

SPOTLIGHT

Distinct roles of two homologous kinesins in mammalian motile cilia

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How do the two kinesin-9 members Kif6 and Kif9 function in mammalian cilia? Ou and colleagues discuss new work from Fang et al. (<https://doi.org/10.1083/jcb.202312060>) showing that Kif6 is an active motor while Kif9 serves as a stationary regulator, both of which are essential for cilia motility.

Cilia are highly differentiated organelles protruding from the surfaces of most eukaryotic cells. Functionally, cilia are classified into two categories: primary cilia involved in perceiving environmental cues and motile cilia possessing intrinsic motility (1). For both types, ciliogenesis relies on intraflagellar transport (IFT) driven by the anterograde motor kinesin-2 and the retrograde motor dynein-2 (2). Within the eukaryotic kingdom, a diverse array of kinesin proteins exist—45 human kinesins across 15 families (3). Notably, several kinesin families have been shown to play crucial roles in ciliogenesis and are essential for the functional attributes of cilia (Fig. 1). For instance, kinesin-13 (Kif24) is involved in ciliary depolymerization, kinesin-4 (Kif7) is critical for ciliary Hedgehog signaling, and kinesin-9 (Kif6 and Kif9) has important but yet undefined roles in motile cilia (2). Despite the importance of Kif6 and Kif9 in ciliopathies and ciliary beating (4, 5), their specific functions in motile cilia and their molecular properties in mammals remain poorly understood. In this study (6), Fang et al. address several critical questions in this area: the roles of Kif6 and Kif9 in motile cilia, whether they function synergistically or redundantly, and how their deficiency contributes to ciliopathies such as hydrocephalus and male infertility. These findings significantly enhance our understanding of the regulation of motile ciliary function.

The kinesin-9 family comprises highly conserved motor proteins found in organisms from single-celled species to humans. Phylogenetic analysis has shown a co-evolutionary relationship between Kif6/Kif9 and motile cilia, suggesting their functional specificity (6). This was further supported by a strong positive correlation between Kif6/Kif9 levels and tissues with motile cilia, as opposed to primary cilia. Notably, ectopic expression of Kif6/Kif9 in cells with primary cilia did not lead to ciliary localization, underscoring their specific roles in motile cilia.

To elucidate the roles of these kinesins, Fang et al. performed three-dimensional structured illumination microscopy (3D-SIM) for super-resolution imaging of cultured mouse ependymal multicilia. They observed robust co-localization of Kif6 with ciliary doublet microtubules (DMTs) and Kif9 with central pair (CP) microtubules (Fig. 1), suggesting distinct roles for the two kinesins. Using grazing incidence (GI)-SIM, full-length Kif6 demonstrated IFT-like movements along DMTs, contrasting with previous findings showing that truncated Kif6 (1–493) lacked motility (5). This indicates that Kif6 acts as an active motor, while Kif9 may regulate ciliary beating at the CP apparatus. Consistent with these observations, purified truncated Kif6 exhibited robust movement along microtubules in vitro, whereas Kif9 showed no

affinity for microtubules. Additionally, using the IFT-B component Ift27 as a marker for IFT complexes, some Kif6-GFP puncta colocalized with Ift27 in both anterograde and retrograde movements while others did not, raising the possibility that Kif6 could mediate transport in motile cilia independently of conventional IFT.

To explore the physiological roles of Kif6 and Kif9, Fang et al. created knockout mice for each and noted distinct phenotypic outcomes. Kif6^{-/-} mice displayed severe growth defects, hydrocephalus, dome-shaped skulls, enlarged ventricles, and high lethality. In contrast, Kif9^{-/-} mice exhibited only mild hydrocephalus and were similar to wild-type mice in viability and appearance, highlighting the differential impacts of Kif6 and Kif9. Kif6^{-/-} mice also showed significant sperm immobility and reduced sperm counts, while both strains had normal sperm morphogenesis. Dissection of ventricle walls from the brains of Kif6^{-/-} and Kif9^{-/-} mice revealed that Kif9^{-/-} ependyma developed normal polarity, while Kif6 deficiency disrupted cerebrospinal fluid (CSF) flow and led to hydrocephalus. This suggests that Kif6 and Kif9 play crucial roles in cilia motility rather than general ciliogenesis.

