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# Cilium assembly and disassembly

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## Abstract

The primary cilium is an antenna-like, immotile organelle present on most types of mammalian cells, which interprets extracellular signals that regulate growth and development. Although once considered a vestigial organelle, the primary cilium is now the focus of considerable interest. We now know that ciliary defects lead to a panoply of human diseases, termed ciliopathies, and the loss of this organelle may be an early signature event during oncogenic transformation. Ciliopathies include numerous seemingly unrelated developmental syndromes, with involvement of the retina, kidney, liver, pancreas, skeletal system and brain. Recent studies have begun to clarify the key mechanisms that link cilium assembly and disassembly to the cell cycle, and suggest new possibilities for therapeutic intervention.

Primary cilia emerge from centrioles through a unique mechanism by which one organelle transforms into another with altogether different properties[1]. Centrosomes are composed of two orthogonally arranged centrioles embedded in proteinaceous material, the pericentriolar matrix, and are present in one or two copies per cell, depending on cell cycle stage (Fig. 1). Centriole duplication occurs in the S phase of the cell cycle, when a new (daughter) centriole assembles perpendicular to the old (mother) centriole. Each centriole is composed of nine microtubule triplets radially distributed with respect to a central lumen[2]. The centrosome, unlike most organelles, is not encased within a membrane. In contrast, the core microtubule structure of the primary cilium, or axoneme, is encased within a membranous sheath continuous with the plasma membrane. Ultrastructural studies have shown that only the mother centriole can give rise to a primary cilium and that the microtubule triplets in centrioles transition to doublets at the distal end of the basal body, which nucleates the axoneme[1].

The primary cilium is observed primarily in quiescent or differentiated cells (Fig. 1). Major insights into the function of the primary cilium came in the 1990s, when studies of the flagellum in the green alga, *Chlamydomonas*, revealed the existence of a conserved intraflagellar transport (IFT) system[3] required for organelle biogenesis. This led to the



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discovery that the mouse homologue of the *Chlamydomonas* IFT88 gene was mutated in a model for polycystic kidney disease (PKD)[4,5], in which the cells lining the urinary tract tubules fail to properly assemble primary cilia. This study provided the first evidence that, despite being immotile, primary cilia clearly have a function. Since then, much has been learned about the function of this extraordinary organelle, and a rapidly growing area of interest is its role as a major conduit for key signal transduction pathways. Signalling associated with primary cilia impacts processes as diverse as calcium flux in the kidney, growth and differentiation, and memory and learning[6–8]. Proteins that contribute to ciliogenesis and human diseases that result from defects in this organelle have been reviewed elsewhere (for example, refs 9–13). In this Review, we focus on exciting new developments in the field, with particular emphasis on the mechanisms that promote the assembly and disassembly of primary cilia and how these processes are subverted in pathological states.

## Cilium assembly

Assembly of the primary cilium begins when cells exit the mitotic cycle in response to mitogen deprivation or differentiation cues, although certain differentiated cell lineages (including lymphocytes, hepatocytes, mature adipocytes and skeletal muscle) lack primary cilia[14–18]. Ciliation can be recapitulated in cell culture through serum withdrawal, and the use of mouse 3T3 fibroblasts and human retinal pigment epithelial (RPE1) cells[19–21], in particular, has been instrumental for the analysis of factors required for cilium assembly. Although the nature of proximate cues able to promote ciliogenesis remains largely unknown, multiple initiating events — both intrinsic and extrinsic to the basal body — accompany the rapid remodelling of the distal end of mother centrioles to basal bodies (which then assemble the primary cilium) in an elegant switching mechanism (Fig. 2).

Ciliogenesis can be divided into distinct phases (Fig. 2), encompassing all the events that occur before and after the basal body docks at the plasma membrane. Time-lapse microscopy has revealed a remarkably dynamic process[22]. Within 10–15 minutes of mitogen withdrawal, small cytoplasmic vesicles thought to originate from the Golgi and the recycling endosome, termed distal appendage vesicles (DAVs), begin to accumulate in the vicinity of distal appendages of the mother centriole, where they seem to dock[23–25]. This is perhaps the first visible sign of the centriole-to-basal-body transition. Vesicular fusion then produces a membranous cap, or ciliary vesicle, on the distal tip of the mother centriole, which Sorokin designated as the primary ciliary vesicle[26]. Extension of the microtubules of the centriole at its distal tip proceeds underneath this cap, and subsequent vesicular trafficking enlarges the cap concomitant with extension of the microtubules, ensheathing the growing axoneme in a double membrane. This nascent cilium then docks to the plasma membrane by fusion with the ciliary sheath, establishing continuity of these compartments.

