New frontiers: discovering cilia-independent functions of cilia proteins

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Abstract

In most vertebrates, mitotic spindles and primary cilia arise from a common origin, the centrosome. In non-cycling cells, the centrosome is the template for primary cilia assembly and, thus, is crucial for their associated sensory and signaling functions. During mitosis, the duplicated centrosomes mature into spindle poles, which orchestrate mitotic spindle assembly, chromosome segregation, and orientation of the cell division axis. Intriguingly, both cilia and spindle poles are centrosome-based, functionally distinct structures that require the action of microtubule-mediated, motor-driven transport for their assembly. Cilia proteins have been found at non-cilia sites, where they have distinct functions, illustrating a diverse and growing list of cellular processes and structures that utilize cilia proteins for crucial functions. In this review, we discuss cilia-independent functions of cilia proteins and re-evaluate their potential contributions to "cilia" disorders.

See the Glossary for abbreviations used in this article.

Cilia proteins and cilia disorders—is there more?

Primary cilia (henceforth called "cilia") are present in almost all cell types, lymphoid cells being one exception. Thus, cilia proteins have the potential to adversely affect numerous organs and tissues when disrupted. Over the past 10–15 years, cilia loss and/or dysfunction have been linked to numerous human disorders, collectively termed ciliopathies. Phenotypes associated with cilia dysfunction are often syndromic and include cystic kidneys, polydactyly, situs inversus, obesity, and encephalocele [1], to name a few. For example, disruption of cilia-based Hedgehog (Hh) signaling has been implicated in polydactyly [2,3] and disruption of cilia-based signaling through

Ca^{2+} or planar cell polarity (PCP) has been linked to cystogenesis [4,5]. However, a growing body of evidence suggests that cilia proteins are, in fact, multifunctional. In addition to their localization to cilia, many localize to and are bona fide components of centrosomes, as well as other cellular organelles and structures. At these non-cilia sites, cilia proteins perform functions distinct from their ciliary roles. Thus, we contend that disruption of cilia proteins—historically named for their localization to cilia, as well as their association with cilia defects and cilia-related disorders— can additionally disrupt spindle poles and an expanding list of cellular structures that impact numerous cellular functions. As a result, it has become increasingly difficult to determine which of these cellular functions and which of the these non-cilia organelles truly contribute to ciliopathies when cilia proteins are disrupted [6,7]. In this review, we first explore the diversity of cilia-independent processes that involve cilia proteins and then discuss alternative hypotheses for the etiology of "ciliopathies".

The centrosome, a common thread between cilia and mitosis

Most vertebrate centrosomes are comprised of two centriole barrels surrounded by pericentriolar material (PCM). A major function of the centrosome is to serve as the primary microtubule-organizing center [8–10] (MTOC) of the cell. The duplicated centrosomes undergo "centrosome maturation" during mitosis by increasing their microtubule nucleation capacity through recruitment of pericentriolar material and associated microtubule-nucleating proteins (such as γ-tubulin). This facilitates chromosome capture and the construction of the mitotic spindle [10]. During interphase (G_1/G_0), the mother/older centriole (basal body) docks at the plasma membrane through its distal appendages and then elongates preexisting microtubules of the centriolar barrel—rather than nucleating microtubules from the PCM as in mitosis—to form the primary cilium [11]. The dual role of centrosomes in the assembly of both cilia and spindle poles provides a common link between the two structures [12] (Fig 1). Thus, centrosome defects have the potential

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Glossary

APC	antigen-presenting cell
APC/C	anaphase-promoting complex/cyclosome
ATM	ataxia telangiectasia mutated
ATMIN	ATM interactor
ATR	ataxia telangiectasia and Rad3 related
BBS	Bardet–Biedl syndrome
Cc2d2A	coiled-coil and C2 domain-containing protein 2A
Ccdc13	coiled-coil domain-containing 13
Cdc20	cell division cycle protein 20
Cep164	centrosomal protein of 164 kDa
Che-1	abnormal chemotaxis
CHK1	checkpoint kinase 1
Cspp1	centrosome and spindle pole-associated protein 1
CYLD	cylindromatosis
DYNLL1	dynein light chain LC8-type1
E2F1	E2F transcription factor 1
GMAP210	Golgi microtubule-associated protein of 210 kDa
Hh	Hedgehog
HNF1β	hepatocyte nuclear factor-1beta
IFT	intraflagellar transport
MTs	microtubules
MTOC	microtubule-organizing center
MVA	mosaic variegated aneuploidy
Nek8	never in mitosis A-related kinase 8
Nphp9	nephrocystin protein 9
Odf1	oral-facial-digital syndrome 1
PCM	pericentriolar material
PCP	planar cell polarity
PKD	polycystic kidney disease
Pkhd	polycystic kidney and hepatic disease
Rb	retinoblastoma protein
SAC	spindle assembly checkpoint
Sdccag8	serologically defined colon cancer antigen 8
Shh	Sonic Hedgehog pathway
TCR	T-cell receptor
TG737	gene symbol for IFT88

Sidebar A: Centrosomes and cilia

Not all centrosomal protein are positive contributors to ciliogenesis. For example, CP110 and Cep97 are highly expressed in proliferating cells during mitosis, but are not expressed during cilia formation [137,138]. Their depletion results in cilia formation during multiple stages of the cell cycle, thus disrupting cell cycle control of cilia assembly specifically at G1/G0 [138]. Conversely, when CP110 and Cep97 are ectopically expressed during interphase, cilia can no longer form [138], suggesting that CP110 and Cep97 negatively regulate ciliogenesis. These two proteins may act as a cell cycle switch that controls centrosome participation on either cell cycle progression or in cilia construction and maintenance.

