

REVIEW



Rab GTPases in cilium formation and function

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ABSTRACT

Cilia are microtubule-based organelles extending from a basal body at the surface of eukaryotic cells. Cilia regulate cell and fluid motility, sensation and developmental signaling, and ciliary defects cause human diseases (ciliopathies) affecting the formation and function of many tissues and organs. Over the past decade, various Rab and Rab-like membrane trafficking proteins have been shown to regulate cilia-related processes such as basal body maturation, ciliary axoneme extension, intraflagellar transport and ciliary signaling. In this review, we provide a comprehensive overview of Rab protein ciliary associations, drawing on findings from multiple model systems, including mammalian cell culture, mice, zebrafish, *C. elegans*, trypanosomes, and green algae. We also discuss several emerging mechanistic themes related to ciliary Rab cascades and functional redundancy.

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Introduction

The compartmentalisation of the endomembrane system into spatially segregated organelles of distinct composition and identity depends on vesicular membrane trafficking processes such as secretion and endocytosis that transport distinct lipids and proteins from donor to target membranes. Rab GTPases are critical regulators of endomembrane organization, controlling many aspects of vesicle identity and transport such as fusion, fission, budding, tethering and motility.^{1–4} Rabs comprise an ancient conserved subfamily of the small Ras-like GTPases, with at least 20 members in the last eukaryotic common ancestor and more than 60 proteins in mammals.^{5,6} Rabs share the basic Ras-like structure of a 6-stranded β sheet, flanked by 5 α helices, and are defined by Rab family (RabF)-specific motifs as well as C-terminal prenyl groups for membrane targeting (geranyl-geranyl or farnesyl).⁷

Rabs function as molecular switches, alternating between cytosolic GDP-bound ‘inactive’ and membrane-associated GTP-bound ‘active’ states. GTP binding induces conformational changes in the switch regions, causing exposure of binding motifs for downstream effector proteins such as molecular motors, vesicle tethering factors and membrane fusion mediators. GTP to GDP exchange is regulated by the Rab’s intrinsic GTPase activity, assisted by GTPase-activating proteins (GAPs), whereas GDP to GTP exchange requires GTP exchange factors (GEF).⁸ Additional regulation is

provided by GDIs (guanine nucleotide dissociation inhibitors) and GDFs (GDI dissociation factors) that regulate Rab cycling between active membrane-bound and inactive cytosolic states.⁹ Because Rab GEFs, GDFs and effectors can localize at distinct membranes, these proteins also provide Rabs with endomembrane-specific functions by trapping their cognate Rab at a target membrane.⁹ Furthermore, multiple Rabs can function within the same pathway, sometimes as part of a Rab cascade; for example, the effector of an upstream Rab can be a GEF of a downstream Rab.^{10,11}

Despite progress, the functions of a large number of Rabs remain unknown or are poorly understood, and in many cases there is scant knowledge of the target effectors, as well as the cognate GEF/GAP/GDI/GDF regulators, some of which are shared with other Rabs. Among the myriad of additional questions, little is known about feedback control within many Rab-driven pathways, nor is there a clear picture of *in vivo* functional redundancy within the Rab family.

In recent years, various Rab and Rab-like proteins have been linked to cilia, which are motile and non-motile microtubule-based organelles extending from eukaryotic cell surfaces (Fig. 1). Like the Rab superfamily, the cilium is ancestral, present in the last eukaryotic common ancestor (LECA), and subsequently lost in some eukaryote branches.^{5,6,12} The canonical cilium consists of a cylinder of 9 doublet microtubules extending from a mother centriole-derived basal body, enveloped

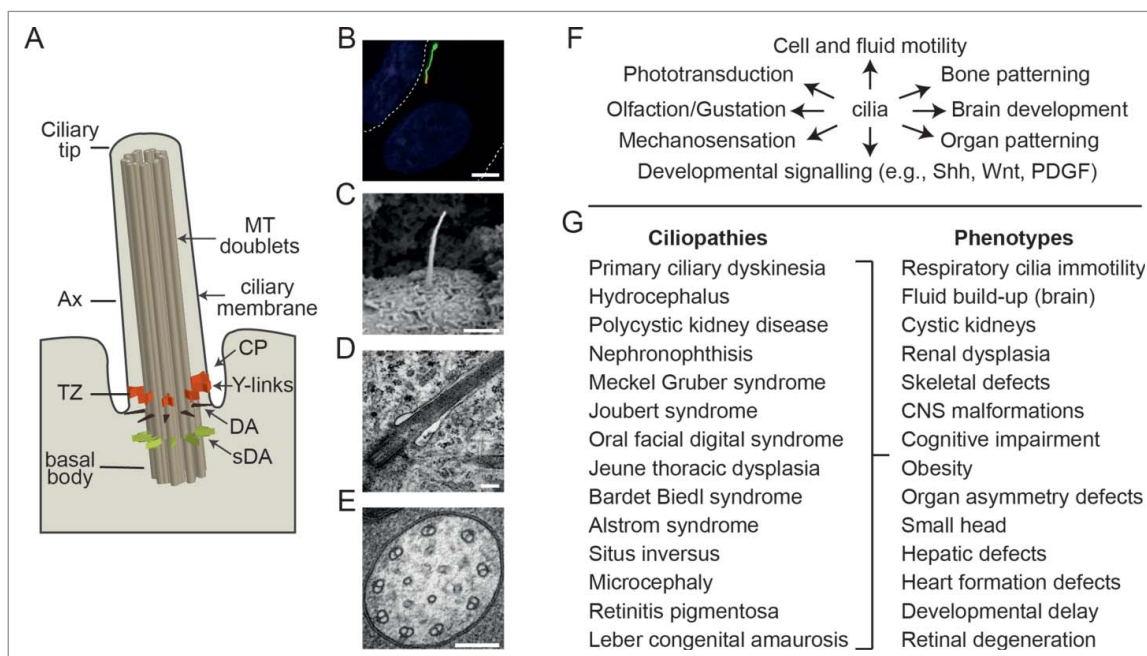


Figure 1. Overview of cilium structure, function and disease associations. (A) Schematic of cilium structure. TZ; transition zone, Ax; axoneme, DA; distal appendages, sDA; subdistal appendages; MT; microtubules, CP; ciliary pocket. (B) Epifluorescence image of a hTERT-RPE1 cell expressing an ARL13B-GFP reporter that stains the ciliary membrane (green). Nucleus stained blue (DAPI). Dotted line denotes the plasma membrane. Scale bar; 5 μm . (C) SEM image of a kidney epithelial cell cilium. Image provided by K. Phelps (Electron Microscopy Unit, UT Southwestern Medical Center). Scale bar; 1 μm . (D) TEM image of the proximal end of a primary cilium on a hTERT-RPE1 cell. Scale bar; 200 nm. (E) TEM cross section of a *C. elegans* sensory cilium showing the 9 doublet microtubules (plus additional inner singlet microtubules). Scale bar; 100 nm. (F) Selection of ciliary-based functions. (G) Ciliopathies and associated phenotypes.

by a specialized patch of plasma membrane called the ciliary membrane¹³ (Fig. 1). In some cells, the basal body lies at the apex of a deep depression in the plasma membrane termed the ciliary pocket.¹⁴ Cilia serve a wide number of functions related to motility and the transduction of physical (light, temperature, touch) and chemical (odorants, taste compounds, biochemical ligands) signals¹³ (Fig. 1). Cilia also coordinate extrinsic signaling pathways required for tissue development and homeostasis (e.g., Shh, Wnt, PDGF α).¹⁵ For example, sonic hedgehog (Shh) signal transduction from the Patched receptor (Ptch) via Smoothened (Smo) to the Gli transcriptional factors occurs within the cilium and requires dynamic transport of signaling intermediates and regulators into (e.g., Smo) and out of (e.g., Ptch) the organelle.^{16–22} Defects in cilia are associated with more than 20 genetically inherited (mostly recessive) diseases, termed ciliopathies, affecting many tissues and organs^{23,24} (Fig. 1). Examples include polycystic kidney disease, *retinitis pigmentosa*, Bardet-Biedl syndrome (BBS) and Meckel Gruber syndrome, which are characterized by cystic kidneys, blindness, bone abnormalities, organ patterning defects, brain abnormalities, infertility and other symptoms. Presently, more than 150 ciliopathy genes are known.

