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REVIEW

Small GTPases and cilia

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ABSTRACT

Small GTPases are key molecular switches that bind and hydrolyze GTP in diverse membrane- and cytoskeleton-related cellular processes. Recently, mounting evidences have highlighted the role of various small GTPases, including the members in Arf/Arl, Rab, and Ran subfamilies, in cilia formation and function. Once overlooked as an evolutionary vestige, the primary cilium has attracted more and more attention in last decade because of its role in sensing various extracellular signals and the association between cilia dysfunction and a wide spectrum of human diseases, now called ciliopathies. Here we review recent advances about the function of small GTPases in the context of cilia, and the correlation between the functional impairment of small GTPases and ciliopathies. Understanding of these cellular processes is of fundamental importance for broadening our view of cilia development and function in normal and pathological states and for providing valuable insights into the role of various small GTPases in disease processes, and their potential as therapeutic targets.

KEYWORDS Small GTPase, cilia, ciliopathy

INTRODUCTION

Cilia act as motile or sensory devices on the surfaces of most eukaryotic cells, and cilia dysfunction results in a variety of severe human pathologies, now collectively termed ciliopathies (Badano et al., 2006; Singla and Reiter, 2006; Fliegauf et al., 2007; Marshall, 2008). Phylogenetically conserved intraflagellar transport (IFT) machinery, which is composed of IFT-A and IFT-B subcomplexes, mediates the bidirectional movement of IFT cargos that are required for the biogenesis, maintenance, and signaling of cilia (Rosenbaum and Witman, 2002; Scholey and Anderson, 2006; Pedersen and Rosenbaum, 2008; Scholey, 2008). However, an understanding of how cilia form and execute their sensory function remains poorly defined.

In the last few years, several lines of emerging evidences suggested that small GTPases could play critical roles in many aspects of cilia formation and signaling. The small GTPase superfamily has numerous members. In this review, we focus on several small GTPases in Arf/Arl, Rab, and Ran subfamilies. We will briefly introduce the properties of small GTPases and the current understanding about cilia and ciliopathies. We will discuss the recent advances that revealed the importance of various small GTPases in the context of cilia, with an emphasis on the underlying molecular mechanism and the general implication in cilia biogenesis and sensory function. Lastly, we will introduce the advantages of applying the genetic model nematode *Caenorhabditis elegans* in cilia-related research. Our goal in this review is to highlight the critical features of small GTPases in cilia-related cellular events in both normal and pathological states.

SMALL GTPases

The small GTPase superfamily is divided into five major subfamilies: Ras, Rho, Rab, Arf/Arl, and Ran, on the basis of sequence and function similarities (Wennerberg et al., 2005). Small GTPases are key regulators of many cellular processes, such as vesicular trafficking, differentiation, cell division, proliferation, migration, cytoskeleton organization, gene transcription, and nuclear assembly (Wennerberg et al., 2005; Lundquist, 2006). Like heterotrimeric G proteins, small GTPases are monomeric G proteins and act as molecular switches, where the switching process relies on GTP hydrolysis. Small GTPases cycle between the active GTP-bound and the inactive GDP-bound states (Boguski and McCormick, 1993; Donovan et al., 2002). The GTP/GDP cycling process is facilitated by guanine nucleotide exchange factors (GEFs) that both stimulate GDP loss and GTP

binding, as well as by GTPase activating proteins (GAPs) that mediate GTP hydrolysis (Cherfils and Chardin, 1999; Donovan et al., 2002; Bernards and Settleman, 2004).

The founding members of small GTPase superfamily are the Ras proteins that were first characterized as human oncogene products (Lowy and Willumsen, 1993). As master regulators of diverse signaling pathways, Ras proteins control cell proliferation, differentiation and survival (Reuther and Der, 2000; Wennerberg et al., 2005). Small GTPases in Rho subfamily mainly serve as key regulators in actin and microtubule cytoskeleton organization, cell cycle progression, cell division, adhesion and gene expression (Jaffe and Hall, 2005). Rab proteins comprise the largest subfamily of small GTPases with more than 60 members in mammals (Pereira-Leal and Seabra, 2001; Zerial and McBride, 2001; Stenmark, 2009). They are believed to control the membrane identity, vesicle budding, uncoating, tethering and fusion through the recruitment of the sorting, tethering and docking factors (Stenmark, 2009). Like the Rab proteins, the ADP-ribosylation factor (Arf) and some Arf-like (Arl) proteins are also involved in vesicular transport (D'Souza-Schorey and Chavrier, 2006; Myers and Casanova, 2008). Ran is the most abundant small GTPase in the cell, which is known for its nucleocytoplasmic transport function of both proteins and RNAs (Weis, 2003).

CILIA AND CILIOPATHIES

Cilia, specialized organelles with a microtubule core (the axoneme), are present on the surfaces of most polarized cells in the human body. There are two main types of cilia: motile and immotile (also known as primary cilia). In general, motile cilia contain 9 pairs of outer microtubule doublet surrounding a central pair (9 + 2 configure). The dynein arms and radial spoke are connected to outer doublets and responsible for generating force and regulating the ciliary beating direction. Primary cilia lack the central pair and present a 9 + 0 arrangement. While the physiologic roles of motile cilia in cell locomotion, sexual reproduction and fluid flow generation have long been recognized, the importance of primary cilia was overlooked until last decade when the correlation between the primary cilia dysfunction and various human genetic diseases was revealed.