The protein composition of centrosomal satellites can be altered by the autophagy machinery, contributing to ciliogenesis [139,140]. An interesting connection between centrosome satellites and the BBSome complex—one of the main contributors to ciliopathy phenotypes—was recently proposed. Bbs4, a member of the BBSome, was shown to interact with the centrosome/satellite proteins Cep290 and Azi1 (Cep131) [141]. Moreover, the ciliopathy protein Ofd1 is a satellite protein that might be responsible for the loss of PCM1 and Cep290 from satellites [142]. Although the mechanisms connecting the components of centrosome satellites to cilia function remain to be elucidated, multiple satellite proteins are essential contributors to ciliopathies.
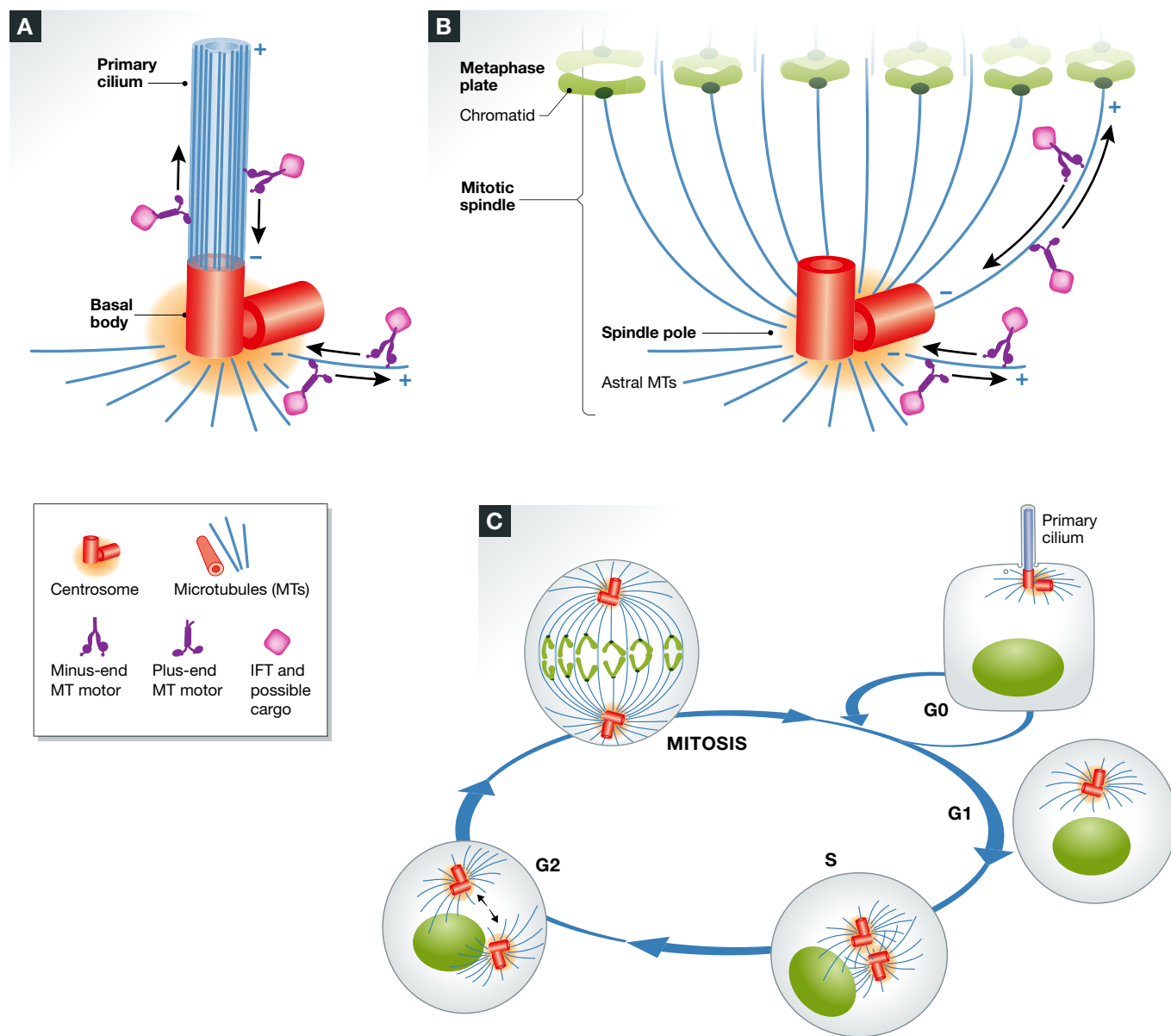
Cilia-independent roles of cilia proteins

Orientation of the mitotic spindle and the axis of cell division

We envision two potential mechanisms for mis-orientation of cell division caused by dysfunction of "cilia proteins". (i) Certain cilia proteins have direct and essential roles in cell division orientation independent from their roles in cilia, and/or (ii) dysfunctional non-canonical Wnt signaling (also known as the PCP signaling pathway) induces a downstream defect in spindle orientation, possibly through both cilia-dependent and cilia-independent mechanisms.

Proteins involved in the intraflagellar transport of molecules into and out of the cilium have been recently shown to also transport microtubule-nucleating/anchoring proteins to centrosomes during their maturation into spindle poles in mitotic cells [17]. Spindle poles are key players in the organization of mitotic spindles and the orientation of the cell division axis [18,19]. In fact, work from our laboratory and others indicates that centrosome protein dysfunction can induce spindle orientation defects [17,20–26]. Of note, depletion of the cilia protein IFT88 [27,28] induces spindle mis-orientation independently of its known roles in cilia formation and maintenance [17,29] (Fig 2). During mitosis, IFT88 is part of a dynein1-driven complex [17] (not ciliary dynein) that transports microtubule-nucleating proteins to spindle poles. IFT88 depletion disrupts this trafficking pathway and inhibits centrosome maturation. This, in turn, decreases the subset of astral microtubules required for spindle positioning [17] and induces spindle mis-positioning.