Cilia form when cells exit the cell cycle and 2 cell type-specific pathways of mammalian ciliogenesis have been described based on seminal electron microscopy work by Sorokin in the 1960s.^{25–27} In the extracellular pathway, the mother centriole migrates to the cell periphery, docks directly with the plasma membrane via its distal appendages, followed by ciliary axoneme and membrane elongation. In the intracellular pathway, the migrating mother centriole first associates with a presumptive Golgi-derived (ciliary) vesicle; this is followed by partial extension of the ciliary axoneme (i.e., intracellular extension) before the elongated ciliary vesicle fuses with the plasma membrane to complete basal body docking.

Many of the key steps in ciliogenesis such as basal body formation and maturation, as well as ciliary microtubule and membrane biogenesis and elongation, are regulated by intracellular transport pathways. The most extensively studied is intraflagellar transport (IFT), which is a bidirectional motility along the ciliary axoneme that traffics protein cargos such as tubulin subunits and signaling molecules into and out of the organelle. Sandwiched between the microtubules and membrane, IFT trains consist of kinesin-2 and cytoplasmic dynein motors, together with IFT-A, IFT-B and BBSome complexes that serve as cargo adaptors.^{28–31} These adaptor

complexes are large macromolecular assemblies, consisting of at least 6 IFT-A, 8 BBSome and 16 IFT-B proteins; furthermore, the IFT-B complex is subdivided into 2 biochemically distinct IFT-B1 (10 proteins) and IFT-B2 (6 proteins) subcomplexes.³²⁻³⁶

Multiple small GTPases of the Arl and Rab families, together with membrane remodelling factors such as EHD1/3, are also critical for ciliogenesis, serving important roles in ciliary vesicle formation, centriole uncapping, ciliary membrane elongation, and IFT regulation.^{35,37-44} Post-ciliogenic maintenance and regulation of cilium composition is also driven by IFT as well as polarized delivery of post-Golgi vesicles and associated cargo to the periciliary membrane, together with endocytic events at the ciliary base (Fig. 2).⁴⁵⁻⁴⁸ Finally, the formation of a compartmentalised cilium of defined composition depends on 'gating' mechanisms at the ciliary base. Specifically, basal body distal appendages and the Y-link connectors in the proximal-most part of the ciliary axoneme (transition zone) physically inhibit vesicles from crossing into the cilium, and membrane and cytosolic TZ diffusion barriers regulate the ciliary entry and exit of at least some proteins.⁴⁹⁻⁵¹

In this review, we examine the roles for Rab (Rab5/8/11/23/28/29) and Rab-like (Rab12/4/5), proteins in establishing and maintaining the ciliary compartment

(see Fig. 2 for overview of Rab ciliary associations). Since Rab ciliary associations are also outlined in several excellent review articles,⁵²⁻⁵⁴ we focus here on more recent findings, and how this new data reflects on models of Rab function related to ciliary formation, function, transport and signaling. We also consider the conservation of Rab protein function in different cell and animal systems and outline some of the important questions that remain unanswered.

Rab8 and Rab11

Rab8's role during ciliogenesis

Rab8, a crucial regulator of exocytosis, was first linked to cilia from studies of the retina, where Rab8 was shown to regulate the transport and entry of rhodopsin to the specialized cilium (outer segment) of photoreceptor cells.⁵⁴⁻⁵⁶ In addition to ciliary transport functions (discussed below), various experimental approaches have linked Rab8 to cilium formation. In human hTERT-RPE1 cells, overexpression of a Rab8 GAP (XM_037557) or Rab8 (GDP) blocks cilium formation.³⁷ Similarly, depletion of a Rab8 GEF (Rabin8) abolishes ciliogenesis in hTERT-RPE1 cells, while RAB8(GTP) overexpression drives ciliary membrane extension.³⁵ In further support of a ciliogenic role, siRNA and morpholino depletion of Rab8 reduces ciliation in cultured mammalian cells and causes a BBS-like phenotype in zebrafish.^{35,37,41,44} In addition, RAB-8(WT) or RAB-8(GTP) overexpression in a subset of *C. elegans* sensory neurons induces cilium length and integrity defects, although a *rab-8* null mutant appears normal.^{57,58} However, somewhat surprisingly, Rab8a and Rab8a/Rab8b (double) knockout mice do not display ciliopathy-like phenotypes or cilia defects (at least for those cilia that were analyzed), suggesting that there may be other Rab GTPases working redundantly with Rab8 *in vivo*.^{59,60} One candidate is Rab10, which is a paralogue of Rab8 previously shown to localize to the ciliary axoneme and base.⁶¹ Indeed, Rab10 depletion in fibroblasts cultured from Rab8a/b double knockout mice leads to a significant reduction of ciliation.⁶⁰

Multiple studies have shown that Rab8 associates with ciliary membranes. In early studies, GFP-tagged Rab8a was found to localize to the primary cilia of hTERT-RPE1 cells.^{35,37} Subsequent studies revealed that GFP-Rab8a localizes to the ciliary base and the growing axoneme but it is lost from the mature cilium.⁴¹ GFP-Rab8a is also recruited to the basal body before axoneme extension suggesting a role for Rab8 at the ciliary vesicle, and several studies used this dynamic localization to analyze early ciliogenesis steps.⁶²⁻⁶⁴ A recent study from the Westlake group using live cell imaging and ultrastructural analyses found that Rab8 is recruited to ciliary

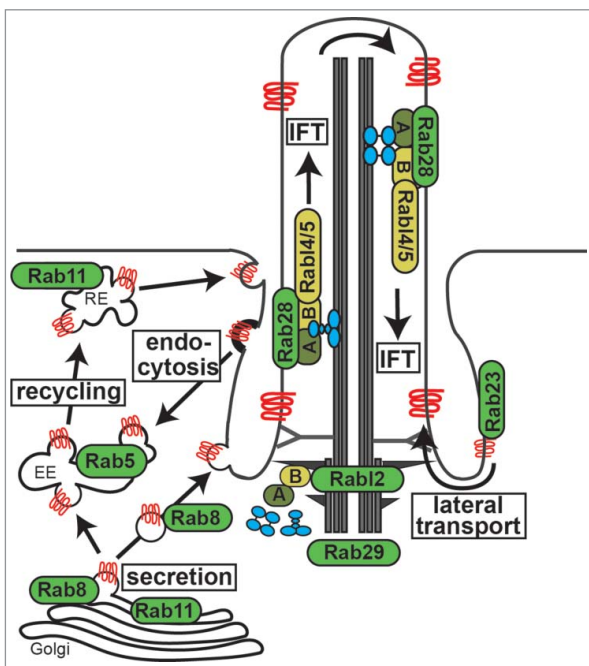


Figure 2. Overview of cilia-related transport functions for Rab proteins. Shown are Rab and Rab-like proteins mapped to major routes of ciliary membrane protein (red) trafficking and IFT (intraflagellar transport) machinery. A; IFT-A complex, B; IFT-B complex, RE; recycling endosome, EE; early endosome. IFT motors (kinesin-2 and IFT-dynein) shown in blue.