Primary cilia serve as sensory organelles and assemble via the IFT, two features that are phylogenetically conserved in all ciliated organisms. IFT is a microtubule-based bi-directional motility that is required for the development of all cilia/flagella (Rosenbaum and Witman, 2002; Pedersen and Rosenbaum, 2008). Pioneering insights into the mechanism of IFT emerged from the observation of microscopic particles moving up and down the length of flagella of the green algae *Chlamydomonas* (Kozminski et al., 1998). The cellular machinery that drives IFT was determined primarily by cellular and biochemical approaches (see references within (Rosenbaum and Witman, 2002)). The IFT machinery in *Chlamydomonas* includes heterotrimeric kinesin-II, which consists of two motor subunits and one non-motor accessory subunit, and retrograde cytoplasmic dynein motors that move IFT particles and cargos to and from the distal tips of cilia. The IFT particle is composed of two subcomplexes (A and B) that contain 16–18 polypeptides. It's well accepted that the IFT complex is assembled at the cilia base to load the cargos, including membrane receptors, structural proteins, and signaling molecules, then transported to the ciliary tip and unloaded the cargos, which are used to build cilia and maintain the sensory function (Rosenbaum and Witman, 2002; Scholey and Anderson, 2006; Pedersen and Rosenbaum, 2008; Scholey, 2008). On the other hand, retrograde movement of the IFT complex not only functions to recycle the IFT machinery to ciliary base, but also is indispensable for the cilia signaling transduction, such as phototaxis in green algae (Lechtreck et al., 2009) and sonic hedgehog (Hh) signaling in mammalian cells (Huangfu and Anderson, 2005; May et al., 2005; Tran et al., 2008).

Consistent with their universal cellular distributions and their roles in a number of pivotal signaling pathways, such as Hh signaling, platelet-derived growth factor receptor (PDGFR) signaling, Wnt signaling, and Planar Cell Polarity (PCP), perturbations in the cilia formation and function give rise to a broad range of genetic disorders, collectively termed ciliopathies (Badano et al., 2006; Gerdes et al., 2009; Goetz and Anderson, 2010). This classification includes polycystic kidney disease (PKD), Bardet–Biedl syndrome (BBS), Joubert syndrome, nephronophthisis (NPHP), Meckel–Gruber syndrome (MKS) and so on (Table 1). The functions of all identified ciliopathy candidates have been suggested to be cilia and/or basal body related; nonetheless, the precise roles remain enigmatic (Eley et al., 2005; Badano et al., 2006; Fliegauf et al., 2007).

SMALL GTPases AND CILIOPATHIES

In the past decade, a large body of evidences revealed that small GTPases are involved in cilia biogenesis and function, as well as ciliopathies.

Arf/Arl

The cellular functions of most Arf/Arl small GTPases are poorly understood. The first direct evidence connected an inherited human ciliopathy with a member of Arf/Arl small GTPases came from Arl6 (also termed BBS3) (Fan et al., 2004). BBS is a genetically heterogeneous human disorder characterized by obesity, polydactyly, mental retardation, retinal degeneration, and renal cyst (Zaghloul and Katsanis, 2009). So far, 14 *BBS* loci have been found in patients. To map the causal gene in *BBS3* patients, Fan et al. first reasoned that the ortholog of *BBS3* should exist in other

Table 1 Clinical feature in the ciliopathies

	PKD	BBS	MKS	JS	NPHP	OFD	SL	ALMS	MKKS
Cystic kidney	+	+	+	+	+	+	+	+	
Heart disease		+						+	+
Hepatic dysfunction	+	+	+	+	+	+	+	+	
Retinal degenaration		+	+	+			+	+	
Mental retardation		+	+	+		+			
Polydactyly		+	+	+		+			+
Diabetes		+						+	
Obesity		+						+	
Left-right asymmetry		+	+	+	+		+		

PKD, polycystic kidney disease; BBS, Bardet–Biedl syndrome; MKS, Meckel–Gruber syndrome; JS, Joubert syndrome/Cerebello-oculo-renal syndrome; NPHP, nephronophthisis; OFD, orofaciodigital syndrome; SLSN, Senior–Løken syndrome; ALMS, Alstrom syndrome; MKKS, McKusick–Kaufman syndrome.

ciliated organisms. In *C. elegans*, it's already known that *daf-19* encodes the sole member of the regulatory factor X (RFX) transcription factor family and acts as the master regulator of ciliogenesis (Swoboda et al., 2000). DAF-19 regulates ciliogenesis by binding to an X-box motif in the promoters of target genes. By analyzing the *C. elegans* genes with X-box in their promoters and then correlating to the human gene homologs in the *BBS3* critical interval, Fan et al., were able to identify *ARL6* as the causal locus for *BBS3* (Fan et al., 2004). A similar comparative genomics approach using *C. elegans* genome as reference also characterized *ARL6* as the gene mutated in *BBS3* patients (Chiang et al., 2004). With the determination of the crystal structure of GTP-bound Arl6 (Wiens et al., 2010), it's found that the altered resides found in *BBS3* patients cluster in or around Arl6 GTP binding motif, suggesting the importance of small GTPase activity in ciliopathy etiology. Furthermore, overexpression of GDP- or GTP-locked forms, leads to anomalies in cilia assembly and Wnt signaling *in vivo* (Wiens et al., 2010). Seven other BBS proteins (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9) and a BBIP10 protein form the core complex called the BBSome (Nachury et al., 2007; Loktev et al., 2008). The BBSome is proposed in promoting cilia membrane biogenesis (Nachury et al., 2007), and the ciliary targeting of IFT cargo proteins (Berbari et al., 2008; Jin et al., 2010). Nachury's group found that $Arl6^{GTP}$ and the BBSome localize to mammalian primary cilia in an interdependent manner, and the ciliary targeting of the membrane receptor somatostatin receptor 3 (SSTR3CTS) is directly mediated by the BBSome (Jin et al., 2010). This finding supports the hypothesis that the abnormal Arl6 small GTPase activity leads to the compromised ciliary entry of critical cargo proteins essential for cilia assembly and signaling and, thus, causes the variety of BBS symptoms.