The non-canonical Wnt/PCP pathway (for canonical Wnt pathway, see Sidebar B) is responsible for ensuring the orientation of the spindle and cell division axis (Fig 2). Inactivation of the renal-specific, PCP-associated transcription factor Tcf2 (also known as HNF1β)—which is essential for the expression of *Pkhd* and *Pkd2* [5], two genes involved in the hallmark ciliopathy polycystic kidney disease (PKD)—causes spindle mis-orientation in cells of kidney tubules [5]. This was the first evidence linking PCP to PKD and, thus, ciliopathies. Moreover, depletion of the core PCP protein,

to disrupt structural and functional aspects of both cilia and mitotic spindles, which could co-contribute to ciliopathy phenotypes.

Many centrosomal protein are required for cilia integrity (see Sidebar A). Pericentrin was originally characterized as a centrosomal protein based on its localization and its role in microtubule nucleation and spindle assembly [13]. Pericentrin was subsequently identified in mouse testes as a component of protein complexes involved in intraflagellar transport (IFT)—the process of transporting material within the cilium—and was shown to be required for ciliogenesis [11,14]. This triggered a second study, which revealed an additional eight centrosomal protein required for cilia integrity, and a third study identified a dozen more [15,16]. These studies identify centrosomal protein control of cilia integrity as a fundamental and conserved feature of centrosomes. Because centrosomes function throughout the cell cycle, centrosome defects could contribute to ciliopathy phenotypes at different cell cycle stages. In contrast, cilia defects occur only in G1/G_0, when cilia are present (Fig 1).

Defective centrosomes are an appealing alternative explanation for ciliopathy phenotypes that appear during development, especially because many cells are actively proliferating at this time and, thus, do not have cilia.

Figure 1. The two lives of the centrosome during the cell cycle.
(A) In interphase, the centrosome functions as a basal body for primary cilia formation. (B) The basal body matures into the spindle poles during mitosis. (C) The centrosome duplicates once per cell cycle, during S phase, and moves to opposite sides of the cell starting at G2 and throughout mitosis. In G0, the centrosome docks at the membrane and templates cilia formation once again.

Vangl2, induces a similar spindle mis-orientation phenotype [30] as well as cilia mis-positioning [31].

The PCP pathway includes the cilia protein inversin, which is linked to the ciliopathy nephronophthisis type 2 [32]. Inversin interacts with disheveled [33], a protein involved in transducing both canonical Wnt and non-canonical Wnt signaling pathways, and acts as a molecular switch between them. Inversin induces the degradation of cytoplasmic disheveled [33], leaving only plasma membrane-localized disheveled and, thus, activating the PCP pathway. Importantly, inversin is localized not only to cilia [32], but also to adherens junctions [34] and the nucleus [34]. Thus, it is unclear which pool of inversin mediates disheveled degradation and modulation of PCP. In addition to the aforementioned cilia- and PCP-independent modulation of cell division orientation [29], it is likely that these ciliopathy proteins contribute to PCP modulation through both ciliary and non-ciliary mechanisms.

Cytokinesis
Several cilia proteins that are linked to Bardet–Biedl syndrome (BBS) were originally shown to form a protein complex called the BBSome. These cilia components (such as Bbs4, Bbs6) also localize to centrosomes/spindle poles and retain their centrosomal

localization throughout mitosis, when the cilium is resorbed and the centrosomes mature into spindle poles [35,36]. Bbs4 interacts with the dynactin subunit p150, which is important for dynein1 function in interphase and mitosis [35]. Notably, p150 resides outside the cilium, suggesting that the p150-Bbs4 complex has a cilia-independent role. In fact, Bbs4 depletion leads to an increase in binucleated and aneuploid cells, consistent with defects in cell division [35]. Another BBS member, Bbs6, localizes to the midbody, an organelle that controls abscission, which is the final step of cytokinesis [36]. Like Bbs4, depletion of Bbs6 causes cell cycle defects that include binucleated cells, supernumerary centrosomes, and elongated cytokinetic bridges, all of which are hallmarks of defective cytokinesis [36]. Moreover, several IFT proteins localize to the cleavage furrow [37]. Among these, IFT27 seems to have an active role in regulating cytokinesis, as its depletion increases cytokinesis failure and multinucleated cell formation [37,38]. These observations indicate that the BBSome and IFT proteins likely act in concert to resolve the cytokinetic bridge during abscission.

Membrane trafficking during spindle pole maturation
Two small GTPases, Rab8 and Rab11, have been implicated in cilia formation. For instance, active Rab11 recruits the Rab8 GEF, Rabin8, to activate Rab8 and induce cilia assembly [39,40]. Rab11 and Rab8 also interact with several well-defined proteins required for ciliogenesis, such as the BBSome, the vesicle tethering complex (exocyst), and the mother centriole appendage protein cenexin [39,41–48]. However, the molecular mechanisms and interplay of these molecular components during ciliogenesis require further investigation.