vesicles after the recruitment of other proteins linked to ciliary vesicle formation such as the endocytic transport regulator EHD1, the ciliary transition zone protein B9D2 and Golgi-associated IFT20.⁴⁴ The latter study also found that Rab8 is dispensable for initial docking of ciliary vesicles to the mother centriole, indicating that this GTPase may function at the slightly later step of ciliary vesicle membrane elongation (Fig. 3A).⁴⁴

Taken together, the various localization and functional data described above supports a role for Rab8 in cilium formation, at least in cultured mammalian cells. Indeed, this scenario is further supported by the growing number of ciliogenic proteins that physically interact with Rab8 and/or its GEF Rabin8, and regulate Rab8 ciliary localisations in some cases. These include the BBSome complex, several centriolar satellite proteins (HOOK2, PCM1, Talpid, Cep290), and several mother centriole/basal body proteins (Cep164, Chibby, ODF2/cenexin).^{35,37,43,65-69} Consistent with a role for Rab8 during early steps of ciliogenesis, some of these Rab8 interactors function during the formation and fusion of ciliary vesicles (Cep164, HOOK2, PCM1, Chibby), and there are several reports showing that overexpression of Rab8(WT), Rab8(GTP) or Rabin8 (but not Rab8(GDP)) rescues cilia loss observed in cells depleted of Cep164, HOOK2, TALPID3, or Cep290.^{43,66,67}

Ciliogenesis regulation via a Rab8/Rab11 cascade

Targeting and activation of Rab8 at the ciliary base is thought to be achieved by a Rab cascade involving Rab8,

Rabin8 (Rab8 GEF) and the recycling endosome-associated GTPase, Rab11.^{41,70} In this model, Rab11 stimulates the GEF activity and mother centriole recruitment of Rabin8, which in turn leads to recruitment and activation of Rab8^{41,71} (Fig. 3A). Recent studies report that the upstream recruitment of activated Rab11 at the mother centriole depends on the appendage protein ODF2/cenexin and a phosphatidylinositol 3-kinase (PI3K-C2) that generates a pool of phospholipid phosphatidylinositol 3-phosphate (PtdIns3P) at the ciliary base.^{72,73} This PtdIns3P pool appears necessary for Rab11 activation and the ciliary Rab cascade because PI3K-C2a mouse knockout cells display Rab8 mislocalization as well as cilium length and Shh signaling defects, and these phenotypes are rescued via overexpression of Rab11(GTP) but not Rab11(WT).⁷³ Downstream of Rab11, the transfer of Rabin8 to the mother centriole is regulated by the NDR2 kinase that phosphorylates Rabin8 at Ser-272; specifically, phosphorylation decreases Rabin8 affinity for phosphatidylserine on Rab11 positive vesicles and increases Rabin8 affinity for the centriolar exocyst component Sec15.⁷⁴ Finally, the amount of free Rab8(GDP) available for Rabin8 activation is negatively controlled by binding with the GDI2 inhibitor and positively controlled by the GDF protein, Dzip1, which stimulates release of Rab8 from GDI2 at the pericentriolar region.⁷⁵ The finding that Dzip1 itself is positively regulated via phosphorylation by GSK3 β , a kinase active in growth arrested cells, also serves to link Rab8 activation and ciliogenesis onset with cell cycle exit.

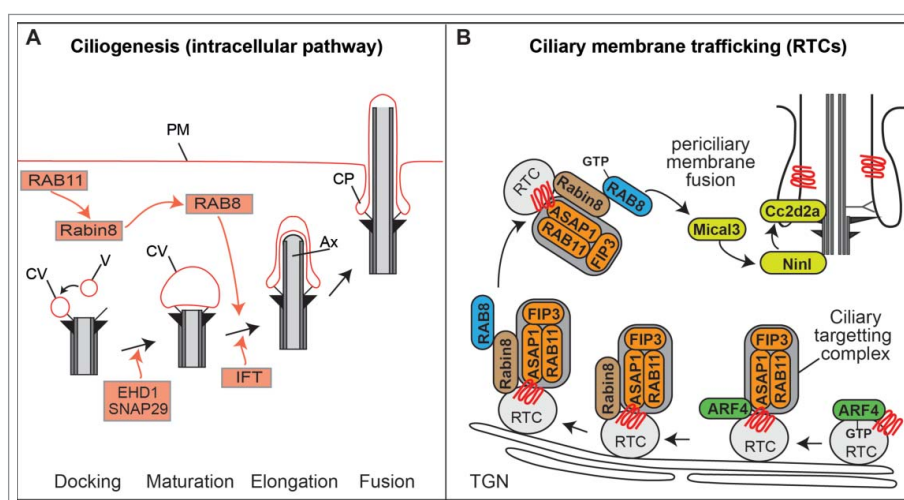


Figure 3. RAB cascades during ciliogenesis and protein transport. (A) Proposed model of RAB11-RAB8 cascade during intracellular ciliogenesis. Shortly after induction of ciliogenesis, Rab11 activates and recruits Rabin8 to the pericentriolar compartment and vesicles (V) dock to the distal appendages of the mother centriole forming small ciliary vesicles (CV). EHD1 together with SNAP29 promotes the fusion of these small vesicles resulting in the maturation of a large CV that caps the distal end of the mother centriole. Rab8, which is recruited and activated by Rabin8, subsequently drives CV membrane extension, and IFT elongates the ciliary axoneme (Ax). This is followed by CV fusion with the plasma membrane (PM), often resulting in the formation of a ciliary pocket (CP). (B) ARF4-RAB11-RAB8 cascade that sorts and delivers rhodopsin transport carriers (RTC) to the periciliary membrane of photoreceptor cells. TGN; trans-Golgi network.

Rab8 and Rab11-mediated regulation of ciliary protein trafficking

From studies in mammalian cells, and especially the photoreceptor cell, the Rab11-Rabin8-Rab8 cascade has also been linked to ciliary membrane trafficking processes that maintain the mature cilium (Fig. 3B). The photoreceptor is a well-established model for ciliary protein transport and findings predominantly from the Deretic laboratory have implicated an ordered Arf-Rab cascade that regulates the formation and delivery of rhodopsin transport carriers (RTC) from initial sorting at Golgi membranes to end-stage fusion at the periciliary membrane. Since this topic is extensively covered in several excellent reviews, we present here a summary of some of the main conclusions.^{47,54,76} Briefly, sorting of Rhodopsin into Golgi/TGN transport carriers is initiated via binding of activated Arf4 (GTP) to the Rhodopsin ciliary targeting sequence. This is followed by the Golgi recruitment of the Arf-GAP ASAP1, which also binds Rhodopsin, and forms part of a ciliary targeting complex with Rab11 and FIP3 (Arf-Rab11 effector). Following inactivation and dissociation of Arf4, ASAP1-Rab11-FIP3 recruits Rabin8 and Rab8, after which RTCs bud from the TGN and eventually fuse with the periciliary membrane in a Rab8 and exocyst complex-dependent manner.^{54-56,76-80} In this model, ASAP1 acts as a scaffold to link Arf GTPases with the Rab11-Rabin8-Rab8 cascade and provides a Rab8 positive ciliary sorting address for Golgi/TGN-derived RTCs⁵⁴ (Fig. 3B). With regard to FIP3, it has been shown that this protein facilitates the orderly assembly and activation of the Rab cascade by disrupting Rhodopsin interaction with the Arf4-ASAP1 ternary complex and enhancing ASAP1-Rab11 binding to Rabin8.⁸⁰

Data from zebrafish photoreceptors has advanced our understanding of how Rab8 facilitates RTC docking at the ciliary base region. Specifically, the ciliary transition zone protein CC2D2A, mutated in Joubert Syndrome, links the transport and fusion of Rab8-positive carrier vesicles at the ciliary base (Fig. 3B). Loss of CC2D2A leads to an accumulation of vesicles in the photoreceptor and mislocalization of Rab8.⁸¹ Further mechanistic insight was obtained from proteomics and genetic interaction studies that identified the centrosome and basal body protein NINL as an interactor of CC2D2A as well as MICAL3.⁸² MICAL3 is a known interactor of Rab8 and was previously reported to be involved in exocytotic vesicle fusion.⁸³ Together, these zebrafish data suggest a model whereby NINL and CC2D2A provide a docking platform for incoming Rab8- and MICAL- positive vesicles at the cilia base (Fig. 3B).

