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TORC1-mediated protein synthesis regulates cilia size and function

Implications for organelle size control by diverse signaling cascades

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The cilium is a cell-surface antenna that is well conserved from single-cell eukaryotes to humans. In recent years, the cilium has garnered immense attention due to revelations of the indispensible role that the organelle plays in orchestrating vertebrate development and homeostasis. This tiny organelle senses extracellular signals and couples them with a plethora of downstream functions, such as growth, division and motility.[1] As such, ciliary dysfunction is intimately associated with numerous disease etiologies, including polycystic kidney disease, obesity and cancer.[2] Despite this, the mechanisms regulating the morphology and function of the cilium, as well as the relationship between ciliary morphology and function, are not well understood.

Emerging work in cell culture, zebrafish and mice suggest that broad and diverse signaling pathways may play a crucial role in regulating ciliary size.[3-7] Some of these pathways seem to execute cilia size changes through shared downstream targets, which suggests the existence of a common mechanism that integrates signaling from numerous inputs. For example, knockdown of fibroblast growth factor receptor 1 (Fgfr1), a component of the fibroblast growth factor (FGF) signaling cascade, results in shortened cilia and left-right body patterning defects in zebrafish[4] (**Fig. 1A**). FGF abrogation results in decreased expression of transcription factors *foxj1* and *rfx2*, which have been previously demonstrated to be essential for motile ciliogenesis.[4,8,9] Similarly, zebrafish that lack DeltaD, a ligand of the Notch pathway, display shortened cilia, left-right body defects and decreased expression of *foxj1* (**Fig. 1A**). However, how ciliogenic transcription factors such as *foxj1* and *rfx2* mediate cilia length changes and the identity of their downstream effectors remains largely unknown.

Alternatively, the delivery rate of cargo necessary for ciliogenesis by intraflagellar transport (IFT) has been suggested to be a mechanism for cilia length control.[10] A small-molecule screen performed in tissue culture revealed that decreased intracellular calcium or increased cyclic AMP (cAMP) signaling results in abnormally elongated primary cilia.[5] These elongated cilia display elevated anterograde IFT particle velocities, which suggests that alterations in IFT kinetics may influence ciliary length. (**Fig. 1B**). In addition, a small-molecule screen performed in *C. reinhardtii* identified a compound that induced flagellar shortening and decreased anterograde IFT speed and frequency.[11]

Interestingly, a genome-wide RNAi screen performed in mammalian cultured cells revealed a role for actin dynamics and endocytic vesicular trafficking in regulating cilia length and ciliogenesis.[6] Silencing of actin-related protein 3 (ARP3), a component of the ARP2/3 complex that initiates actin filament polymerization, resulted in elongated primary cilia and increased ciliogenesis (**Fig. 1C**). In addition, silencing of phospholipase A2, group III (PLA2G3), a secreted phospholipase that has been implicated in vesicular trafficking by inducing membrane curvature, also elongated primary cilia and increased ciliogenesis (**Fig. 1D**).

We have recently demonstrated a novel role for target-of-rapamycin (TOR) signal transduction in modulating cilia size and function through translational regulation.[12] Inspired by the observation of abnormally long cilia in the renal epithelium of tuberous sclerosis complex (TSC) models, we investigated TOR signaling as a downstream mechanism for TSC-mediated cilia elongation.[3] In zebrafish, activation of TOR complex-1 (TORC1) signaling increases ciliary length through S6 kinase 1 (S6K1)-mediated protein synthesis of cilia precursors (**Fig. 1E**). Conversely, rapamycin inhibition of TORC1 decreases ciliary length by repressing S6K1-mediated translation. Surprisingly, we observed that this mechanism is seemingly independent of IFT speed, as inhibition of TORC1-mediated translation did not alter anterograde particle velocity (unpublished data). Rather, TORC1 inhibition diminishes the synthesis of ciliary precursors in the cytosol, thus representing a unique and novel mechanism for cilia length control. However, the identity of such precursors remains to be elucidated. We further demonstrated that ciliary length is critical for ciliary function. In the zebrafish Kupffer's vesicle, motile cilia that are too long or short display slow beating frequencies and fail to generate the proper fluid flow that is required for establishing embryonic left-right body asymmetry. Moreover, ciliary length is also tightly correlated with ciliary motility in a wild-type population of Chlamydomonas