The same GTPases that function in ciliogenesis are also bona fide endosome components that play important roles in endosome recycling [39,49]. The centrosome has recently been shown to be involved in organizing and mediating the recycling branch of endosomal trafficking [50]. More specifically, the recycling endosome interacts with the subdistal appendages of the mother (older) centriole. These appendages are also required for assembly of the ciliary vesicle, a structure that forms at the mother centriole before the centriole docks at the plasma membrane to form the cilium [51–55]. Rab8 and Rab11 move into and out of the centrosome area, a process possibly mediated by an interaction with the subdistal appendage protein cenexin [50]. Thus, the two GTPases function in very different processes (cilia formation and endosome recycling), making it difficult to discern which contributes to cilia disorders. We speculate that the interaction between the recycling endosome and its endocytic machinery on the mother centriole appendages may facilitate the organization of the Rab11–Rab8 GTPase cascade, ensuring the initiation of cilia assembly at the right time during the cell cycle. Moreover, Rab11 depletion leads to defects in membrane trafficking toward spindle poles, which contributes to defects in spindle mis-orientation [20] (Fig 2). Other appendage proteins have been implicated in this membrane trafficking process. For example, Cc2d2A [56] modulates Rab8 localization and function in photoreceptor cells [42], suggesting a centrosome–endosome interaction. Importantly, Cc2d2A mutations are associated with the Meckel and Joubert syndromes, which are well-known ciliopathies [57,58].

Polarized transport toward the immune synapse
Intraflagellar transport protein IFT20—a member of ciliary IFT complex B—also has cilia-independent roles. IFT20 is expressed in
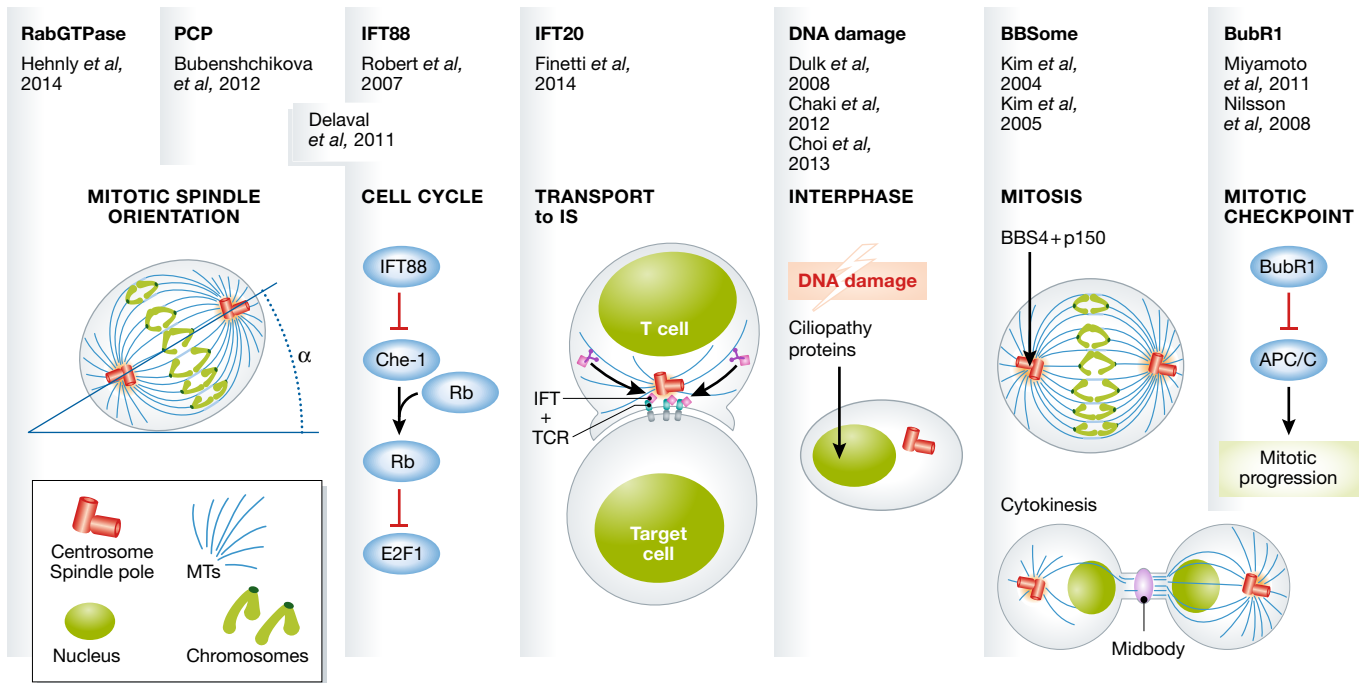
Figure 2. Primary cilia-independent functions of cilia proteins.
Cilia proteins participate in non-ciliarly events, such as mitosis, immunological synapse (IS) formation, DNA damage, cytokinesis, and chromosome segregation.

Sidebar B: Canonical Wnt signaling, cilia, and asymmetric division

Mouse embryos that cannot form cilia have been shown to be hyper-responsive to activation by canonical Wnt ligands [143], expressing increased levels of active β-catenin [118,144]. However, mouse embryos lacking IFT88, IFT172, Kif3a, or dynein heavy chain—all of which inhibit cilia formation—express canonical Wnt target genes in the appropriate tissues as well as control embryos [145]. Strikingly, all these mutant embryos that lack cilia have intact canonical Wnt signaling pathways. Moreover, there is no defect in switching between canonical and non-canonical Wnt signaling under these conditions [145].

Exposure to exogenous canonical Wnt during mitosis was recently shown to orient the spindle for asymmetric cell division, leading to the asymmetric distribution of stem cell properties to the daughter cells [146]. The lack of cilia in mitosis argues for cilia-independent mechanisms of Wnt/centrosome interconnection. For example, Axin, a negative regulator of Wnt signaling, and β-catenin, a major downstream target of canonical Wnt signaling, can be found at the centrosome [147]. GSK3β—an inhibitor of β-catenin signaling—regulates SMAD proteins, which are components of the BMP signaling pathway. The GSK3β-mediated degradation of SMAD occurs at the centrosome [148], leading to the idea that β-catenin stabilization at the centrosome occurs through Plk1-Nek2-dependent phosphorylation of β-catenin at multiple sites [149]. Destabilized β-catenin is targeted for proteasomal degradation, and the ciliopathy proteins Bbs and Ofd1—which localize to the centrosome satellites—bind the proteasome lid and control degradation-related signaling pathways [150].