In addition to rhodopsin in photoreceptor cells, Rab8 is also implicated in transport of other ciliary proteins. Overexpression of Rab8(GDP) decreases the ciliary entry

kinetics of the membrane proteins SmoA1 and Kim1 as well as the soluble protein EB1.⁸⁴ Furthermore, Rab8 was shown to interact with the ciliary targeting sequence of fibrocystin and polycystin1, and regulate their transport to the ciliary membrane.^{85,86} Mammalian Rab8 also regulates the ciliary trafficking of polycystin 2;⁸⁷ indeed, a similar finding was very recently reported for Rab11,⁸⁸ which could suggest that a Rab11-Rab8 cascade may deliver polycystin 2 to cilia. Interestingly, a recent study reported that Rab8 together with transportin 1 (Importin β 2) regulates the lateral diffusion of membrane proteins from the plasma membrane to the ciliary membrane.⁸⁹ In this model, Rab8(GDP) and ciliary membrane proteins (fibrocystin, rhodopsin and others) form ternary complexes with transportin1, which facilitates the translocation to the ciliary compartment across the diffusion barrier at the ciliary base. Once in the cilium, Rab8 is activated by its ciliary GEF leading to dismantling of the ternary complex and release of the ciliary membrane proteins. Thus, Rab8 appears to regulate vesicular and non-vesicular modes of membrane protein trafficking into the cilium. Also, the recent Rab8-transportin 1 study supports the notion that nucleocytoplasmic transport regulators such as transportin 1 facilitate trafficking of proteins into cilia, across the permeability barrier at the ciliary base, and that Rab proteins may partake in this process (see also Rab23 section below).^{90,91}

Rab29

In a recent study in cultured mouse cells, GFP-Rab29 was found to localize near the ciliary base, and Rab29 depletion resulted in shorter and fewer cilia, as well as a defect in targeting a Smoothed GFP reporter to the ciliary membrane.⁹² It was also found that Rab29 biochemically interacts with Rab8, Rab11 and IFT20 in ciliated cells.⁹² Interestingly, Rab29 also regulates the assembly of the immune synapse, which is a specialized cell junction between T-cells and antigen presenting cells.⁹² Indeed, the immune synapse and cilium share structural and regulatory features underpinning their biogenesis and function such as a plasma membrane-docked centriole and Rab8-Rab11-IFT20/52/57/88 protein transport machinery.⁹³ As a 'frustrated cilium',⁹⁴ discoveries in the context of immune synapse can inform the biology of the cilium and the specific functions of cilia-related Rabs such as Rab8, Rab11 and Rab29.

Rab23

Rab23 was first described as a regulator of sonic hedgehog signaling in the mouse dorsal neural tube.⁹⁵ Since then, multiple studies show Rab23 to be an inhibitor of

cilium-based Shh signaling, acting at a similar step to IFT, downstream of Smoothed and upstream of the Gli transcription factors.^{16,84,96-99} Rab23 function is also implicated in autophagy, planar cell polarity, nodal signaling and cancer cell invasion.^{98,100-102}

A number of studies have found that Rab23 reporters localize to primary cilia and flagella, and in mammalian cells, this localization depends on GTP binding and the phosphorylated state of the GTPase.^{84,99,103,104} Thus, in addition to previously suggested transport functions at the plasma membrane, Rab23 may regulate transport events within the cilium itself.¹⁰⁵ It is unlikely, however, that these events include direct regulation of IFT because Rab23 depletion or loss does not affect cilia/flagella formation and length in various cultured mammalian cells lines, zebrafish (Kupfers vesicle), trypanosomes, and null mice (nodal cilia).^{98,99,103,104} Despite these observations, overexpression of mammalian Rab23(GDP) in cultured cells reduces ciliation and cilium length, whereas overexpression of Rab23 (GTP) increases cilium length.^{37,99} In agreement with these observations, overexpression of a Rab23-specific GAP (EVI5like) in hTERT-RPE1 cells also reduces ciliation.³⁷ Although caution must be excised when interpreting overexpression phenotypes, these data raise the possibility that Rab23 serves a ciliogenesis-related function, perhaps in a redundant capacity with other ciliary Rabs.

There is now mounting evidence that Rab23 regulates the transport of ciliary proteins. Data from 2 studies suggest that Rab23 negatively regulates the ciliary levels and transport of Smoothed, which correlates with Rab23s role as a Shh signaling inhibitor. In the first study (MDCK cells), Rab23 depletion or Rab23(GDP) overexpression was found to increase SmoA1 entry kinetics into cilia, whereas the opposite occurs in cells expressing Rab23(WT).⁸⁴ In the second study (NIH3T3 cells), Rab23 depletion caused a modest elevation of steady-state ciliary SmoA1 levels.⁹⁹ This study also found that Rab23 promotes the ciliary entry and tip accumulation of the KIF17 IFT-associated motor, and facilitates the formation of a ternary complex with KIF17 and its proposed ciliary import regulator, importin β .^{90,99} On the basis of epistasis data indicating independent functions for Rab23 and Ran, it was suggested that Rab23 regulates KIF17 transport toward the cilium, whereas the ciliary/cytoplasmic Ran gradient subsequently delivers KIF17 into the cilium.^{90,99} Thus, while Rab23 restricts ciliary targeting of Smo, the opposite is true for KIF17. How these 2 functions relate to each other in the context of Shh signaling remains to be determined. Also, since Rab23 and Rab8 are both linked to importin β -regulated ciliary trafficking, it will interesting to determine whether these GTPases function in common ciliary import pathways with nucleocytoplasmic proteins.^{89,99}

RAB23 was also recently reported to traffic the D1 dopaminergic receptor (D1R) and the somatostatin receptor 3 (SSTR3) to mammalian cilia.¹⁰⁴ In this study, photoactivatable reporters revealed that D1R laterally diffuses from the plasma membrane into the ciliary membrane via a mechanism involving its C-terminal tail, together with Rab23, KIF17 and IFT complex B.¹⁰⁴ As further evidence of its ciliary targeting capacity, fusions of Rab23(GTP) and non-ciliary receptors are ectopically targeted to cilia, as are Rab23 fusions with a normally mislocalised D1R variant (Δ 381-395).¹⁰⁴ Furthermore, Rab23(GTP) fused to D1R(Δ 381-395) bypassed, at least in part, the ciliary targeting requirement of KIF17 and IFT-B.¹⁰⁴ From this work, the authors conclude that Rab23 functions in an integrated pathway with KIF17 and IFT-B to target D1R from the plasma membrane to the ciliary membrane.

In summary, Rab23 is required for transport-based regulation of ciliary signaling, but is mostly dispensable for ciliogenesis (Fig. 2). Ciliary roles for Rab23 are further supported by phylogenetic analyses showing Rab23 loss in some organisms lacking cilia, including most organisms lacking motile flagella.^{6,103} In addition, Rab23 is mutated in Carpenter syndrome, which features ciliopathy-related symptoms such as craniofacial defects, cardiac abnormalities, obesity, digit and limb defects, hydrocephalus and mental retardation.¹⁰⁶⁻¹⁰⁹ Despite progress, there are many unanswered questions related to: (1) the nature of the Rab23 GEF/GAP regulators and effectors in the ciliary context, (2) the precise mechanistic relationship between Rab23, KIF17, Rab8 and Importin β /Ran transport pathways, and (3) how Rab23 functionally interacts with known ciliary transport pathways to inhibit Shh signaling.