## Cilia membrane assembly and trafficking

Recently, significant progress has been made in understanding the trafficking of vesicles to the nascent cilium. Rab GTPases are integral to membrane trafficking, and their activity is under the strict control of a corresponding pair of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), which convert Rabs into an active (GTP-

bound) or inactive (GDP-bound) state, respectively. Rab proteins regulate distinct steps in membrane trafficking through the control of vesicularization of the donor membrane and the ensuing fusion with the acceptor membrane. Thus, the localization of GEFs and GAPs in distinct vesicular compartments can direct membrane trafficking. Rab8a has been implicated in ciliary membrane assembly[22,27] and, in growing RPE1 cells, it is localized to cytoplasmic vesicles and the Golgi/*trans*-Golgi network — but serum starvation induces its rapid redistribution to the mother centriole[22,27]. After the formation of DAVs, the Ehd1 protein is recruited, converting these small vesicles to the larger ciliary vesicles, which then elongate through continuous fusion with Rab8-positive vesicles to produce the primary cilium membrane[25].

How is Rab8 activated and directed to the distal appendages of the mother centriole in response to a ciliogenic signal? Rab11-positive recycling endosomes may transport the GEF, Rabin8, an activator for Rab8, and the membrane-tethering complex, TrappII, to this location[22]. Rab8 activation therefore coincides with the fusion of Rab11- and Rab8- positive vesicles and the activation of the ciliary assembly program. Insight into the function of Rab8/Rabin8 at the primary cilium came from an analysis of proteins interacting with the BBSome complex, which includes Bardet–Biedl syndrome protein 4 (BBS4), a protein that localizes to the basal body of the primary cilium[28]. Defects in some or all of the proteins in the BBSome complex are associated with the human ciliopathy Bardet–Biedl syndrome, although mutations in BBS genes generally perturb ciliary function rather than ciliogenesis *per se*[29]. Rabin8 associates with the BBSome at the centrosome and the base of the primary cilium. Thus, ciliogenesis is driven by sustained activation of Rab8 through two different sources of Rabin8: the Rab11-positive recycling endosome and the BBSome. As cilia lack ribosomes, growth and maintenance of the axoneme also requires two macromolecular IFT complexes, IFT-B and IFT-A, which transport hundreds of proteins towards (anterograde) or away (retrograde) from the ciliary tip, respectively[29]. In addition, the BBSome functions as an adaptor for IFT during ciliary export[29–31]. Transport within the cilium also requires kinesin and dynein motors that, together with IFT complexes, are necessary for the localization of key ciliary membrane proteins such as G-protein-coupled receptors (GPCRs), one of which (Smoothened, Smo) functions in the Hedgehog (Hh) signalling pathway. Defects in both IFT-A and IFT-B subunits result in multiple ciliopathies, attesting to an essential role for IFT in cilium assembly (Fig. 2).

## Basal body docking

Elegant studies have also begun to clarify the succession of events that occur at the distal ends of basal bodies and during distal-appendage-mediated vesicle docking and maturation[25,32] (Fig. 2). Components intrinsic to the basal body undergo dramatic remodelling during the early stages of cilium assembly. One element involved in this switch is CP110, a distal-end protein essential for centriole duplication and length regulation[33–36]. Asymmetric destruction of CP110 and its interacting partner Cep97 on mother centrioles may be an obligate event in the initiation of ciliogenesis[37], and the silencing of either gene results in the aberrant assembly of primary cilia in proliferating cells or the formation of extra-long centrioles in non-ciliated cells[37].

Extension of the ciliary axoneme occurs after the anchoring (or docking) of basal bodies to intracellular vesicles or the plasma membrane via distal appendages (Fig. 2). The assembly of distal appendages on mother centrioles involves the orchestrated recruitment of five proteins (Cep83, Cep89, Cep164, SCLT1 and FBF1)[32]. At least one distal appendage protein, Cep164, helps dock vesicles through interactions with Rab8 and Rabin8, and loss of Cep164 or another ciliopathy-associated distal centriolar protein, Talpid3, abrogates the recruitment of ciliary vesicles[23,24]. All five proteins are required for ciliogenesis, and beyond docking to membranes, the distal appendages are assembly points for the recruitment of IFT proteins[38] and other components required to build the cilium and selectively import proteins through the transition zone, which acts as a ciliary gate to the cilium[29].