Although these examples suggest that canonical Wnt signaling depends on the centrosome rather than on cilia, the older centrosome may carry membrane from a previously assembled cilium [151], which might be recognized by the Wnt pathway. Therefore, cilia could indirectly influence the centrosome, an intriguing concept that requires further investigation. Collectively, these studies underscore the idea that canonical Wnt signaling might depend on centrosomes rather than cilia.

lymphoid cells, one of the few cell types that does not form cilia [59]. T lymphocytes contact antigen-presenting cells (APCs) through the formation of a specialized, TCR-rich membrane region at the APC contact site, known as the immunological synapse. TCRs are targeted to the immune synapse through a combination of polarized membrane recycling, lateral diffusion, and cytoskeleton-driven movement [60]. In response to T-cell activation, IFT20 interacts with components of the TCR/CD3 complex and is required for clustering these complexes at the synapse through polarized recycling (Fig 2). More specifically, IFT20 is involved in Rab GTPase-mediated recycling of the TCR [61]. In this context, IFT20 interacts with two other IFT-B complex members, IFT88 and IFT57 [59], suggesting that the trimeric complex, rather than IFT20 alone, participates in TCR/CD3 trafficking to the immune synapse [61]. Together, these data define a conserved role for IFT20 in intracellular trafficking in both ciliated and non-ciliated cells.

Over the years, the Sonic Hedgehog pathway (Shh) [62,63] has been linked to the presence and function of cilia. Shh receptors and downstream targets are well known to locate at the cilia [64], and the intensity of Shh signaling seems to depend on functional cilia [65]. Not surprisingly, ablation of the essential centriole protein Sas4 in mice results in cilia and Shh signaling defects [66], demonstrating how interconnected centrosome and cilia function can be. Nevertheless, Shh receptors can also function outside of cilia [67]. Interestingly, the Shh pathway was recently shown to function in non-ciliated immune cells that retain centrosomes [68], suggesting

that centrosomes, and not cilia, are essential for this process. Another recent study showed that cilia formation in lymphocytes could be induced if the centrosome protein CP110 was removed by another centrosome protein, centrin2 (see also Sidebar A) [69]. This system may be a powerful strategy to dissect the contribution of the centrosome versus cilium in Shh signaling. For example, the centrosome can dock at the plasma membrane, which is one of the early steps in cilia assembly, but is unable to form a cilium in lymphoid cells. Whether centrosome docking at the plasma membrane is sufficient for Shh signaling remains to be elucidated. If centrosome docking is prevented, perhaps by distal appendage disruption [16,70], is Shh signaling disabled? Additional studies are required to determine the molecular mechanism governing Shh signaling and the role of the cilium versus centrosome in this process.

Cell cycle regulation and cell proliferation

Recent studies in non-ciliated and ciliated cells demonstrate a role for IFT proteins in cell cycle progression. For instance, depletion of IFT88 leads to a mitotic delay, whereas overabundance of IFT88 induces G1 arrest. More specifically, IFT88 over-expression induces G1 cell cycle arrest by blocking the interaction of Che-1 with the tumor suppressor Rb, freeing Rb to bind and repress E2F1, a transcription factor whose activation is required for the G_1/S transition [71] (Fig 2). The mitotic delay and defects in spindle assembly in IFT88-depleted cells [17] require further analysis to identify critical signaling molecules that are possibly transported by IFT88 during mitosis to ensure timely cell cycle progression. Interestingly, studies in *Chlamydomonas* demonstrated that an additional IFT protein, IFT27, is required for cell cycle progression [38]. How and whether IFT27, IFT88, and/or other IFT proteins act in combination to regulate cell cycle progression remains an open question. It is currently difficult to delineate the complex relationship between cilia and the cell cycle. For example, the presence of cilia may delay reentry into the cell cycle [72,73]. However, we propose that the role of IFT88 in regulating mitotic progression [17] and G1 cell cycle arrest in non-ciliated cells [71] is independent of the role of IFT88 in cilia function.

Cell proliferation defects are one of the major underlying causes of human cancer. Notably, several ciliopathy proteins are associated with different types of cancer. Loss of cilia has been linked to cancer by many independent groups [74–82]. For instance, TG737—the gene that encodes the IFT88 protein—is required for ciliogenesis, and its mutation leads to over-proliferation of liver progenitor cells in mice [83]. Based on the high rate of TG737 mutations in human liver tumors and tumor cell lines, and the rescue of phenotypes following TG737 re-expression, the gene has been classified as a tumor suppressor [84]. These studies strongly link ciliopathy proteins to cell over-proliferation/cancer.

DNA damage response (DDR)

The characterization of new roles of cilia and centrosomal protein in DNA damage repair is an emerging area of research. Cilia, centrosomes, and the DDR are linked in several ways: (i) the DDR pathway functions at the centrosome, (ii) ciliopathy proteins function in the nucleus during DDR, and (iii) DDR and cilia share a common regulatory protein (see below).

The ATR–CHK1 pathway is a well-characterized mediator of the DNA damage response. CHK1 localizes to the spindle pole, where it regulates mitotic progression [85,86]. Another protein that was

originally identified as a centrosome satellite protein, Ccdc13, has been implicated in both cilia formation and the DDR [87]. These findings suggest that checkpoint regulation in response to DNA damage may occur through the centrosome.