Rab5

RAB5 is a central regulator of early endosome formation and associated protein sorting, driving key processes such as membrane tethering, fusion and motility.¹¹⁰ Despite these critical functions, surprisingly little is known about the role of Rab5 in ciliary processes. However, as outlined below, insight is now beginning to emerge from various studies.

A number of observations indicate that Rab5 functionally associates with cilia-related membranes. In *C. elegans* sensory neurons, GFP::RAB-5-marked early endosomes and additional endocytic regulators localize at the ciliary base, within the so-called periciliary membrane compartment (PCMC). An additional minor pool of RAB-5 is also found within at least some ciliary axonemes.¹¹¹⁻¹¹³ In support of RAB-5 functions near the ciliary base, disruption of a *C. elegans* p38 MAPK ortholog (PMK-3) that extracts inactive RAB5 from membranes

causes elevated PCMC accumulations of RAB-5.^{113,114} PCMC-associated RAB-5 also colocalises with OSTA-1, which is an organic solute transporter α homolog that regulates cilium morphology, ciliary membrane protein localisations, and the dendritic trafficking of RAB-5-associated endosomes.¹¹⁵ In addition, *C. elegans* RAB-5 colocalises at the ciliary base with ESCRT protein orthologues (STAM1, HRS) required for the ciliary localization and signaling functions of TRP channel polycystin 1/2 orthologues (LOV-1, PKD-2).¹¹¹ Consistent with the nematode findings, mammalian Rab5 is found on internalized cilium-derived early endosomes together with the ciliogenesis regulator, inositol 5-phosphatase (OCRL1).^{116,117} Furthermore, mammalian Rab5(GDP) overexpression slows the ciliary recovery of the apical receptor Kim1, indicating that Kim1 ciliary targeting involves Rab5-mediated recycling from apical membranes.⁸⁴ Together, these data provide good evidence that endosomal Rab5 functions at or near the ciliary base where many ciliogenic and ciliary signaling factors reside. Such findings are also consistent with the ciliary pocket being a unique site of endocytosis, enriched for clathrin coated pits, endocytic vesicles and endosomal compartments.¹¹⁸⁻¹²⁰

Although RAB5 appears to participate in ciliary endomembrane processes, its requirement for ciliogenesis remains unclear. In cultured mammalian cells, overexpression of Rab5(GTP) or Rabaptin5 (Rab5 GEF co-activation factor) reduces ciliation, whereas this does not occur in cells overexpressing Rab5(GDP) or depleted for Rabaptin5.^{121,122} These observations suggest that Rab5 is an inhibitor of ciliogenesis in cultured cells, and this notion is further supported by the very recent finding that RAB5(GDP) overexpression blocks cilium disassembly.¹²² In contrast, overexpression of RAB-5(WT), RAB-5(GDP) or RAB-5(GTP) does not affect the integrity and length of most examined cilia in *C. elegans*.^{112,113} The one exception is the AWB cilium, which possesses branching and membrane expansion defects in worms overexpressing RAB-5(GTP) or RAB-5(GDP).¹¹² Consistent with a mostly non-ciliogenic role for nematode RAB-5, cilium structures appear normal in worms depleted of RAB-5 (RNAi) and in worms with disrupted RAB-5 GEFs (RABX-5 or RME-6).^{112,113} Interestingly, loss-of-function mutations in these RAB-5 GEFs suppress the cilium structure defects of G-protein α -disrupted worms,¹¹³ which could indicate that RAB-5 negatively regulates ciliogenesis in this context, similar to what is described above for mammalian RAB5. Taken together, the mammalian and *C. elegans* data indicate that RAB5 is largely dispensable for ciliogenesis, although it serves as a negative regulator of cilium formation and resorption in some contexts.

Consistent with the above model, disruption of genes involved in the earliest steps of clathrin dependent endocytosis (CDE), upstream of Rab5, cause only modest or no effects on cilium structure. In AP2 (clathrin adaptor complex 2) or clathrin heavy chain-depleted mammalian cells, cilium formation is normal (IMCD3 cells) or only modestly affected (RPE1 cells).^{118,123} Similarly, cilium structure defects are only observed for a subset of cilia in *C. elegans* AP2 subunit gene mutants.¹¹² Thus, like RAB5, regulators of early CDE events are not globally required for ciliogenesis. However, and again like RAB5, the earliest steps of CDE are required for ciliary protein localization and signaling functions. For example, in AP2-disrupted worms, the ciliary localisations of multiple proteins (e.g., GPCRs and IFT components) are partially disrupted, IFT rates are abnormal, and AP2 subunit genes interact with known ciliary transport genes such as *bbs-8* to regulate ciliary membrane volume and homeostasis.¹¹² In mammalian cells, several studies show that CDE events at the ciliary base or pocket regulate Shh and TGF- β ciliary signaling.^{120,123-125} Together, the data discussed here for upstream components of presumptive RAB-5 pathways is consistent with the model that *C. elegans* RAB-5 is not a major regulator of ciliogenesis, but rather a regulator of ciliary protein trafficking and ciliary membrane homeostasis.

Rabl4 (IFT27)

Rabl4 belongs to the Rab-like (Rabl) clade of proteins distinguished from the canonical Rab family based on distinct sequence features such as the lack of a C-terminal prenylation motif.^{5,126,127} Although Rabl4 is not a classical Rab, it nonetheless retains all 5 Rab-specific consensus sequences (F1-F5) and it has been suggested that Rabl4 was part of the ancestral Rab family in LECA.^{6,127} Until the recent discovery of ciliary associations, nothing was known about Rabl4 functions in any cellular context.

In 2005, Lucker and colleagues identified a 27kDa protein, termed IFT27, that biochemically associates with a salt stable IFT-B complex core in *Chlamydomonas*.³⁴ IFT27 was subsequently identified as the algal ortholog of Rabl4 and shown to biochemically interact with IFT-B, localize to cilia and undergo IFT in multiple systems.¹²⁸⁻¹³⁴ In agreement with its lack of a prenylation motif, flagellar IFT27 biochemically partitions with the aqueous phase in *Chlamydomonas*, indicating that IFT27 function may not depend on ciliary membrane associations.¹²⁸ However, the ciliary localization, IFT movement and IFT-B association of IFT27 is dependent on GTP binding, as well as nucleotide-independent heterodimer formation with the IFT25 phosphoprotein.^{129,130,132-138}

IFT25/27 is thought to associate with the IFT-B1 core module via a surface-exposed patch adjacent to the GTP-binding site that interacts with the C-terminal coiled coil region of the IFT81/74 complex.^{130,139-141} Thus, RabL4 is a conserved *bona fide* IFT protein that forms a stable complex with IFT25, tethered to the IFT-B1 module. Consistent with its ciliary association, RabL4 is lost from the genomes of organisms that lack cilia.^{6,127}

Despite a conserved biochemical association with the IFT-B particle, IFT27 is dispensable for cilium formation in some cell types but not others. In *Chlamydomonas* and trypanosomes, IFT27 depletion or overexpression of putative GDP- or GTP-locked variants (trypanosomes) results in truncated flagella and cell division/growth defects.^{128,133} In IFT27-depleted trypanosomes, the phenotype resembles a retrograde IFT defect, consisting of flagellar accumulation of IFT-B proteins and flagellar loss of IFT-A and IFT dynein proteins.¹³³ This led to the model that IFT27 controls the flagellar import of retrograde IFT machinery by regulating its docking to IFT trains.¹³³ In contrast, most ciliary structures are normal in IFT27 (and IFT25) knockout mice, with normal ciliary levels of IFT-A, IFT-B and IFT dynein, despite a neonatal-lethality phenotype.^{132,134,138,142} Interestingly, the one exception is the mouse sperm flagellum, whose formation relies on an intact IFT25/27 complex.¹⁴³ The ciliogenesis and neonatal lethality phenotype of IFT25 and IFT27 null mice contrasts with the strong ciliogenesis defects and mid-gestation lethality phenotype of mice with mutations in other IFT-B genes.^{144,145} Thus, in higher organisms, the IFT25/27 heterodimer serves as a functionally distinct submodule of IFT-B1 that is not required for ciliogenesis and IFT in somatic cells.