This study by Fang et al. systematically elucidates the distinct roles of Kif6 and Kif9 in mammalian cilia. Kif6 functions as an active cargo transporter along DMTs, while Kif9 serves as a non-motile regulator of

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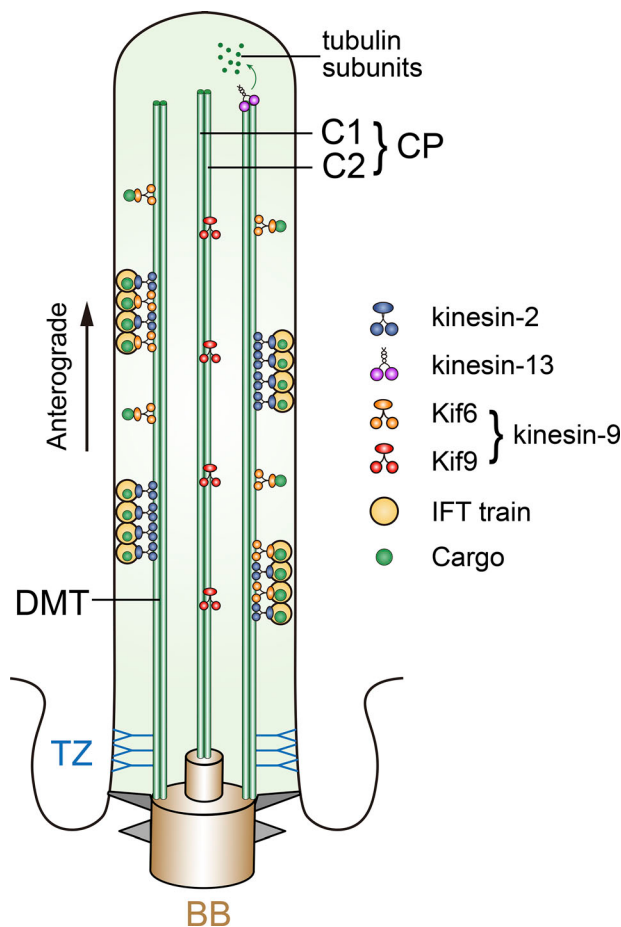


Figure 1. Functional repertoire of motile ciliary kinesins. Ciliogenesis and cilia functionality depend on multiple ciliary kinesins, such as the anterograde motor kinesin-2 mediating intraflagellar transport (IFT) and the kinesin-13 involved in cilia depolymerization. The findings from Fang et al.'s study demonstrate that the kinesin-9 family member Kif6 can also mediate cargo transportation through IFT or independently of IFT to promote cilia motility, while the other kinesin-9 member Kif9 is stationary on CP apparatus as a regulator of cilia motility. DMT, doublet microtubule; CP, central pair; TZ, transition zone; BB, basal body.

ciliary beating at the CP (Fig. 1). Consequently, Kif6 deficiency results in more severe phenotypes related to motile cilia than Kif9 deficiency, including growth defects and impaired sperm motility. These findings enhance our understanding of ciliary function and have implications for motile cilia-related disorders, suggesting potential avenues for targeted therapeutics.

This study lays the groundwork for future research on how kinesin-9 family members fine-tune cilia motility. The processive movements of Kif6 with IFT puncta imply that other kinesins may also mediate

IFT movements. Conversely, the Kif6 movements independent of IFT raise questions about the specific cargo it transports. While Kif9 remains stationary on the CP apparatus as a discrete puncta, its interaction with the CP requires further investigation. Advanced methodologies, such as proximity labeling-based interactome analysis (7), may help address these questions.

In a broader perspective, this study provides several unprecedented insights into cilia biology. Given that cilia are highly differentiated across species and tissues (2, 8, 9), it is likely that many well-characterized

ciliary genes are pleiotropic and perform multiple functions. For instance, Kif9 was demonstrated as an active motor in *Xenopus* (10); however, in this study, it appears to lack motor activity in mice and instead serves as a non-motile regulator of cilia motility. Moreover, whether Kif6/Kif9 functions redundantly with other proteins remains to be further illustrated. Technologies such as tissue-specific conditional knockout of ciliary genes (11), spatio-temporal interaction analysis (7), or functional protein labeling (12) may help address these questions.

In conclusion, the discovery of the distinct roles of active motor Kif6 and non-motile regulator Kif9 provides new insights into the regulation of mammalian cilia motility by additional ciliary kinesins. The identification of robust Kif6 movements along DMTs enhances our understanding of intraciliary cargo transport and highlights the need to uncover unidentified cargo essential for ciliary functions. Additionally, deficiencies in either Kif6 or Kif9 disrupt CSF flow by multiciliated cells and result in hydrocephaly in mice, emphasizing their critical roles in motile cilia and serving as a foundation for further mechanistic investigations.

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