Interestingly, undocked centrioles retain CP110 even after serum withdrawal. Thus, the activation of signalling pathways that induce ciliogenesis is restricted by spatial cues. Certain cell types that do not assemble cilia, such as cytotoxic T lymphocytes (CTLs), can use similar signalling pathways to execute their unique functions[17]. In these cells, the distal appendages of mother centrioles dock at the plasma membrane, analogous to cilium assembly, forming a protrusion at the immunological synapse necessary for CTL secretion and target killing, without producing an axoneme or primary cilium. Notably, Cep97 and CP110 persist at mother centrioles during synapse formation, suggesting that these cells implement an ‘abortive’ ciliogenesis program.

An important step towards the identification of proteins that promote ciliogenesis came from a genetic screen in the mouse to identify regulators of the Hh signal transduction pathway, which depends on the integrity of the primary cilium. This screen identified a Tau tubulin kinase 2 (TTBK2)-null mouse mutant, which lacks primary cilia and exhibits defects in Hh signalling[39]. This was not due to defects in basal body anchoring; rather, loss of TTBK2 abrogated the recruitment of IFT proteins essential for anterograde and retrograde trafficking and axoneme extension and maintenance, and *TTBK2*-null basal bodies consequently lacked axonemes. Further, since CP110 was retained on basal bodies in null cells, it was inferred that TTBK2 (which is recruited by Cep164[40] and localizes at the distal end of the basal body) is necessary for the removal of CP110 from the mother centriole. Interestingly, spinocerebellar ataxia type 11, a neurodegenerative disorder, is caused by dominant, null mutations in the *TTBK2* gene[41,42]. But there are still several gaps in our knowledge of this mechanism. It remains unclear what triggers TTBK2-mediated phosphorylation and how CP110 destruction is restricted to the mother centriole. The identity of proteases that asymmetrically destroy CP110 prior to axoneme growth is still unknown. Whether TTBK2 targets exist beyond the distal appendage protein (Cep164) and Cep97 and how the phosphorylation of these proteins drives ciliogenesis is unknown — although TTBK2 may also play a role in assembly of distal appendages[40,43]. One priority will be to determine how inductive cues activate this kinase. It is tempting to speculate that a feedback mechanism exists, in which docking of ciliary vesicle triggers the activation of TTBK2[32]: engagement of vesicles by Cep164 might trigger conformational changes in Cep164, TTBK2, or both, stimulating kinase activity and promoting phosphorylation of key substrates, thereby ‘flipping the switch’ that initiates cilium assembly.

Phosphatidylinositol (PtdIns) homeostasis and phosphorylation at the basal body are also linked with TTBK2 regulation and ciliogenesis[44]. Intriguingly, the balance of two activities — a PtdIns kinase (PIPKIγ) and an opposing phosphatase (INPP5E) — fine-tunes PtdIns(4)P and PtdIns(4,5)$P^2$ levels. PtdIns(4)P negatively regulates recruitment of TTBK2 (thereby promoting CP110 persistence and suppressing ciliogenesis) by modulating its interaction with Cep164 (Fig. 2). Mutations in INPP5E are found in Joubert syndrome, indicating additional roles for phospholipids, which appear to be compartmentalized within the cilium once it is assembled[45–47].

In addition to TTBK2, depletion of a second basal-body-associated kinase, MAP/microtubule affinity-regulating kinase 4 (MARK4), results in arrest after vesicle docking but prior to axoneme extension[48]. It will be interesting to identify both the activators and substrates of MARK4, as this network could collaborate with TTBK2 to promote remodelling of the distal end of the basal body and growth of axonemal microtubules. An exciting possibility is that the tumour suppressor LKB1 (liver kinase B1; also known as serine/threonine kinase 11, STK11) — an activator of mTOR (mammalian target of rapamycin) and AMPK (AMP-activated protein kinase) as well as MARK4, which localizes to centrosomes and cilia[49–52] — plays a role here. *STK11*-knockout cells exhibits abortive cilium assembly that is relieved by inhibition of HDAC6[52], which plays a key role in cilium resorption, suggesting that cilium loss in these cells is due to excessive organelle disassembly.