Although most cilium structures are normal, IFT27 null mice display cilium-associated developmental defects in digit formation, bone, left-right asymmetry, neural tube patterning and cardiac formation.¹³² Similar abnormalities occur in IFT25 knockout mice, although the phenotypic severity and penetrance is somewhat reduced.¹³⁸ Associated with these tissue level phenotypes are defects in cilium-associated sonic hedgehog signaling.^{132,138,142} Specifically, IFT27^{-/-} fibroblasts treated with SAG (Shh agonist) possess a reduced capacity to upregulate Gli1/Ptch1 expression and redistribute the Gli2 transcription factor to the ciliary tip; also, Smo/Ptch1/Gpr161 abnormally accumulate in the cilia of untreated IFT27^{-/-} cells.^{132,134,142} Furthermore, expression of a constitutively active Smo variant (SmoM2) causes Gli2 to localize all along IFT27-disrupted cilia, rather than just the tip, indicating that IFT27 is not required for Gli2 ciliary entry but rather its transport to, or association with, the ciliary tip.¹³² Thus, IFT27 regulates the ciliary distribution and removal of Shh signal mediators.

Mechanistically, these mammalian IFT27 functions appear to be linked to the BBSome and associated recruitment factors (Lztfl1 and ARL6) based on: (1) BBSome-associated proteins abnormally accumulate in IFT27 (and IFT25) disrupted cilia, (2) Shh signaling mediators (Smo, GPR161) accumulate within BBSome and ARL6 disrupted cilia, (3) ARL6, and specifically the nucleotide free form, biochemically interacts with IFT27 and not other IFT proteins, and (4) BBSome ciliary exit kinetics are retarded in IFT27-disrupted cells.^{132,134,146} In one model, Lztfl1 couples IFT-B-associated IFT25/27 with the BBSome, which in turn interacts with Smo at the ciliary membrane to facilitate its removal from the organelle via retrograde IFT.¹³² In a second complementary model, IFT25/27 released from IFT-B at the ciliary tip serves as a GEF to activate ARL6, which in turn drives assembly and recruitment of the BBSome (and associated cargo) at the ciliary tip membrane, followed by BBSome association with retrograde IFT trains. These models (shown in Fig. 4A) are consistent with a role for the *Chlamydomonas* BBSome in flagellar export of signaling molecules, the observation that a portion of IFT25/27 is biochemically separated from IFT-B, and the pathogenic association of IFT27 with Bardet-Biedl syndrome.^{129,131,147,148} In relation to the second model, it is important to note that although the IFT25/27 complex stabilises nucleotide-free ARL6, its ability to induce GDP release from ARL6 is very limited. While this could be explained by a requirement of other factors to reconstitute full GDP-release activity,¹³⁴ it remains to be shown whether IFT27 is a true ARL6 GEF.

Taken together, the available data establishes IFT- and BBSome-associated roles for Rabl4 (IFT27) in regulating cilium structural integrity (protists), function and developmental signaling. Such functions are consistent with the loss of Rabl4 from the genomes of non-ciliated organisms.⁶ Of great interest for future work will be to identify the mechanisms regulating the activity of Rabl4 itself. Similar to other small ciliary GTPases like Arl13b, Rabl4 possesses a poorly conserved switch II catalytic glutamine, which might explain why stabilised IFT27 (and Arl13b) possesses very low intrinsic GTPase activities.^{39,130,149} Low GTPase activity also indicates that IFT27 GTP hydrolysis probably requires a GAP regulator that remains to be identified.¹³⁰ Also, it is noteworthy that stabilised IFT27 has relatively weak micromolar affinity for GDP, indicating that GDP to GTP exchange may not require a GEF.¹³⁰

Rabl5 (IFT22)

RAB-like 5 (Rabl5) displays a high degree of sequence divergence from canonical Rab proteins, lacking, for

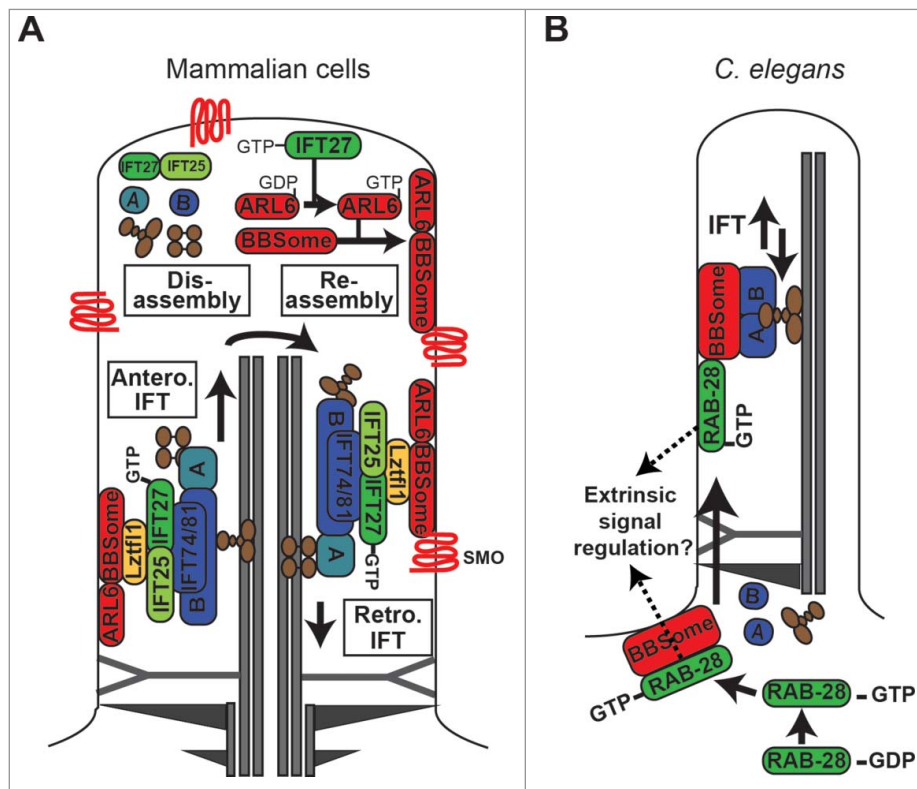


Figure 4. Models of RABL4 (IFT27) and RAB28 association with IFT and the BBSome cargo adaptor. (A) IFT27 forms a submodule of IFT-B that associates with the BBSome via Lztf11. At the ciliary tip, where IFT trains are remodelled for retrograde transport, IFT27 acts as a GEF to activate ARL6, which in turn directs the BBSome to the ciliary membrane for assembly into retrograde IFT trains that remove signaling proteins such as Smoothened (Smo) from the cilium. IFT complexes (A and B) shown in blue. IFT motors (kinesin-II and cytoplasmic dynein) shown in brown. (B) In *C. elegans*, activated RAB-28 is targeted to the periciliary membrane via the BBSome where it incorporates into IFT trains as cargo. RAB-28 is proposed to serve cell non-autonomous functions by regulating extrinsic signaling to a nearby glial cell.¹⁶⁴ Note that it remains to be shown if RAB-28 directly interacts with the BBSome. For simplicity, only one microtubule doublet is shown in the cartoon.

example, several the Rab consensus sequences (F4, F5).^{5,6,126,127,150} In fact, multiple studies place Rab15 closer to Ras/Rho/Ran family members than Rabs.^{5,6,127} Like Rab14, the cellular functions of Rab15 were completely unknown before ciliary associations were uncovered.