Moreover, some cilia and centrosomal protein localize to the nucleus and are directly linked to DDR (Fig 2). For example, centrin2 [88–90] and Cep164 [91] are directly involved in DNA damage-related events, such as excision repair. Mutations in a centrosome-localized ciliopathy protein, Sdccag8, lead to DDR and activation of the DDR kinase ATM [92]. Similarly, mutations in the centrosome protein pericentrin result in DDR defects [93]. In addition, the ciliopathy protein Nek8/Nphp9 cooperates with the ATR–CHK1 DNA damage pathway to regulate CDK levels [94]. Consistent with previous findings, Nek8 appears to play role in DNA stability and proliferation [95], which correlates with altered expression of Nek8 in human tumors [96], thus providing an intriguing link between DNA damage and ciliopathies [95].

Finally, both DDR and cilia might be regulated by a protein known as ATMIN. ATMIN is a co-factor for ATM in the response to DNA damage [97] and also a transcriptional regulator of ciliary dynein, DYNLL1 [98]. Intriguingly, it does not regulate cytoplasmic dynein (DYNLL2) [99]. Thus, the regulation of both cilia and DDR is likely tightly connected to the cell cycle.

Spindle assembly checkpoint regulation

BubR1 was recently shown to be essential for primary cilia formation in Medaka fish [100]. BubR1 is an important component of the spindle assembly checkpoint (SAC, below). During the G_1/G_0 phase of the cell cycle, BubR1 induces ubiquitin-mediated proteasomal degradation of Cdc20, which allows the activation of anaphase-promoting complex/cyclosome (APC/C) by Cdh1 [100]. In ciliated cells, in addition to regulating cell cycle progression, APC/C^{CDH1} targets disheveled for destruction [101], thus enabling ciliogenesis.

However, BubR1 is best known as a kinetochore and spindle pole protein that regulates the SAC (Fig 2). In this context, BubR1 prevents progression through mitosis until all kinetochores make stable, bipolar attachments to microtubules. This occurs partly through BubR1-induced Cdc20 degradation, which prevents the activation of the APC/C [102]. When active, the APC/C catalyzes the degradation of cyclin B and securin, two proteins that are essential for maintaining metaphase [103]. During both SAC maintenance and ciliogenesis, BubR1 targets Cdc20 for degradation, thereby ensuring that downstream events proceed properly, including the maintenance of essential metaphase proteins and of APC/C^{CDH1} activity. The interplay between BubR1 and inversin, in disheveled degradation, is unknown, as is the pool of inversin implicated in this process. These parallels again identify a single molecule that regulates two distinct processes, namely mitosis and ciliogenesis.

Additional examples

Studies showing that the cilia protein IFT20 also localizes to the cis-Golgi complex via the centrosome/Golgi protein Gmap210 [104,105] provide further support to the notion that cilia proteins have cilia-independent roles. Three other cilia-localized [106–110] proteins that are associated with kidney disease (polycystin-1, polycystin-2, fibrocystin) have been implicated in the amplification of centrosomes [111–113]. Moreover, the cilia proteins polycystin-2 and

fibrocystin localize to the mitotic spindle [114], although the functional relevance of this localization is unclear.

In summary, we have provided multiple examples of cilia proteins that have cilia-independent functions in diverse cellular localizations and processes, including spindle orientation, cytokinesis, cell proliferation, cell cycle progression, checkpoint control, immune synapse integrity, cancer, Golgi organization, and endosome trafficking.

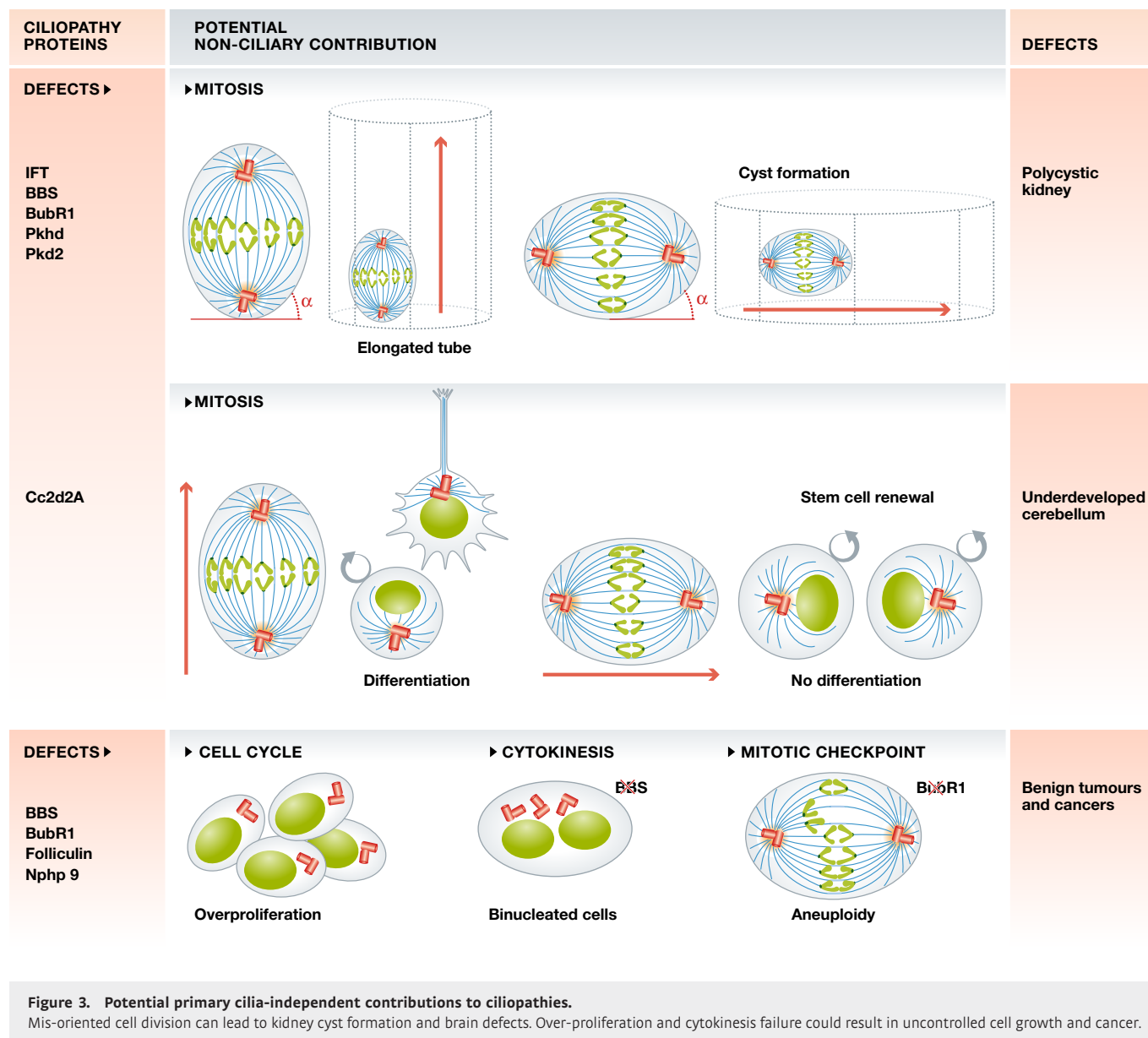## Possible cilia-independent contributions to ciliopathies