The second Arl small GTPase identified as a ciliopathy candidate is *Arl13b*, one of causal loci for ciliopathy Joubert Syndrome disorder. Joubert syndrome is the most common inherited cerebellar malformation syndrome and characterized by congenital cerebellar ataxia, hypotonia, oculomotor apraxia, and mental retardation, cystic kidney, polydactyly. Unlike the common ~25 kDa small GTPase, Arl13b is an atypical small GTPase with an elongated C terminus which contains a coiled-coil domain and a proline-rich domain. Similar to *BBS3*, mutations found in JS patients interfere with the GTP binding activity of Arl13b. *Arl13b* mutant mice (*hennin)* showed coupled defects in cilia structure and Hh signaling, and mimicked the mutant phenotypes in human patients (Caspary et al., 2007). Due to the lethality of *hennin* mice, *C. elegans* is used as an alternative model to gain mechanistic insights into the *in vivo* function of Arl13b protein. Studies from the Blacque's laboratory and our laboratory revealed a role for ARL-13, the *C. elegans* ortholog of human Arl13b, at ciliary membranes, where it regulates ciliary transmembrane protein localizations and anterograde IFT assembly stability (Cevik et al., 2010; Li et al., 2010). Moreover, our laboratory's work showed that ARL-3, the *C. elegans* homolog of human Arl3, acts antagonistically with ARL-13 to regulate IFT integrity and ciliogenesis (Li et al., 2010). Specifically, the absence of ARL-13 causes destabilized IFT complex and IFT-A and IFT-B tend to dissociate in anterograde IFT transport; whereas ARL-3 depletion can partially rescue ciliogenesis defects in *arl-13* mutants by stabilizing the association between IFT-A and IFT-B.

Our work revealed that ARL-3 acts through an HDAC6-dependent pathway (Li et al., 2010). HDAC6 is a unique member of the histone deacetylase family because it deacetylates not only histone (Grozinger et al., 1999), but also α-tubulin (Hubbert et al., 2002), Hsp90 (Kovacs et al., 2005), and coractin (Zhang et al., 2007). Inhibition of HDAC6 enzymatic activity causes increased kinesin-1-cargo complex transportation along the microtubule in neuronal cells (Reed et al., 2006). Interestingly, activation of HDAC6 was found to promote cilia disassembly and loss of HDAC6 activity selectively stabilizes cilia in human retinal epithelial cells

(Pugacheva et al., 2007). Currently, HDAC6 is thought to regulate microtubule-dependent cellular processes by deacetylating α-tubulin. However, this model is not true at least in *C. elegans* ciliogenesis pathway because MEC-12, the only α-tubulin capable of being acetylated, does not express in ciliated cells in *C. elegans* (Fukushige et al., 1999). Another HDAC6 substrate Hsp90 was shown to associate with β-tubulin and may be involved in stabilizing tubulin polymerization in cilia (Takaki et al., 2007). However, a *null* allele of *daf-21*, the *C. elegans* homolog of *Hsp90*, possesses normal cilia in our analysis (data not shown). These data ruled out the possibilities of α-tubulin and Hsp90 as the candidates in ARL-3-HDAC-6 pathway in the process of cilia formation in *C. elegans*. Based on our model, we propose that HDAC-6 probably influences the acetylation level of an unknown adaptor protein in IFT complex. Intriguingly, HDAC6 was found physically associated with a subunit of the BBSome, BBIP10 (Loktev et al., 2008), suggesting a potential functional crosstalk between Arl3 and Arl6/BBS3 pathways in cilia.

The cilia-related function for Arl3 and Arl6 may be highly conserved across species. A comparative genomics study indicated that Arl3 and Arl6 are the only two small GTPases presented in the genome of all ciliated organisms (Avidor-Reiss et al., 2004). An *Arl3* homolog product was found to target to the *Leishmania* flagella and proved to be essential for flagellum biogenesis (Cuvillier et al., 2000). *Arl3*$^{-/-}$ mice failed to thrive after birth, and all died by 3 weeks of age (Schrick et al., 2006). Although *Arl3* is not identified as a ciliopathy gene, *Arl3*$^{-/-}$ mice do exhibit classic phenotypes of a ciliopathy such as retinal degeneration, cyst formation in kidney, liver and pancreas as well as epithelial cell proliferation (Schrick et al., 2006). It is also reported that Arl3 localized predominantly to connecting cilia in mammalian rod and cone photoreceptors (Grayson et al., 2002). Arl3 has been shown to interact with the X-linked retinitis pigmentosa protein (RP2), which is demonstrated to act as the GAP for Arl3 (Grayson et al., 2002). RP2 localizes to the basal body and the associated centriole at the base of the photoreceptor cilium. Mutations in RP2 lead to a clinically and genetically diverse group of retinal dystrophies characterized by progressive photoreceptor degeneration. In photoreceptor cells, RP2 is proposed to regulate Arl3 small GTPase activity and facilitate the vesicle transport from Golgi to the base of the photoreceptor connecting cilia (Evans et al., 2010).