The first functional association with the cilium was uncovered by Schafer and colleagues who found that the sole Rab15 ortholog (termed IFT-associated protein 2 or IFTA-2) in *C. elegans* is expressed exclusively in ciliated cells and undergoes IFT.¹⁵⁰ This study also found that a predicted IFTA-2(GDP) variant is excluded from the cilium, indicating that GTP-binding regulates the GTPase's association with IFT trains.¹⁵⁰ Consistent with these findings, Rab15 is also found in the ciliary and flagellar axonemes of mammalian cells and protists (*Chlamydomonas*, trypanosomes); also, trypanosome Rab15 undergoes IFT and accumulates at the basal body and axoneme of IFT-B and IFT-A gene depleted cells, respectively.^{37,126,150,151} Thus, Rab15 is a conserved IFT-associated protein, similar to Rab14.

Work in *Chlamydomonas* has revealed that Rab15 biochemically associates with IFT complexes. Specifically, the 22kDa Rab15 ortholog (called FAP9 or IFT22) co-sediments with IFT81 (but not IFT139), indicating association with IFT-B, but not IFT-A, complexes.¹⁵¹ This conclusion is further supported from immunoprecipitation and IFT-B1 reconstitution experiments showing that *Chlamydomonas* IFT22 binds to the IFT81/74 coiled-coil region, similar to the interaction observed for IFT25/27.¹⁴⁰ However, despite a common binding region, IFT22 does not directly interact with IFT25/27, nor is IFT22 required for the formation of a stable IFT81/74/27/25 tetrameric complex.^{140,152} Therefore, although IFT27 and IFT22 both associate with IFT-B1, they likely occupy distinct functional submodules of the assembly.

Despite the low overall sequence homology between Rab15 with Rab14, the shared association with IFT-B implicates related functions for these GTPases. Indeed, like Rab14, Rab15 is conserved in ciliated organisms versus non ciliated organisms, lacks C-terminal prenylation and

switch II glutamine residues, and biochemically partitions with the aqueous (rather than the membrane) flagellar fraction in *Chlamydomonas*.^{126,150,151,153} Furthermore, and again similar to Rab14, trypanosome Rab15 depletion causes a retrograde IFT-like phenotype, comprised of a short bulbous flagellum with accumulation of electron dense material that includes IFT172.¹²⁶ Consistent with this finding, *Chlamydomonas* IFT22 depletion causes IFT-A/B protein redistribution to the flagellum, although flagellum length remains normal.¹⁵¹ Thus, like Rab14, protist Rab15 appears to regulate retrograde IFT, as well as flagellum structural integrity in trypanosomes.

Rab15 function is different in metazoans. In *C. elegans*, a predicted null mutant of the worm ortholog (called IFTA-2) displays normal ciliary localisations and IFT movement of IFT-A/B and an IFT-dynein light intermediate chain protein, as well as normal cilium structures.¹⁵⁰ Despite these observations, IFTA-2 loss causes defects in cilium-associated signaling pathways related to lifespan and the formation of the alternative ‘dauer’ developmental stage.¹⁵⁰ Therefore, like mammalian Rab14, Rab15 appears dispensable for cilium formation in *C. elegans*, but necessary for cilium-mediated signaling.^{132,134,150} Interestingly, the ciliary localisations of 2 key lifespan and dauer formation signaling molecules (DAF-2, AGE-1) are not affected in IFTA-2-disrupted worms;¹⁵⁰ thus, unlike mammalian Rab14, there is no evidence at this point that nematode Rab15 regulates the ciliary levels of signaling molecules.

Rab28

Rab28 is considered a peripheral member of the Rab superfamily, possibly forming an atypical Rab subfamily (Group VI) with its closest homolog, Rab14.^{5,6,127,154} Rab28 possesses several unusual features including divergent switch 1 sequences and the ability to undergo an abnormally large nucleotide-dependent conformational change.¹⁵⁵ Although relatively unstudied compared with other Rabs, Rab28 is reported to control Glut4 trafficking, NF- κ B nuclear transport, endosomal sorting (trypanosomes), and plant germination.¹⁵⁶⁻¹⁵⁹

The first ciliary connection was reported by Roosing and colleagues in 2013. In this study, Rab28 was found to localize at the ciliary basal body and rootlet of rat photoreceptor cells, and nonsense mutations (R137X, E189X) in *RAB28* were identified in 2 families with the non-syndromic retinal ciliopathy, cone-rod dystrophy (CRD).^{160,161} Subsequent studies identified additional CRD families with homozygous splice donor site (exon 2) and missense (C217W) *RAB28* mutations¹⁶² and additional Rab28 localisations to the mouse photoreceptor outer segment (modified cilium).¹⁶³

Consistent with the mammalian localization data, our laboratory in collaboration with the Leroux group recently reported that the sole ortholog in *C. elegans* is expressed almost exclusively in ciliated cells, accumulates at the periciliary membrane and ciliary axoneme, and undergoes IFT.¹⁶⁴ We also found that RAB-28s ciliary membrane and IFT associations depend on GTP binding and the BBSome IFT cargo adaptor.¹⁶⁴ Consistent with RAB-28 being an IFT cargo, rather than a core component of the IFT machinery, *rab-28* null mutants display apparently normal cilium structures and sensory functions, as well as normal localisations and transport behaviors of IFT proteins and the BBSome.¹⁶⁴ Together, these findings establish RAB-28 as the third IFT-associated Rab alongside Rab14 and Rab15, and suggest that nematode RAB-28 functions as an IFT cargo, downstream of the BBSome, to regulate a discrete set of ciliary pathways (Fig. 4). Although mammalian Rab28 ciliary functions are not yet described, the nematode findings could indicate that Rab28 and Rab14 function at distinct steps of a common BBS-related pathway, upstream (Rab14) and downstream (Rab28) of the BBSome.^{132,134,164} This scenario is consistent with the relatively severe BBS phenotype of patients with *RABL4* (IFT27) mutations compared with the restricted CRD phenotype of patients with likely null mutations in *RAB28*.^{148,160,161}

An additional interesting finding from nematode studies is that RAB-28 may serve cell non-autonomous functions in neighboring glial cells (Fig. 4). In worms overexpressing RAB-28(GDP) or RAB-28(GTP), defects occur in the non-ciliated glial (sheath) cell that forms the supporting channel for the amphid ciliary bundle. Specifically, RAB-28(GTP) overexpression dramatically enlarges the channel, whereas RAB-28(GDP) overexpression slightly reduces channel size and causes large matrix filled vesicles (MFVs) to accumulate in the sheath cell.¹⁶⁴ These phenotypes appear to be cell non-autonomous effects because RAB-28 is not expressed in the sheath.¹⁶⁴ In one model, based on seminal work from the Shaham group,¹⁶⁵⁻¹⁶⁷ MFV delivery to the sheath cell membrane, and therefore the expansion of the amphid pore, is regulated by RAB-28.¹⁶⁴ Thus, excessive RAB-28 activation would enhance MFV delivery to cause an abnormally large channel, whereas the opposite occurs when RAB-28 is inactivated. Although we don't know how ciliary RAB-28 might signal to glia, one possibility is that the GTPase regulates release of ciliary ectosomes carrying glial cell morphogenic factors. In support of this idea, the ciliary base region (where activated RAB-28 localizes) has been proposed as one potential site of nematode extracellular vesicle (EV) release and RAB-28 expression is upregulated in EV-releasing cells.¹⁶⁸⁻¹⁷⁰

Rabl2

Rabl2 sequences are significantly diverged from most Rab proteins, with some studies considering it to be more 'Ran-like' than 'Rab-like'.^{6,127} However, Rabl2 retains all 5 Rab consensus motifs and therefore Rab-like functions for RABL2 cannot be fully excluded.^{5,171} Although very little is known about its cellular functions, there is evidence that Rabl2 serves cilia-related roles.