The fact that the centrosome is linked to both ciliogenesis and mitosis can explain the function of some proteins (such as IFT, BBS, BubR1) during different cell cycle stages. It is logical to think that the cell would use similar elements to regulate both processes. Thus, future studies are required to test whether cilia-independent pathways contribute to the pathogenesis currently attributed solely to cilia disruption. Below, we discuss possible contributions of non-ciliary functions to the pathogenesis associated with "ciliopathies".

Mis-orientation of spindles and the axis of cell division

These effects have been linked to kidney disease [5,115,116], developmental delays, and underdeveloped cerebellum [117], the very same phenotypes associated with cilia dysfunction. To generate proper kidney tubules, spindle orientation is strictly regulated to occur in the longitudinal axis of the tubule, to ensure that tubules are elongated and not widened [5] (Fig 3). In ciliopathy models of PKD, the intrinsic polarity of kidney duct cells is lost [5], leading to cyst formation. Mis-oriented cell divisions are observed not only in cystic kidney tubules, but also prior to cyst formation (during duct formation), suggesting that mis-oriented cell division could be the underlying cause of cystic kidneys [5]. This paradigm indicates that centrosome disruption could induce defects in structural and functional aspects of both cilia and mitotic spindle poles/centrosomes, either of which or both could contribute to ciliopathies. Improper regulation of spindle orientation has been suggested as a mechanism of kidney cyst onset in patients with PKD. This was originally proposed when mitotic spindle mis-orientation was identified in kidney cysts that lacked IFT20 [115]. Counterintuitively, deletion of IFT140, which causes defects in Wnt signaling, does not affect spindle orientation but still induces cyst formation [118]. Thus, cystic kidneys seem to result from cilia-dependent as well as cilia-independent processes [118,119].

Bardet–Biedl Syndrome

Ciliopathy phenotypes are associated with mutations in at least 14 different BBS genes [120,121]. BBS presents with nine or more diverse ciliopathy phenotypes, including retinopathy (damage to retina), polydactyly (supernumerary digits), cystic kidneys, situs inversus (mis-positioning of major visceral organs), hypoplastic (underdeveloped) cerebellum, hydrometrocolpos (distension of uterine cavity), obesity, and hepatic dysfunction. As described above, some of the proteins associated with ciliopathies are organized at centrosomes/spindle poles and when mutated or depleted cause cytokinetic defects, mitotic failure, multipolar spindle formation, and cell cycle perturbation [35,36]. All of these defects could lead to benign tumor formation and cancer (Fig 3), which are associated

Figure 3.   Potential primary cilia-independent contributions to ciliopathies.
Mis-oriented cell division can lead to kidney cyst formation and brain defects. Over-proliferation and cytokinesis failure could result in uncontrolled cell growth and cancer.

with BBS ciliopathy [122]. Moreover, cell division abnormalities are associated with brain development [21,25,123,124], (Fig 3), and thus, mutations in the BBSome could contribute to hypoplastic cerebellum via both ciliary and mitotic defects.

BubR1 depletion
This causes left–right asymmetry, polycystic kidney disease, and aberrant cerebral development [100], again phenotypes associated with cilia disorders (Fig 3). Strikingly, mutations in BubR1 in humans are responsible for mosaic variegated aneuploidy (MVA), a rare disorder with complex symptoms such as microcephaly, polycystic kidneys, and malignancies, among others [100,125].

Birt–Hogg–Dube syndrome and Nephronophthisis (NPH)
These are two other ciliopathies with possible cilia-independent underlying mechanisms (Fig 3). Birt–Hogg–Dube syndrome is caused

by mutations in the tumor suppressor gene folliculin, which localizes to cilia, centrosomes, and spindle poles. Folliculin mutations disrupt the onset of ciliogenesis and cause spindle mis-orientation, putting patients at high risk of kidney cancer, cysts, and benign skin tumors [22]. Nephronophthisis (NPH) [126] results from defects in cilia-associated nephrocystin proteins (Nphp 1–12). For example, Nphp 9 is linked to the development of breast cancer and strongly promotes proliferation through the activation of the transcription regulator Taz [81]. Collectively, these studies suggest that various mechanisms lead to the human disorders, including cilia defects as one of the causes, but indicating that other pathways could co-contribute to the etiology of these disorders.