Taken together, Arl3, Arl6, and Arl13b are three conserved small GTPases implicated in human ciliopathies or vertebrate ciliopathy models. To date, the cellular effectors of Arl3, Arl6 and Arl13b are poorly understood. However, experiments on various Arl mutants with altered GTP binding capacity indicate that normal ciliogenesis requires the GTPase activities of the three ciliary Arls. Identification of the putative GEF, GAP and other Arl binding effectors will be critical for fully dissecting their roles in cilia biogenesis and signalings. It will be also interesting to investigate how the three Arl proteins coordinate their functions in cilia. The abilities of small GTPases to bind different partners and effectors and cycle between an inactive GDP-bound state and an active GTP-bound state make them promising candidates for molecular switches that regulate decisive steps in cilia biogenesis and/or signal transduction. The severe phenotypes related to cilia dysfunction in the aforementioned *Arl* ciliopathy models support this assertion. Thus, determining the functions of the Arls and their *bona fide* effectors will advance our understanding of cilia formation and function, as well as of ciliopathy pathogenesis.

Other than Arl proteins, Arf4 is the only reported Arf protein that shows cilia-related function (Mazelova et al., 2009a). Arf4 binds specifically to rhodopsin VxPx motif, a functional cilia targeting motif that existed in several cilia membrane proteins (Deretic et al., 2005), including polycystin-2 (PC2) (Geng et al., 2006), a protein affected in autosomal dominant polycystic kidney disease (ADPKD), and the cyclic nucleotide-gated (CNG) channel CNGB1b subunit in olfactory neurons (Jenkins et al., 2006). Upon the binding of Arf4 with the rhodopsin VxPx motif, a complex including Arf4, Arf4 GAP protein ASAP1, Arf4 effector FIP2, and another small GTPase Rab11, forms to regulate the transport of rhodopsin from the Trans-Golgi-Network (TGN) to photoreceptor cilia (Mazelova et al., 2009a). If Arf4 plays a pivotal role in sorting rhodopsin out of the TGN and into carrier vesicles that are targeted to the cilium, it would be interesting to test whether Arf4 also mediates the ciliary targeting of other ciliary sensory receptors such as PC-2, CNG, and Smoothened (Smo).

Rabs

Rab proteins form the largest family of small GTPases superfamily. Given the general role of Rab proteins in mediating membrane trafficking and imparting identity to the subdomains of the cell surface, it is likely that specific Rab protein functions in membrane trafficking to primary cilia. The first study that linked the function of Rab small GTPases to cilia came from analyses of Rab8 in photoreceptor cells. It has been found that, in photoreceptor rod cells, more than 10% of the total cellular Rab8 protein localizes on the TGN and may participate in rhodopsin cilium entry (Deretic et al., 1995). This finding was later confirmed by the fact that mutant Rab8 causes defects in the trafficking of rhodopsin-carrying post-Golgi vesicles to the outer segment of retinal photoreceptor, and this process is mediated by the exocyst and SNARE proteins (Moritz et al., 2001). Other than rhodopsin, Rab8 is also implicated in cilium entry of other cilia membrane proteins. For example, Rab8 physically associates with and mediates the ciliary targeting of the cilia membrane protein fibrocystin, the gene product of human ciliopathy ARPKD (Follit et al., 2010); additionally, the GTPase activity of Rab8 is required for the cilium entry of Hh signaling receptor Smo (Boehlke et al., 2010). Furthermore, a mechanistical insight

about Rab8's cilium-related role was obtained by the studies from Nachury and his colleagues. Nachury et al. proposed that the BBSome recruits Rab8 via its GEF, Rabin8, to direct post-Golgi vesicles fusion at the cilia base (Nachury et al., 2007). Rabin8 acts to promote the GTP loading on Rab8 and $Rab8^{GTP}$ may drive the docking and fusion of exocytic vesicles to the base of the ciliary membrane. Notably, Omori et al. demonstrated that Rabaptin5, a well studied Rab5 effector involved in endocytosis (Stenmark et al., 1995), functions together with Rab8 and IFT component Elipas/DYF-11 as a bridging system between the IFT complex and the IFT cargo that assemble at the cilia membrane (Omori et al., 2008). Additionally, CEP290, the gene product of a ciliopathy Joubert Syndrome causal locus, localizes around the cilia base and interacts with Rab8 (Tsang et al., 2008). Depletion of CEP290 interferes with localization of Rab8 to cilia and compromises the normal ciliogenesis (Kim et al., 2008).

