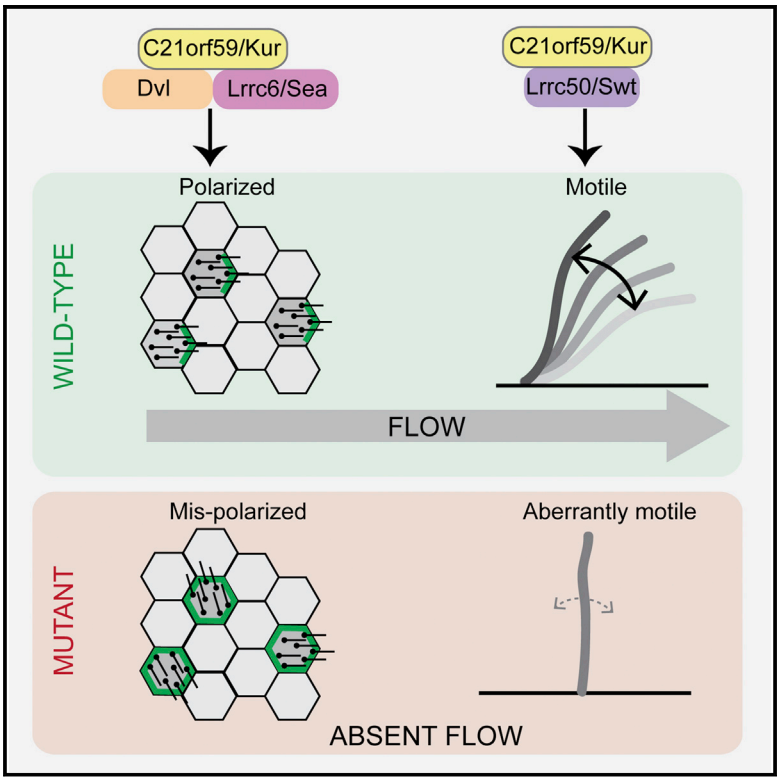
Report

Cell Reports

c21orf59/kurly Controls Both Cilia Motility and Polarization

Graphical Abstract



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In Brief

Jaffe et al. report a dual role for the C21orf59 protein in cilia motility and polarization. Zebrafish and *Xenopus* mutants revealed motility and planar cell polarity (PCP) as well as cilia positioning defects. C21orf59 made several interactions with PCP components and was required for proper Prickle2 localization.

Highlights

- *kurly* (*kur*) mutants exhibit defects characteristic of motile cilia dysfunction
- *c21orf59* is mutated in *kur* and is needed for dynein arm localization/cilia motility
- CRISPR/Cas9 with homologous recombination in *Xenopus* shows C21orf59 regulates PCP
- C21orf59 interacts with various PCP components to correctly polarize motile cilia

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c21orf59/kurly Controls Both Cilia Motility and Polarization

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SUMMARY

Cilia are microtubule-based projections that function in the movement of extracellular fluid. This requires cilia to be: (1) motile and driven by dynein complexes and (2) correctly polarized on the surface of cells, which requires planar cell polarity (PCP). Few factors that regulate both processes have been discovered. We reveal that C21orf59/Kurly (Kur), a cytoplasmic protein with some enrichment at the base of cilia, is needed for motility; zebrafish mutants exhibit characteristic developmental abnormalities and dynein arm defects. *kur* was also required for proper cilia polarization in the zebrafish kidney and the larval skin of *Xenopus laevis*. CRISPR/Cas9 coupled with homologous recombination to disrupt the endogenous *kur* locus in *Xenopus* resulted in the asymmetric localization of the PCP protein Prickle2 being lost in mutant multiciliated cells. Kur also makes interactions with other PCP components, including Dishevelled. This supports a model wherein Kur plays a dual role in cilia motility and polarization.

INTRODUCTION

In multicellular organisms, motile cilia are present on the surface of many cell types. Their primary function is the generation of fluid flow across epithelial surfaces and, in so doing, motile cilia are required for a variety of developmental and physiological processes. As such, abnormalities in cilia-driven flow generation result in primary ciliary dyskinesia (PCD), a condition that involves situs abnormalities, airway clearance defects leading to bronchiectasis, and infertility (Fliegauf et al., 2007; Norris and Grimes, 2012). In order to generate robust fluid flow, cilia must be properly motile. This requires a poorly understood pathway in which dynein arms are loaded into the cilium (Kobayashi and Takeda, 2012; Omran et al., 2008). Cilia must also be correctly positioned on the apical surface of cells (single-cell polarity), and they must be coordinately polarized across the entire tissue (tissue polarity). These roles involve the planar cell polarity (PCP) mechanism (Boutin et al., 2014; Hashimoto et al., 2010; Ohata et al., 2014; Park et al., 2008) and there also exists a feedback between cilia position and flow (Wallingford and Mitchell, 2011). To further our understanding of this link, it will be critical to discover novel components that impact cilia motility and ciliated cell PCP.

The zebrafish and *Xenopus* model systems have been used to understand the components and mechanisms required for cilia-generated fluid flow (Becker-Heck et al., 2011; Hjeij et al., 2014; Kishimoto et al., 2008; Mitchell et al., 2007; Panizzi et al., 2012; Tarkar et al., 2013; Zhao et al., 2013). Here, we describe two zebrafish alleles of a mutant called *kurly* (*kur*) that disrupt *c21orf59*, a gene recently implicated in human PCD (Austin-Tse et al., 2013). We show that Kur is involved in cilia-associated developmental processes and is required for initiating cilia motility via recruitment of outer dynein arms (ODAs). Moreover, Kur interacts with multiple PCP components including Disheveled (Dvl) and is needed for correct cilia positioning in the zebrafish kidney. Experiments in *Xenopus*, including the generation of mosaic mutants by CRISPR/Cas9 and homologous recombination-based gene targeting, further support a role for Kur in cell polarity and tissue-level cilia polarity. Together, these results demonstrate that Kur plays a dual role in cilia motility and cilia positioning.

RESULTS AND DISCUSSION

kur Mutants Exhibit Defects Associated with Improper Cilia-Mediated Flow Generation

kur^{tj271} and kur^{tm304}, two zebrafish mutants isolated from forward genetic screens (Haffter and Nüsslein-Volhard, 1996), exhibit body curvature defects and kidney cysts in larval stages (Figure 1A) (Sullivan-Brown et al., 2008). The two mutations fail to complement in *trans* heterozygotes, indicating that they impact the same gene. Intriguingly, the penetrance of $kur^{tm304/tm304}$ mutants varied with temperature, displaying progressively more severe defects when embryos were raised at 25°C, 28°C, and 32°C, respectively (Figures 1A, 1B, and 1D). Although $kur^{tj271/tj271}$ mutants died as larvae, $kur^{tm304/tm304}$ mutants raised at the permissive temperature (25°C) often resolved their mild body curvature defects by 5 days post fertilization (dpf) or earlier (Figures 1A and 1B). Though kidney cysts were apparent in $kur^{tm304/tm304}$ embryos raised at permissive temperature, they