## Cilium disassembly

In contrast with cilium assembly, much less is known regarding mechanisms underlying disassembly of this organelle in normal or pathological conditions (Fig. 3). Moreover, how cilium disassembly is linked to cell cycle progression remains largely unanswered. Experiments in cultured mammalian cells suggest that cilia disassemble in a biphasic manner[19,21], with the first, major ‘wave’ occurring in the G1 phase shortly after mitogen stimulation of quiescent cells (Fig. 1) and a second wave prior to mitosis. These initial studies identified several key regulators of cilium disassembly (Fig. 3): the scaffolding protein HEF1 (also known as NEDD9) and calcium–calmodulin activate Aurora A kinase, which in turn phosphorylates and stimulates the histone deacetylase HDAC6, promoting the de-acetylation of modified, stabilized tubulins within the axoneme[21,53]. Additional studies will be needed to identify the mechanisms through which HEF1 (NEDD9) is upregulated and recruited to basal bodies following mitogen stimulation. Recent studies have uncovered another critical effector of HDAC6 activity, cortactin, which promotes actin polymerization[54]. Given that acetylation of cortactin abrogates its interaction with filamentous actin and that actin polymerization counteracts cilium assembly, de-acetylation of cortactin could further explain how HDAC6 activation promotes cilium disassembly by enhancing actin polymerization. Apart from its role as an HDAC6 regulator, Aurora A also activates INPP5E[55], which would favour high levels of PtdIns(4)P and suppress ciliogenesis through a second mechanism[44].

Two additional activators of Aurora A at the basal body, trichoplein and Pitchfork (Pifo), also play a role in cilium disassembly[56,57] (Fig. 3). Similarly to CP110, trichoplein

disappears from mother centrioles during ciliogenesis, and its depletion promotes aberrant cilium assembly in proliferating cells[57]. The process of ubiquitin-mediated protein degradation is required for cilium disassembly[58], and trichoplein proteolysis is critical for the initiation of axoneme extension, indicating a similar requirement during cilium assembly[59,60]. Interestingly, a newly discovered protein, nuclear distribution element (NDE)-like 1 (Ndel1), functions in the trichoplein–Aurora-A pathway, and similarly to trichoplein depletion, loss of Ndel1 triggers aberrant ciliogenesis in growing cells[61]. Further, Ndel1 blocks ciliogenesis prior to axoneme extension by stabilizing trichoplein at mother centrioles, perhaps by protecting trichoplein from ubiquitin-mediated destruction. The requirement for Pifo in cilium disassembly is illustrated by the observation that *Pifo* haplo-insufficiency leads to the persistence of cilia in mitosis, abolishes the liberation and duplication of centrioles, and leads to mitotic defects[56]. Heterozygous mutant *Pifo* mice and patients exhibit ciliopathy-related manifestations, attesting to the biological consequences of perturbing cilium disassembly.

Cilium disassembly requires the destabilization and de-polymerization of axonemal microtubules, and two members of the Kinesin-13 family of de-polymerizing kinesins, Kif2a and Kif24, are implicated in cilium disassembly[62–64] (Fig. 3). Kif2a localizes to proximal ends of both centrioles as well as the sub-distal appendages of the mother centriole, and it is phosphorylated and activated by the G2/M-phase kinase Plk1[63]. Activated Kif2a promotes the de-polymerization of ciliary microtubules, provoking cilium disassembly shortly following a proliferative signal. Kif2a presents an interesting prototype for exploring the role of aberrant cilium disassembly in disease. Premature chromatid separation syndrome (PCS; also known as mosaic variegated aneuploidy syndrome, MVA) is a rare autosomal recessive disorder characterized by a high risk of cancer and symptoms associated with ciliopathies[63]. Fibroblasts from PCS patients exhibit constitutive activation of Plk1 and Kif2a, as well as reduced ciliogenesis, suggesting that aberrant activation of a disassembly pathway could, in part, underlie the pathology.