Upstream of Rab8 signaling cascade is its GEF, Rabin8. Intriguingly, the GTP-bound Rab11 is found to physically interact with Rabin8 and regulate ciliogenesis, and $Rab11^{GTP}$ can stimulate the GEF activity of Rabin8 toward Rab8 (Knödler et al., 2010). Rab11 and the BBSome bind to the same region of Rabin8, and activated Rab11 seems to promote the association of the BBSome with Rabin8 (Knödler et al., 2010). As mentioned above, Rab11 and Arf4 form a ternary complex with Arf effector FIP3, and the Arf GAP ASAP1, and are all required for the packaging of rhodopsin into carrier vesicles from isolated Golgi membranes (Mazelova et al., 2009a). Taken together, these observations propose a working model in which Rab11 and Arf4 acts at the TGN to recruit and activate Rab8, and the activated Rab8 is anchored to the cilia base by CEP290 and mediate the docking and fusion of TGN vesicles to cilia membrane (Fig. 1).

Recently, Rab10 emerges as a new Rab involved in ciliogenesis. Rab10 is another mammalian homolog of yeast Sec4p that is closely related to Rab8 and has likewise been associated with cilia-related membrane transport pathways. Studies showed that Rab10 physically interacts with the exocyst complex protein Sec8 and colocalizes with the exocyst at the cilia base (Babbey et al., 2010). The exocyst, an evolutionarily conserved octameric protein complex (He and Guo, 2009), has increasingly been implicated to play a role in cilia. Proteomic analysis indicates most exocyst proteins can be found in the mouse photoreceptor cilium (Liu et al., 2007). Sec10 has been shown to be necessary for ciliogenesis and dysfunction of Sec10 leads to cyst formation in kidney epithelial cells (Zuo et al., 2009). Two other exocyst components, Sec6 and Sec8, localize at the cilia base in MDCK cells (Rogers et al., 2004; Babbey et al., 2010). The exocyst complex has been suggested as an effector for Rab8 in cilia targeting of rhodopsin (Mazelova et al., 2009b). However, knockdown of Rab10 didn't produce consistent defects in ciliogenesis (Babbey et al., 2010). Considering the sequence homology between Rab8 and Rab10 and that they both use the exocyst as the effector, it could be possible that Rab10 and Rab8 are redundant players regulating the same cellular process at the cilia base. Interestingly, a number of other small GTP binding proteins have been demonstrated to directly interact with the exocyst. For example, Sec15 is the downstream effector of Sec4p in yeast and of Rab11 in higher eukaryotes that regulate the corresponding membrane trafficking stages (Guo et al., 1999; Zhang et al., 2004; Wu et al., 2005; Oztan et al., 2007); ARF6 binds Sec10 directly and uses it as downstream effector (Prigent et al., 2003; Fielding et al., 2005). These evidences suggest that the exocyst seems to function as a hub for receiving regulatory information from various small GTPase-meditated pathways. Thus, it would be interesting to examine whether and how the exocyst-associating small GTPases coordinate their functions in ciliogenesis.

By overexpressing 39 predicted human Rab GAPs, Yoshimura et al. found that XM_037557, TBC1D7, and EVI5like (the GAPs of Rab8, 17, and 23, respectively) are involved in primary cilium formation (Yoshimura et al., 2007). Little is known about the cellular function of Rab17. While Rab23 has been discovered as a negative regulator of Hh signaling (Eggenschwiler et al., 2006), and mutations in Rab23 lead to Carpenter's syndrome, which has overlapped phenotype with ciliopathies (e.g. polydactyly and obesity) (Jenkins et al., 2007). A recent study showed that Rab23 is present in primary cilia and depletion of Rab23 or expression of dominant-negative Rab23 increases the fraction of Hh receptor Smo inside cilia, suggesting a role of Rab23 in Smo turnover in the cilium (Boehlke et al., 2010).

Two Rab like proteins Rab-like 5 (IFTA-2) and Rab-like 4 (IFT27) are involved in cilia/flagellar function in *C. elegans* and *Chlamydomonas reinhardtii*, respectively (Schafer et al., 2006; Qin et al., 2007). Rabl4 and Rabl5 are unique Rab-like small GTPases because they lack the C-terminal prenylation motifs, which are essential membrane targeting signals for all Rabs. Therefore, Rabl4 and Rabl5 must function differently when compared to the canonical Rabs. Accordingly, unlike other Rabs that regulate membrane trafficking in ciliogenesis, both Rabl4/IFT27 and Rabl5/IFTA-2 appear to be components in IFT machinery and exhibit both anterograde and retrograde IFT motility in cilia. Rabl4/IFT27 is found to be instrumental in maintaining the stability of IFT complexes and contributes to flagella assembly (Qin et al., 2007). However, disruption of Rabl5/IFTA-2 shows no defect in cilium formation, but rather affects specific cilium signaling activities (Schafer et al., 2006). This observation raises an intriguing hypothesis that Rabl5/IFTA-2 may act as an adaptor protein for anchoring the signaling molecules whose signaling transduction is dependent on the IFT transport.