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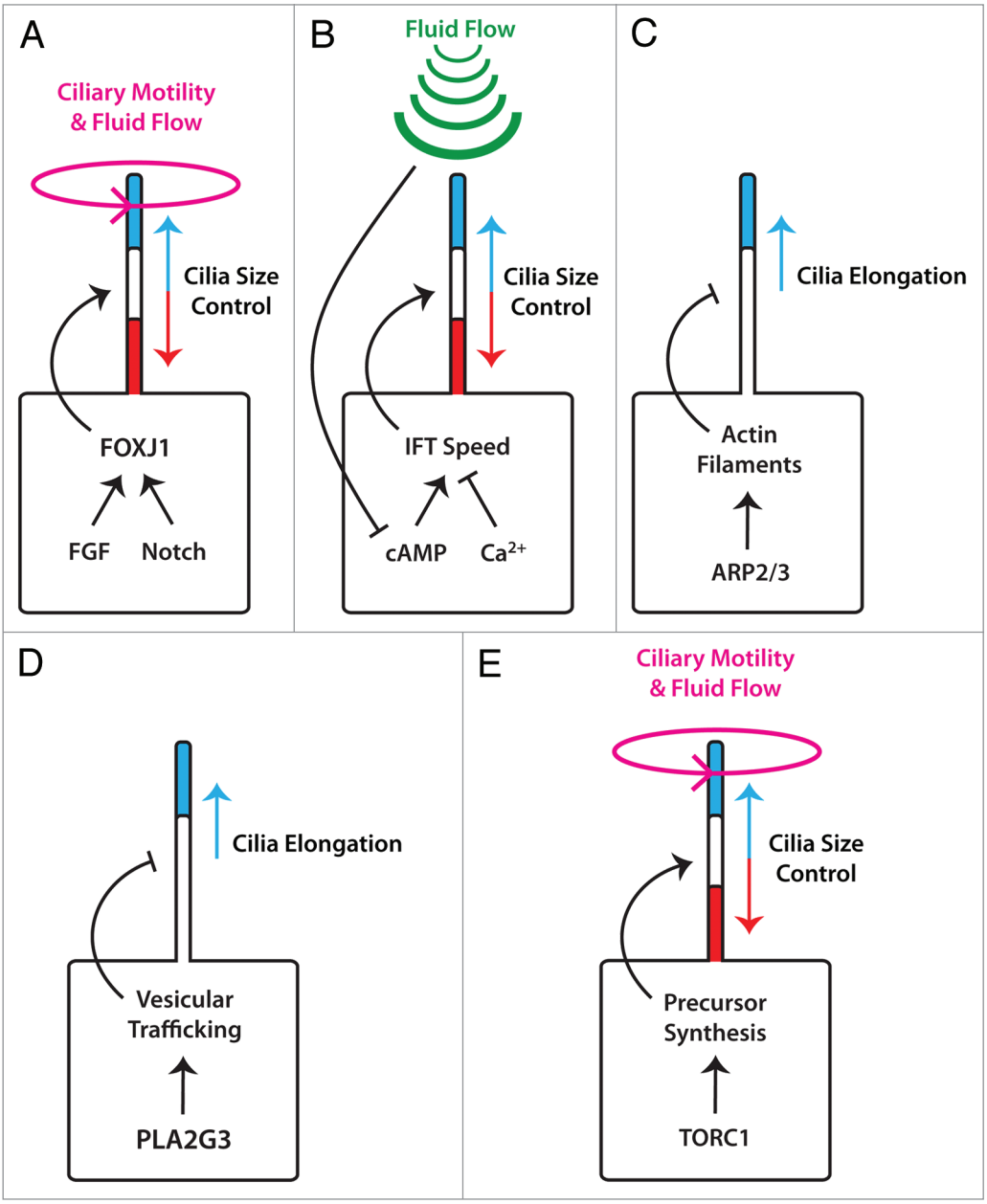

Figure 1. Diverse mechanisms of cilia length control in vertebrates. (A) FGF and Notch signaling influence cilia length, motility and cilia-directed fluid flow via the ciliogenic factor *foxj1*. (B) cAMP signaling and intracellular calcium levels modulate cilia length through changes in anterograde IFT speed. Ciliary bending by fluid flow shortens cilia via cAMP inhibition. (C) The ARP2/3 complex negatively regulates the elongation of cilia by promoting actin filament polymerization. (D) The phospholipase PLA2G3 inhibits ciliary elongation by influencing endocytic vesicular trafficking. (E) TORC1-dependent translation of ciliary precursors regulates cilia length, motility and cilia-directed flow.

cells with a natural distribution of flagellar lengths.

Together, these studies begin to address an overlooked question in cell biology: since cellular size is carefully defined and regulated by robust mechanisms, are subcellular organelles also regulated in this manner? Strikingly, our findings suggest that this is indeed the case, as cellular and subcellular size may be regulated in part by common and shared mechanisms, with TOR signaling being a prominent example. As a major nutrient sensor and metabolic regulator, it is not surprising that TOR may play an integral role in size control at numerous levels for the cell. However, as diverse signaling cascades have also been implicated in ciliary size control, TOR likely represents merely one instrument within an orchestra: numerous parallel and complementary organelle size control mechanisms are likely to coexist. Elucidation of this potential size control network will have profound implications for cellular and subcellular function and, consequently, core biological processes and disease etiologies.

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