In 2012, it was reported that mice carrying a hypomorphic mutation (Mot) in Rabl2 are sterile, possessing modestly truncated sperm tails and reduced sperm motility.¹⁷¹ Consistent with a ciliary role, Rabl2 localizes to the sperm tail mid-piece (proximal segment of flagellum) and immunoprecipitates IFT-B1 proteins (IFT27/81/172).¹⁷¹ In addition, the study identified 5 candidate Rabl2 effectors, which localize at the sperm tail principal piece and the more proximal mid piece.¹⁷¹ These localisations are reduced in Rabl2(Mot) sperm, which led to the proposal that Rabl2 delivers effector proteins into the sperm flagellum up to the point of the annulus (distal-most end of mid-piece).¹⁷¹ Together, these data implicate ciliary and IFT-associated functions for Rabl2 and agree with a recent phylogenetic analysis indicating that Rabl2 is an ancestral ciliary component.¹⁷² Furthermore, the suggested IFT association reflects a role for Rabl2 during flagellum formation, since IFT is absent in mature mouse sperm.¹⁷³

In a recent follow-up study, the O'Bryan group reported that Rabl2 knockout mice develop adult onset obesity and fatty livers (steatosis), as well as impaired glucose and lipid metabolism.¹⁷⁴ Although reminiscent of a ciliopathy pathology, these mice lack hallmarks of cilia-related obesity such as hyperphagia and leptin resistance, which lead to the conclusion that Rabl2 is not a global ciliary regulator.¹⁷⁴ Indeed, this study found that Rabl2 loss alters hepatic mitochondrial fatty acid oxidation and mitochondrial transport along microtubules, suggesting that Rabl2 serves a non-ciliary function in addition to the previously reported role in sperm flagella.^{171,174} Interestingly, Rabl2 colocalises with specific IFT proteins (IFT172) along hepatic cytoplasmic microtubules, indicating that a cytosolic Rabl2 function may involve interaction with a distinct IFT complex.¹⁷⁴

Very recent studies from the Jackson and Nakayama groups now reconcile and extend the previous findings in mice.^{175,176} Specifically, these works show that RABL2 localizes at the base of the *Chlamydomonas* flagellum and human RPE1 cell primary cilia, using a recruitment mechanism that involves CEP19, FOP and CEP350.^{175,176} These studies also show that activated RABL2B binds the IFT-B complex via the IFT74/81 heterodimer, regulates its entry into the cilium, and that RABL2 disruption affects cilium and flagella

formation.^{175,176} Furthermore, Rabl2 knockout mice display retinal degeneration and digit abnormalities, which together with the described previously obesity and sperm defects, suggests that Rabl2 may in fact serve broad cilia-related functions in mammals.^{171,174,175} Together, all the various data supports a conserved role for RABL2 at the base of motile and primary cilia in regulating IFT-B complex entry into cilia.

Concluding remarks

In this review, we outline the cilium-associated functions of various Rab and related Rab-like proteins. From the available data, we make the following general points.

First, the cilia-related Rabs are not globally required for ciliogenesis in the manner observed for other ciliary trafficking modalities such as IFT. For example, despite ciliogenic requirements for Rab8 in cultured mammalian cells and zebrafish, and Rabl2/4/5 in protists, loss of ciliary Rab function does not affect ciliary structures in worms (Rab8, Rab28, Rabl5), zebrafish (Rab23), and mice (Rab8, Rabl4/5). Instead, the ciliary Rabs could regulate ciliogenesis in specific subsets of metazoan cells not yet analyzed; alternatively, as shown for Rab8 and Rab10 in mice, the ciliary Rabs may function redundantly with other Rabs to regulate ciliogenesis by sharing common effectors, for example.

Second, like other Rabs, the ciliary Rabs can function within GTPase cascades to control early ciliogenesis events in cultured mammalian cells (Rab11-Rab-8), post-ciliogenic sorting and delivery of RTCs in photoreceptors (ARF4-Rab11-Rab8), and turnaround of the BBSome at the ciliary tip (Rabl4-Arl6). These exciting findings raise important questions with regards to the conservation of such cascades *in vivo*, and the tissue-specific relevance of the ARF-Rab TGN sorting cascade for ciliary membrane proteins other than Rhodopsin. Indeed, a very recent study in rat kidney cells (NRK1) showed that overexpression of RAB11(GTP) or RAB11(GDP) does not affect cilium formation, suggesting that ciliogenesis is not under the strict control of a Rab11-Rab8 cascade.⁸⁸ Furthermore, regulation of mammalian fibrocystin ciliary steady-state levels appears to require Rab8, but not Arf4.^{85,177} Similarly, mammalian Arf4 was reported in a recent study to be dispensable for polycystin 1 (PC1) ciliary localization, despite previous findings that the ciliary targeting of a PC1-chimera is dependent on Rab8 and Arf4.^{86,178} The *in vivo* significance of the ARF-Rab cascade is also brought into light by another recent study, which shows that Arf4 deletion in the retina does not cause rhodopsin trafficking or photoreceptor degeneration.¹⁷⁹ It also remains to be determined if Rab11 functions with Rab8 to regulate PC1 and fibrocystin ciliary targeting.

Third, it is interesting that 4 atypical Rabs, Rab12/4/5 and Rab28, are all linked to IFT-related processes, perhaps forming distinct cargo adaptor submodules of IFT-BBSome assemblies, or in the case of Rab12, providing a mechanism for IFT-B entry into cilia. Indeed, these GTPases appear to have co-evolved with the cilium, and therefore IFT, and are typically lost in the genomes of organisms lacking cilia. An additional interesting feature of Rab12/4/5 is their lack of a prenylation modification motif in the C-terminus, indicating ciliary functions not directly related to the ciliary membrane.

Fourth, we know very little about the regulatory proteins (e.g., GEFs, GAPs, GDIs, GDFs) that control Rab function in the ciliary context. Also, how the ciliary Rabs collaborate with other ciliary transport and ciliopathy modules remains poorly understood, although some progress has been made.^{175,176,180}

Fifth, 3 of the 8 known Rab/Rabl ciliary proteins (Rab23, Rab14, Rab28) are already known to cause ciliopathy or ciliopathy-related (Carpenters syndrome) diseases. Based on their phylogenetic restriction to ciliated organisms, together with their apparent non-essential requirement for ciliogenesis, Rab12 and Rab15 represent excellent candidates for mutation analysis in ciliopathy patient studies.

Sixth, it is necessary to address the essential nature of some of the ciliary Rabs (Rab5, Rab11). To determine their cilium-specific functions, whole organism, tissue and/or cell specific knockouts (e.g., via CRISPR) will need to be used to derive specific Rab alleles (e.g., GDP- and GTP- locking mutations). It will also be necessary to use knock-in Rab reporters (e.g., GFP tagged) to overcome the potential pitfalls of Rab reporter overexpression.

Finally, the cilium represents a new paradigm for uncovering novel Rab functions, mechanisms of action and disease associations. Indeed, findings for Rabs in the ciliary context can inform the functions of Rabs in other subcellular contexts, and vice versa, and therefore facilitate a greater overall understanding of Rab-mediated membrane trafficking.

Disclosure of potential conflicts of interest

No conflicts of interest were disclosed.

Acknowledgments

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