Centrosomal proteins and disease
Perhaps the most compelling data for the role of centrosomal proteins in ciliopathies is the significant number of mutations that

have been recently described in them. The fast-growing list of ciliopathy disorders is based on newly identified protein mutations that are associated with ciliopathy phenotypes (see Sidebar C). For example, mutation of the centriolar protein Cep120 results in skeletal ciliopathy [127]; mutations in the centrosome protein Poc1A result in microcephaly and cilia defects [128]; and defects in Poc1B cause syndromic retinal ciliopathy [129]. Depletion of Poc1B also results in photoreceptor defects [130]. Cspp1 is yet another example of a centrosomal protein with mutations linked to two different ciliopathies, Joubert and Meckel–Gruber syndromes [131]. Intriguingly, at least two subdistal appendage proteins of the mother (older) centriole are linked to ciliopathy disorders [56,132]. First, Sdccag8, a protein that co-localizes with ninein at the subdistal appendages of the mother centriole, was found to be mutated in nephronophthisis-related ciliopathies [132]. Second, lack of Cc2d2A, which is also localized to subdistal appendages [56], leads to a reduction in subdistal appendage proteins—such as Odf2 and ninein—along with ciliary defects [56]. Investigation of the relationship between Cc2d2A and Sdccag8 proteins could uncover the mechanisms whereby centrosomal subdistal appendages contribute to cilia defects. A relationship between centrosome anchoring to the plasma membrane—an early step in ciliogenesis—and ciliopathies was recently demonstrated in cylindromatosis (CYDL) null mice [133]. Centrosomes were unable to dock at the plasma membrane, which correlated with ciliopathy phenotypes. Earlier studies showed that distal appendages promote ciliogenesis [70], which could explain the effects of CYLD deletion. Mutations in Cep83, a major component of distal appendages, cause ciliary defects and nephronophthisis [70], further supporting a role of distal appendages in ciliopathies. Cep164 is another component of mother centriole distal appendages involved in cilia assembly [16]. Overall, these data are in agreement with the previously elucidated roles of centrosomal proteins in cilia formation [15] and indicate that centrosomal defects contribute significantly to ciliopathies.

However, the obvious role of distal appendages in cilia formation is no longer the only centrosome–cilia connecting axis. Centrosome substructures—such as subdistal appendages, PCM, and centriolar satellites—contain proteins involved in ciliopathies. As discussed above, the centrosome is the common element between cilia and mitotic spindle poles. The fact that defects in centrosome subdistal appendage proteins—like Sdccag8 or Cc2d2A—contribute to ciliopathy phenotypes and have important roles in MT organization, rather than in the structural organization of the cilium, implies that centrosome defects could contribute to ciliopathies in both ciliary and non-ciliary capacities. Defects in MT organization and nucleation lead to mitotic spindle mis-orientation [17], and Cc2d2A$^{-/-}$ cells lack or have abnormal subdistal appendages [56], suggesting that Cc2d2A mutants can mis-regulate cilia or lead to mitotic defects. Cc2d2A mutations are associated with Meckel–Gruber [134] and Joubert [135] syndromes, both of which present with cystic kidneys, which have been proposed to be at least partly caused by mitotic spindle mis-orientation.

Conclusions

The discovery that cilia proteins are present at a growing number of cellular organelles and structures that collectively perform diverse cellular functions opens new frontiers in our quest to understand the etiology of ciliopathies. Moreover, their multifunctionality could account for the syndromic nature of cilia disorders. In fact, the phenotypes caused by dysfunction of cilia proteins at cilia-independent cellular sites can often more accurately account for the generation of organ defects in cilia disorders. The eight examples provided in the previous section illustrate the potential contribution of cilia-independent phenotypes to cilia disorders.

We can now begin to test which of these cilia-independent functions contribute to the etiology of cilia disorders. For example, do they co-conspire to cooperatively generate ciliopathies, or can (at least some) so-called ciliopathies be entirely independent of cilia function? For example, mitosis, ciliogenesis, and PCP have all been implicated in spindle mis-orientation during cyst formation. It is currently difficult to determine whether one or more of these processes dominates, or if all contribute and, if so, which pathway is upstream of the others. For example, does PCP regulate mitotic spindle orientation through cilia, as has been suggested [5]? Or does PCP regulate spindle orientation through cilia proteins that function directly in mitotic spindle assembly and orientation, as shown for IFT88 [17]? Do cilia truly transduce PCP signals, or do they instead require PCP signals for their genesis, as has been suggested by a study in which some PCP pathway members are required for ciliogenesis [136]? These and other questions (see Sidebar D) can now be addressed directly by generating "separation of function" mutations and other strategies.

With the increased recognition that many cilia proteins perform more than one function, we argue that the contradictory findings surrounding the roles of cilia, PCP signaling, and mitosis in ciliopathies are due to the multifaceted role of proteins originally defined

as cilia proteins, and subsequently shown to function at different cellular sites and different stages of the cell cycle.

In closing, ciliopathies are a class of disorders originally grouped together based on the ciliary localization and function of their causative proteins and the observation that cilia are lost or dysfunctional in tissues from afflicted patients. However, different ciliopathy syndromes manifest in different organs, with varying severity, and at different stages of life, raising questions about the true etiology of ciliopathies. We believe that these differences can be explained in part by the important roles of cilia proteins at sites outside the cilium. Consistent with this idea, recent work on cilia overwhelmingly indicates that ciliopathies are caused by disruption of a number of different cellular functions alone, or in combination. It has become increasing clear that ciliopathies could be caused by a complex set of disrupted functions (such as spindle orientation, cilia assembly/signaling, cell polarity, DNA damage) mediated by cilia proteins with multiple functions and localization patterns.

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Conflict of interest

The authors declare that they have no conflict of interest.

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