

## Tubby proteins prove their adaptability

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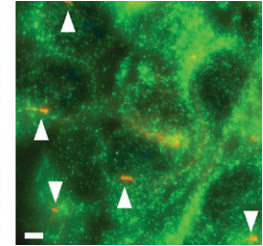
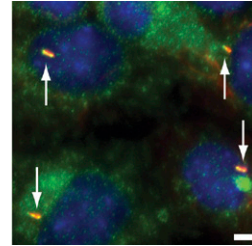
Study reveals that Tubby family proteins help deliver GPCRs and other integral membrane proteins into cilia.

Primary cilia are enriched in a variety of integral membrane proteins, many of which have important roles in cell signaling. How these proteins are trafficked to the ciliary membrane is largely unknown, but Badgandi et al. now reveal that the Tubby family of phospholipid-binding proteins delivers a range of membrane proteins into cilia by linking them to the intraflagellar transport machinery (1).

More than 20 G protein-coupled receptors (GPCRs) have been shown to localize to primary cilia, and defects in the localization of these proteins are thought to underlie many of the pathologies associated with ciliary dysfunction in “ciliopathies” such as Bardet-Biedl syndrome (2). But these receptors may not all be targeted to the ciliary membrane in the same way; researchers have identified several distinct “ciliary localization sequences” that direct the trafficking of different GPCRs. “How can all these GPCRs with all these different localization motifs be targeted to cilia? Can they all be ‘talking’ to a single trafficking protein?” asks Saikat Mukhopadhyay, from University of Texas Southwestern Medical Center in Dallas.

As a postdoc, Mukhopadhyay discovered that the Tubby family protein TULP3 delivers three different GPCRs into cilia by binding to both the plasma membrane phospholipid PI(4,5)P<sub>2</sub> and the intraflagellar transport A (IFT-A) complex that ferries cargo along the cilia’s microtubule-based axoneme (3, 4). Mukhopadhyay and colleagues, led by Hemant Badgandi, investigated whether TULP3 has a broader role in ciliary trafficking and found that knocking down the protein disrupted the localization of at least 13 other ciliary GPCRs (1). Surprisingly, TULP3 was also required for the ciliary trafficking of the TRP channel proteins polycystin 1 and 2, as well as a reporter construct based on the single-pass transmembrane protein fibrocystin. “So we saw three different classes of integral membrane proteins being trafficked by TULP3,” Mukhopadhyay says.

Badgandi et al. found that the various ciliary localization sequences from the GPCRs



**Focal Point** Saikat Mukhopadhyay (left), Hemant Badgandi (right), and colleagues reveal that TULP3 and TUB, two members of the Tubby family of phospholipid-binding proteins, serve as adaptors that target a diverse set of integral membrane proteins into primary cilia by linking them to the intraflagellar transport machinery. The proteins’ cargoes include multiple G protein-coupled receptors having a variety of ciliary localization sequences, the single-pass transmembrane protein fibrocystin, and the channel proteins polycystin 1 and 2. Polycystin 2 (green), for example, localizes to cilia (red) in wild-type cells (left, arrows) but fails to enter the ciliary membrane in cells lacking *Tulp3* (right, arrowheads). Photos courtesy of the authors.

or fibrocystin were sufficient to redirect a plasma membrane protein to cilia in a TULP3-dependent manner (1). TULP3’s C-terminal tubby domain mediated the protein’s association with the different ciliary localization sequences, though whether it directly binds to these targeting motifs remains unclear. Mutating residues in the tubby domain that are crucial for PI(4,5)P<sub>2</sub> binding abolished TULP3’s association with these ciliary localization sequences, suggesting that the protein finds its cargoes by being recruited to the plasma membrane. TULP3’s N-terminal domain can then bind to the IFT-A complex at the base of the cilium, acting as an adaptor to deliver the cargo into the ciliary membrane. Once there, Mukhopadhyay says, low PI(4,5)P<sub>2</sub> levels may trigger TULP3’s release.

**“We saw three different classes of integral membrane proteins being trafficked by TULP3.”**

*Tulp3* is ubiquitously expressed but, in the brain, the predominant Tubby protein is the family’s founding member, *Tub*. Defects in the localization of GPCRs to neuronal cilia have been linked to common ciliopathy symptoms such as learning difficulties and obesity. Several ciliary GPCRs are known to be mislocalized in the brains of *Tub*-knockout mice (5, 6)—which are, themselves, obese—and Badgandi et al. found that TUB works in

the same way as TULP3 to deliver a subset of GPCRs into neuronal cilia (1).

As well as studying how defects in this process lead to obesity, Mukhopadhyay and colleagues are interested in the importance of trafficking fibrocystin and the polycystins into cilia. Mutations in the genes encoding these proteins cause polycystic kidney disease, but whether these proteins need to be delivered into cilia remains uncertain. Mukhopadhyay hopes to address this question by examining conditional knockout mice whose kidneys lack *Tulp3*.

In addition, Mukhopadhyay wants to investigate how the tubby domains of TULP3 and TUB associate with so many different ciliary localization sequences. “How can such diverse sequences talk to the same protein?” he wonders. Though nuclear import and export receptors can interact with a range of different sequences, additional proteins may be required to link tubby domains to their ciliary cargoes.

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