Although no Rab is identified as ciliopathy gene yet, they are indeed involved in different steps in ciliogenesis from membrane trafficking, vesicle fusion, to IFT machinery

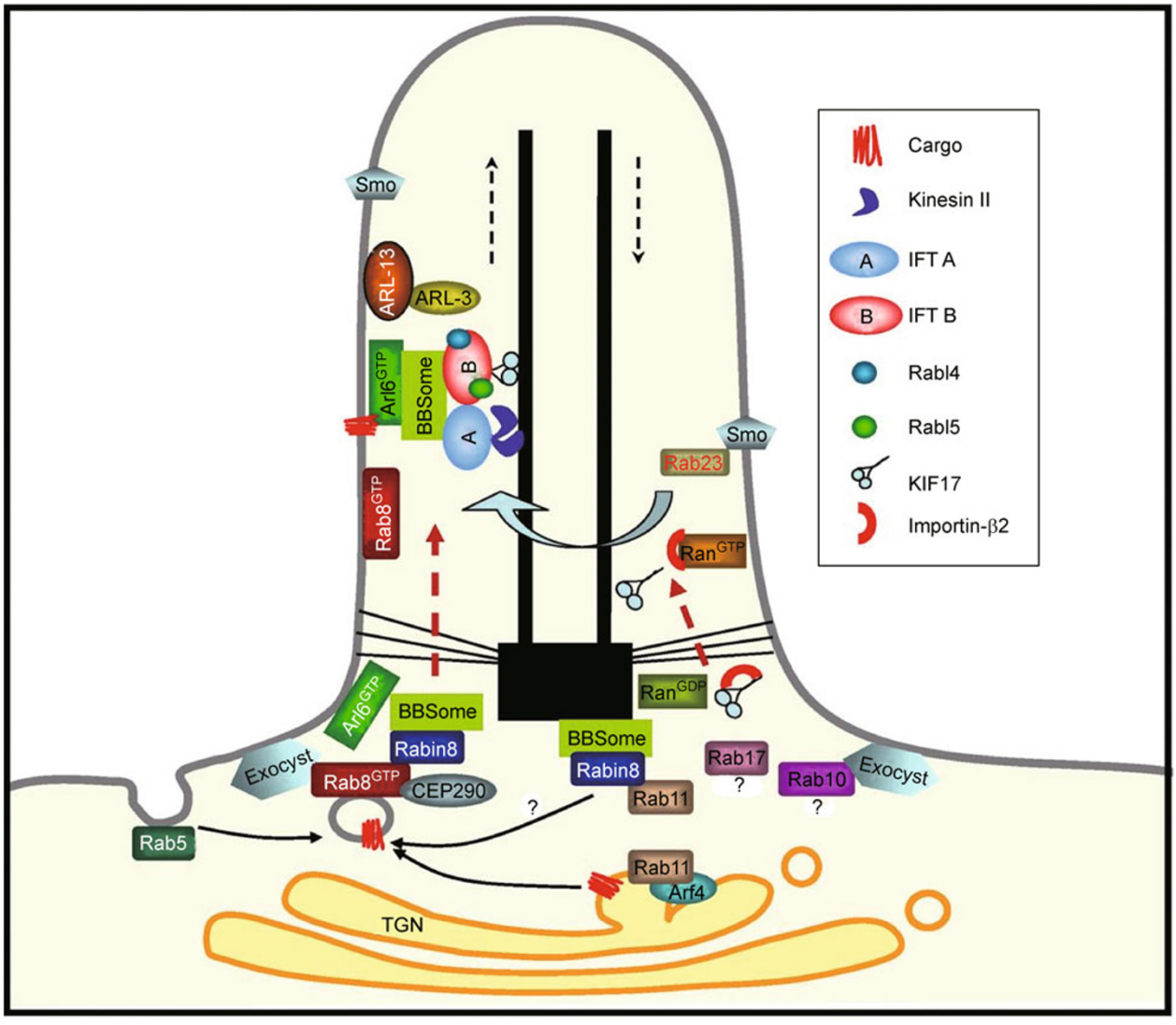

Figure 1. A model for the roles of different small GTPase proteins in cilia. Various GTPases are involved in ciliary protein transport. After activation by the Rabin8, the GTP-bound Rab8 small GTPase is proposed to facilitate docking and fusion of vesicles bearing transmembrane proteins near the ciliary membrane. The BBSome probably first docks onto the intraflagellar transport (IFT) machinery present at the transitional fibers and then moves into the cilia along with $Rab8^{GTP}$. Rab 11 stimulates the GEF activity of Rabin8 toward Rab8 and act upstream of Rab8. Smo enters the ciliary compartment via Rab8. Upon exit from the cilium, Smo is recycled back by Rab23. ARL-13 and ARL-3 are proposed to coordinate the IFT complex integrity. RanGTP/GDP gradient is critical for ciliary protein import.

regulation. And functional correlations between Rabs and ciliopathy gene products are also demonstrated in several studies. The future work on the molecular mechanism underlying Rabs in cilia will definitely enhance our understanding of ciliogenesis as well as of ciliopathy pathogenesis.

Ran

The small GTPase Ran plays roles in nuclear transport, mitotic spindle assembly and nuclear envelope assembly (Zheng, 2004; Stewart, 2007; Clarke and Zhang, 2008). It has become clear that many of these processes rely on Ran^{GTP} binding proteins of the importin-β superfamily (Stewart, 2007). Two independent studies showed that Ran and importin proteins are present in ciliary proteomes (Gherman et al., 2006; Liu et al., 2007). Consistent with this, Dishinger et al. found that Ran and importin-β2 regulate the ciliary entry of kinesin2 motor KIF17 in a similar manner of nuclear transport (Dishinger et al., 2010). GTP-bound Ran is present on primary cilia. The ciliary localization signal (CLS) identified in the KIF17 is similar to classic nuclear localization signals (NLSs) and is necessary and sufficient for cilium targeting.