A second microtubule de-polymerizing kinesin, Kif24, was identified by virtue of its association with CP110 at the distal end of centrioles[62]. As for CP110 loss, ablation of Kif24 results in inappropriate assembly of cilia in proliferating cells, although it does not regulate centriole length. The microtubule de-polymerizing activity of Kif24 is enhanced by Nek2- mediated phosphorylation[64]. Nek2 is expressed during S and G2 phase, ensuring that Kif24 is active in cells that lack cilia. Notably, the ability of Nek2 and Kif24 to suppress cilium assembly can be temporally distinguished from the Aurora-A–HDAC6 pathway, suggesting that Nek2–Kif24 activation ensures the irreversibility of the disassembly process after S phase commences[64]. These studies suggest that Kif24 acts to safeguard against the extension of de-polymerized microtubules, preventing the aberrant assembly of cilia prior to mitosis. Thus, Kif2a and Kif24 are activated by kinases expressed in S/G2 (Nek2) or G2/M (Plk1) phase, further linking cell cycle progression with the maintenance of a deciliated state necessary for mitosis (Figs 1 and 3). Interestingly, this mode of regulation could represent an ancient, conserved program, as relatives of Kif24 and Nek2 — as well as Aurora A — play a role in axonemal assembly and disassembly in flagellated and ciliated species[65–71].

Cilia can also be removed by severing mechanisms. For example, in *Chlamydomonas*, deciliation is facilitated by the action of a microtubule-severing enzyme, katanin, which separates basal bodies from axonemes prior to mitosis[72,73]. In neurons, deciliation can occur through an actomyosin-dependent process of apical abscission, wherein the cilium is pinched off from the centrosome[74]. As abscission could drastically alter the reception of proliferative and differentiative cues by curtailing Hh signalling[74], it will be important to investigate whether this deciliation mechanism is more generally used by other cell types during normal proliferation and differentiation and in pathological states such as cancer.

Whereas these experiments begin to suggest mechanisms for cilium disassembly in mammalian cells, previous studies have mainly examined total populations as opposed to single cells, making it difficult to understand the factors that control equilibria or directionality of the assembly–disassembly process. Thus, it will be important to follow the process in real time *in vivo* and in single cells. Kinases activated in S/G2/M phase regulate a number of targets linked to cilium disassembly. It will be important to determine whether misregulation of cell cycle kinases causes loss of cilia indirectly due to cell cycle perturbations or directly through defective cilia assembly (or a combination of both). It will also be essential to understand the contributions of each of the disassembly pathways in diverse mammalian tissues using animal knockouts. One surprising result of such an approach is the normal development of *HDAC6*-null mice despite hyper-acetylation of tubulin[75]. This suggests that additional, unidentified enzymes could function redundantly with HDAC6 to de-acetylate axonemal microtubules (or bypass mechanisms could be operative), and that HDAC6 inhibition may not be toxic — possibilities that must be considered during drug development. In this regard, the Aurora-A–HDAC6 pathway could be lineage-specific, since inhibitors of either Aurora A or HDAC6 led to inefficient assembly of cilia in only a subset of stem cell lines[76].

Two proteins that localize to the centriole or transition zone (TZ), Nde1 and Tctex-1, respectively, also link cilium disassembly with S phase entry. Nde1, a paralogue of Ndel1, is highly expressed in mitotic cells but depleted during quiescence, and silencing of Nde1 in cultured cells and zebrafish leads to marked enhancement of cilium length and a delay in S phase entry[77]. Nde1 levels are controlled by CDK5 phosphorylation in G0/G1 cells, which targets Nde1 for ubiquitylation (and subsequent destruction) by the F-box protein Fbw7, a tumour suppressor[78]. Ablation of *Nde1* in mice leads to microcephaly, possibly due to proliferative delays provoked by the persistence of cilia in progenitor neurons[77,79]. Likewise, Tctex-1 ablation led to persistent ciliation and a G1/S phase block[80]. These studies point to a mechanism wherein cells sense the resorption of the cilium, and cell cycle progression may be constrained by its removal. It will be essential to identify additional regulators of cilium disassembly to support this model and to elucidate how the integrity of this restrictive mechanism is sensed by the cell cycle machinery.

