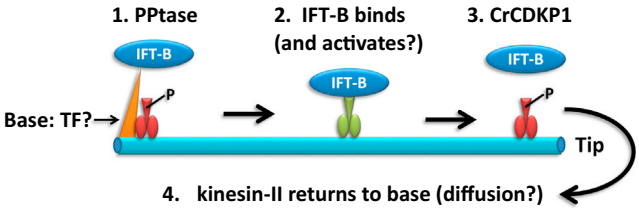

Figure 1. Phosphoregulation of IFT Kinesin-II
Model depicting the findings of Liang et al. and some open questions regarding regulation of kinesin-II by FLA8 phosphorylation. At the ciliary base, possibly at the transition fibers (TFs), an unknown phosphatase (PPtase) dephosphorylates FLA8 (step 1). This allows IFT complex B (IFT-B) to bind to kinesin-II (step 2), which then translocates to the ciliary tip. It remains to be determined whether kinesin-II motor activity is stimulated by dephosphorylation, IFT-B binding, another mechanism, or a combination of events. When kinesin-II reaches the tip, CrCDKP1 phosphorylates FLA8 (step 3), causing IFT-B to dissociate from kinesin-II. If kinesin-II doesn't return to the base via IFT, it might diffuse back (step 4). CrCDKP1 may have additional functions at the tip, e.g., activation of dynein-mediated retrograde transport to return IFT particles to the base of the cilium.

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Lipids Guide the Way: Targeting Proteins to the Chloroplast Outer Envelope Membrane

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Correct delivery of peptides to appropriate subcellular organelles requires distinct trafficking and targeting mechanisms. In this issue of *Developmental Cell*, Kim et al. (2014) demonstrate that AKRA2, a targeting receptor for chloroplast outer envelope membrane proteins, binds chloroplast-specific lipids to ensure proper delivery of cargo to the chloroplast outer envelope.

Organelle biogenesis and function in all eukaryotic cells rely on highly specific targeting pathways to direct thousands of proteins from the cytosol to the proper subcellular compartment. Over the past two decades, the machinery and targeting signals responsible for the import of proteins across boundary membranes into the ER, mitochondria, peroxisomes, and chloroplasts have been extensively studied (Wickner and Schekman, 2005). In each case, intrinsic