Like nuclear transport, high levels of Ran^{GTP} are present in the cilium and the ciliary-cytoplasmic gradient of Ran regulates the ciliary entry of kinesin motor KIF17 (Dishinger et al., 2010). This is the first evidence demonstrating that Ran^{GTP}/ Ran^{GDP} gradient across the ciliary/cytoplasmic barrier regulates ciliary import. Whether and how this pathway regulates the entry of other motors and their cargoes at the cilia base need to be further characterized.

PERSPECTIVES

Cilia are specialized organelles whose biogenesis and maintenance require the precisely spatiotemporal integration of cytoskeleton dynamics and polarized membrane trafficking. It's not at all surprising to see that more and more small GTPases, a group of key switches involved in membrane and cytoskeleton-related cellular processes are found to play roles in ciliogenesis (Fig. 1 and Table 2). However, most of our understandings about the correlation between small GTPases and cilia are still in their infant stage. As we move forward, the molecular mechanisms underlying the ciliary roles of small GTPases must be further pursued to define the framework in this new research field. To achieve this long-term goal, we need first determine the full parts list of small GTPases involved in cilia biogenesis and function. Although Yoshimura and his colleagues have examined all Rab small GTPases by checking if the overexpression of a particular Rab GAP can compromise the ciliogenesis (Yoshimura et al., 2007), this approach is actually indirect and non-conclusive due to several pitfalls: for example, unidentified Rab GAPs may exist; GAP overexpression level may be not efficient enough to block Rab^{GTP} activity; two or more Rabs may play redundant roles in one step in ciliogenesis; some Rabs may play a negative role in ciliogenesis. Direct analysis on individual small GTPase, including Rabs, Arfs/Arls, and beyond, by high-throughput or case-by-case approach, need to be done in the future.

Studies of small GTPase families have demonstrated that numerous proteins bind to small GTPases to control their localization, activity, and downstream signaling pathways. The interactors include enzymes involved in posttranslational modifications, guanine nucleotide exchange factors (GEFs),

Table 2 Small GTPases and cilia

Small GTPases	Organism	Localization	Function	References
Arl13b	*C. elegans* *Zebrafish* *Human, mouse*	middle segment of cilia Cilia Cilia	Cilia formation	Cevik et al., 2010; Li et al., 2010; Duldulao et al., 2009; Cevik et al., 2010; Hori et al., 2008
Arl6	*C. elegans* *Human*	Ciliated cell and cilia Cilia (ATP-bound form)	Cilia formation	Fan et al., 2004; Jin et al., 2010
Arl3	*Leishmania* *C. elegans* *Mouse*	Flagella Ciliated cell and cilia Cilia	Flagella and cilia formation	Cuvillier et al., 2000; Li et al., 2010; Zhou et al., 2006
Arf4	*X. laevis.*	Golgi	Ciliary protein targeting	Deretic et al., 2005; Mazelova et al., 2009a
Rab8	*C. elegans* *Human, mouse*	Cell soma and dendrites Cilia, Golgi	Regulate ciliary membrane sorting or trafficking, cilia formation	Kaplan et al., 2010; Nachury et al., 2007
Rab11	*Human*	Golgi and ciliary base	Act upstream of Rab8, cilia formation	Knödler et al., 2010
Rab23	*Human, canine*	Cilia	Smo turnover in the cilium	Yoshimura et al., 2007; Boehlke et al., 2010
Rab10	*Mouse, rat, Canine*	Cilia base	Redundant players of Rab8?	Babbey et al., 2010
Rabl4	*Chlamydomonas Reinhardtii*	Flagella	IFT	Qin et al., 2007
Rabl5	*C. elegans*	Cilia	Affect specific cilia signaling activities	Schafer et al., 2006
Ran	*Human, mouse and canine*	Cilia (GTP-bound Ran)	Ciliary proteins import	Dishinger et al., 2010

GAPs, and effectors (such as adaptors, motors, kinases, and phosphatases) that bind specifically to the active form of small GTPases (Cherfils and Chardin, 1999; Bernards and Settleman, 2004; Winter-Vann and Casey, 2005; Stenmark, 2009). Biochemical identification and characterization of the binding partners of the cilia-related small GTPases would not only ensure the delineation of the poorly defined functional networks but also significantly enhance our understanding of the ciliary crosstalks among distinct small GTPases; for example, the regulation of the cilium entry of ciliary proteins by Rab11, Rab8, and Arf4 (Nachury et al., 2007; Mazelova et al., 2009a; Knödler et al., 2010), and the coordination of IFT integrity by ARL-3 and ARL-13 (Li et al., 2010). It's notable that multiple small GTPases could bind to different sites of the same effector, as proved by Rabin8 binding to both Rab11 and Rab8 in the regulation of rhodopsin cilary targeting (Knödler et al., 2010), and evidences from other non-cilia related events (Vitale et al., 1998; de Renzis et al., 2002; Sinka et al., 2008; Hayes et al., 2009).

Additionally, an important next step will be to generate transgenic animal models to study the *in vivo* functions of cilia-related small GTPases. Because of the essential role of cilia in early embryonic development and tissue pattern formation, it is prohibitively difficult to study the *in vivo* function of ciliogenic genes in live mammalian models (Nonaka et al., 1998; Essner et al., 2002). Accordingly, *Arl3*$^{-/-}$ and *Arl13*$^{-/-}$ mice fail to thrive after birth (Schrick et al., 2006; Caspary et al., 2007). In this regard, the highly conserved ciliopathy genes, ciliogenesis pathway, and cilia sensory function of *C. elegans* make this organism a simple but powerful model for characterizing the physiologic roles of ciliary small GTPases in their native cellular environment (Barr, 2005). Many genes required for the formation, maintenance, and function of *C. elegans* sensory cilia have mammalian counterparts that, when mutated, cause ciliopathies (Barr, 2005) (Table 3). *C. elegans* is the only established multicellular eukaryotic model whose viability and normal development are not dependent on the existence of cilia. Thus, using of *C. elegans* enables the exploration of cilia biology in living animals. Recently, *C. elegans* has been successfully used to provide valuable insights into many ciliopathies, including ADPKD (Barr and Sternberg, 1999; Qin et al., 2001; Barr et al., 2001; Hu and Barr, 2005; Jauregui and Barr, 2005; Ou et al., 2005; Bae et al., 2006; Hu et al., 2006; Hu et al., 2007; Bae et al., 2008; Jauregui et al., 2008; Knobel et al., 2008), BBS (Ansley et al., 2003; Blacque et al., 2004; Blacque et al., 2005; Chen et al., 2006; Mak et al., 2006; Ou et al., 2007; Mukhopadhyay et al., 2008; Williams et al., 2010), Joubert Syndrome (Cevik et al., 2010; Li et al., 2010), MKS (Williams et al., 2008; Bialas et al., 2009; Williams et al., 2010), and NPHP (Jauregui and Barr, 2005; Wolf et al., 2005; Jauregui et al., 2008; Williams et al., 2008; Williams et al., 2010). *C. elegans* has many experimental advantages for analyzing the *in vivo* function of cilia genes: for example, various behavioral assays enable rapid testing of cilia sensory function; the existing battery of fluorescence-protein-labeled ciliary markers; and the ability to perform time-lapse microscopy of GFP-tagged IFT components or cargos, which make *C. elegans* a tractable model for the study of IFT machinery and cargo protein transport (Orozco et al., 1999; Signor et al., 1999a; Signor et al., 1999b; Snow et al., 2004; Qin et al., 2005); moreover, powerful genetics toolkits (e.g., genome-wide mutagenesis screens, transgenesis (Mello and Fire, 1995), and RNAi (Tabara et al., 1998)) are available to promptly identify and characterize genes involved in cilia formation and function.

Most ciliopathies share two common features: (1) cilia-localized gene products and (2) similar clinical manifestations, such as kidney cysts (reviewed in (Watnick and Germino, 2003). Determining the function and crosstalk of these ciliary proteins is pivotal to understanding disease pathology. Exploring the roles of small GTPases in ciliogenesis and ciliopathies has been advanced significantly by recent studies. The findings of these GTPases together with their regulators and effectors in ciliogenesis will broaden our understanding of how cilia develop and function in normal and

Table 3 The evolutionary conservation of ciliopathy loci

Disease	Loci	Worm homologs	Expression
ADPKD	*PKD1*, *PKD2*	*lov-1*, *pkd-2*	Ciliated cells
ARPKD	*PKHD*	*no*	
Bardet-Biedle syndrome (BBS)	*BBS1-12*	bbs-1,2,3,4,5,7,8,9,11	Ciliated cells
Joubert syndrome	*AHI1*, *NPHP1*, *CEP290*, *MKS3*, *RPGRIP1L*, *ARL13B*, *CC2D2A*, *InPP5E*	*nphp-1*, *mks-3*, *arl-13*, *unc-26*, *k07g5.3*, *c09g5.8*	Ciliated cells
Meckel-Gruber syndrome (MKS)	*MKS1, MKS3, CEP290, NPHP6, CC2DA*	*mks-1, mks-3*	Ciliated cells
Nephronophthisis	*NPHP1-9*	*nphp-1*, *2*, *4*, *7*, *8*, *9*	Ciliated cells
Oral-facial-digital syndrome	*OFD-1*	*ceofd-1*	Ciliated cells
Retinitis pigmentosa	*RPGR, RP1, RP2*	*cerp2*	Ciliated cells

pathological states and provide us new insights into the molecular basis of different ciliopathies. Most importantly, the fact that small GTPase itself and many of its effectors are enzymatically active molecules makes this group of proteins highly promising candidates for future therapeutic invention or phenotype reversion in ciliopathy treatment.

ABBREVIATIONS

ADPKD, autosomal dominant polycystic kidney disease; Arf, ADP-ribosylation factor; Arl proteins, Arf-like (Arl) proteins; BBS, Bardet–Biedl syndrome; CLS, ciliary localization signal; CNG, cyclic nucleotide-gated; GAPs, GTPase activating proteins; GEFs, guanine nucleotide exchange factors; IFT, intraflagellar transport; MKS, Meckel–Gruber syndrome; NLSs, nuclear localization signals; NPHP, nephronophthisis; PCP, Planar Cell Polarity; PDGFR, platelet-derived growth factor receptor; PKD, polycystic kidney disease; PC2, polycystin-2; RFX, regulatory factor X; TGN, trans-Golgi-network

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