## A role for the cilium in cell cycle control and human cancer

As discussed above, ciliogenesis is tightly coordinated with the cell cycle (Fig. 1). With a few exceptions[81], the cilium and mitotic spindle assemble in a mutually exclusive manner, and by tethering the centrosome to the plasma membrane, the cell is deprived of a mitotic

organizing centre (MTOC), thereby restricting proliferation. This observation has significant implications for mammalian growth and development. For example, one important yet untested implication of the ‘cilium versus centrosome’ model is that the cilium could function in a tumour suppressive capacity. In contrast to defects in cilium assembly, which are linked to ciliopathies, aberrations in cilium disassembly have not been widely linked to disease (with the exceptions described above). However, human cancer may be associated with the loss of primary cilia, and given the parallels with ciliopathies, it may be worthwhile to consider these diverse pathologies simultaneously when devising therapeutic strategies.

The presence or absence of cilia — and therefore integrity of signalling — can be instructive for cell growth. For example, certain ciliary signalling proteins, such as Smo (a key regulator of Hh signalling) or platelet-derived growth factor receptor (PDGFR), are proto-oncogenes that are aberrantly activated at cilia in cancer[82,83]. Remarkably, whereas some types of cancer (basal cell carcinoma and medulloblastoma) are dependent on cilia[84,85], other cancers, including melanoma and breast, pancreatic, renal, and prostate cancer, exhibit loss of cilia, most likely during the early stages of oncogenesis[86–90]. The mechanisms by which cancer cells lose their cilia are unknown, as is how cilia loss affects signalling and tumour growth. It is notable that proteins associated with cilium disassembly — Aurora A and Nek2 — are widely deregulated in human cancer through overexpression or gene amplification. Inhibitors of these kinases, which have many of the hallmarks of oncoproteins, as well as HDAC6 inhibitors, are currently in clinical trials[91]. Furthermore, expression of an oncogenic form of PDGFR-α found in gastrointestinal tumours is linked to cilium disassembly through Aurora A activation[82]. Therefore, inhibitors of these kinases could be useful for the treatment of both tumours and ciliopathies[92].

Oncoproteins and tumour suppressors clearly play antagonistic roles in cilium assembly. Indeed, the function of multiple tumour suppressors — including VHL, PTEN, p53, tuberous sclerosis proteins (TSC1 and TSC2) and adenomatous polyposis coli — are linked to normal ciliary assembly and function[93–98]. Mutations in the von Hippel–Lindau tumour suppressor gene (*VHL*) predispose patients to renal cysts and clear cell renal cell carcinoma, and loss of pVHL, combined with ablation of a second tumour suppressor protein (p53 or PTEN), provokes ciliary loss, kidney cyst formation and neoplastic growth[93,94]. PTEN localizes near basal bodies, and its loss leads to severely diminished cilium assembly, most likely as a result of accelerated organelle disassembly[95]. Moreover, the transition zone protein NPHP4, which is mutated in ciliopathies, modulates the Hippo signalling pathway, which regulates cell proliferation[96]. Thus, tumour suppressor function is tied to normal cilium biogenesis and function, and drugs that target downstream effectors of these proteins — such as the mTOR signalling pathway, which is aberrantly activated in TSC mutants[97] — will be an avenue for future exploration.

As described above, the proteins that catalyse cilium disassembly may provide an avenue for drug discovery, since restoration of the primary cilium in tumour cells could promote quiescence[64], especially if two or more pathways were simultaneously targeted. It will be important to distinguish the contributions of aberrant regulation of cell cycle proteins (such as Nek2, Plk1 and Aurora A) to mitotic defects in chromosome segregation, spindle pole behaviour and other centrosome-associated processes from their effects on cilium assembly

and function. Teasing apart these diverse functions will require additional information from proteomics and molecular genetics.

## Future directions

Going forward, it will be essential to understand the inductive cues that link ciliogenesis to the cell cycle, mitogen deprivation and autophagic induction[99]. Super-resolution microscopy[100] and proteomic approaches that combine proximity-based labelling[101,102] with gene editing will undoubtedly accelerate progress. Given the pivotal role that cilia play in diverse aspects of human biology from development to disease, it will be vital to shed light on unanswered questions regarding the earliest steps in cilium assembly. It will be essential to decipher the signals that mediate the onset of basal body maturation and cilium assembly as well as the remodelling events at the distal ends of basal bodies and vesicles that facilitate capture and growth of early ciliary vesicles and extension of the nascent axoneme. Likewise, it will be important to elucidate the cues that directly promote cilium disassembly, as these mechanisms may be relevant to the early steps of tumorigenesis and may reveal targets for drug therapy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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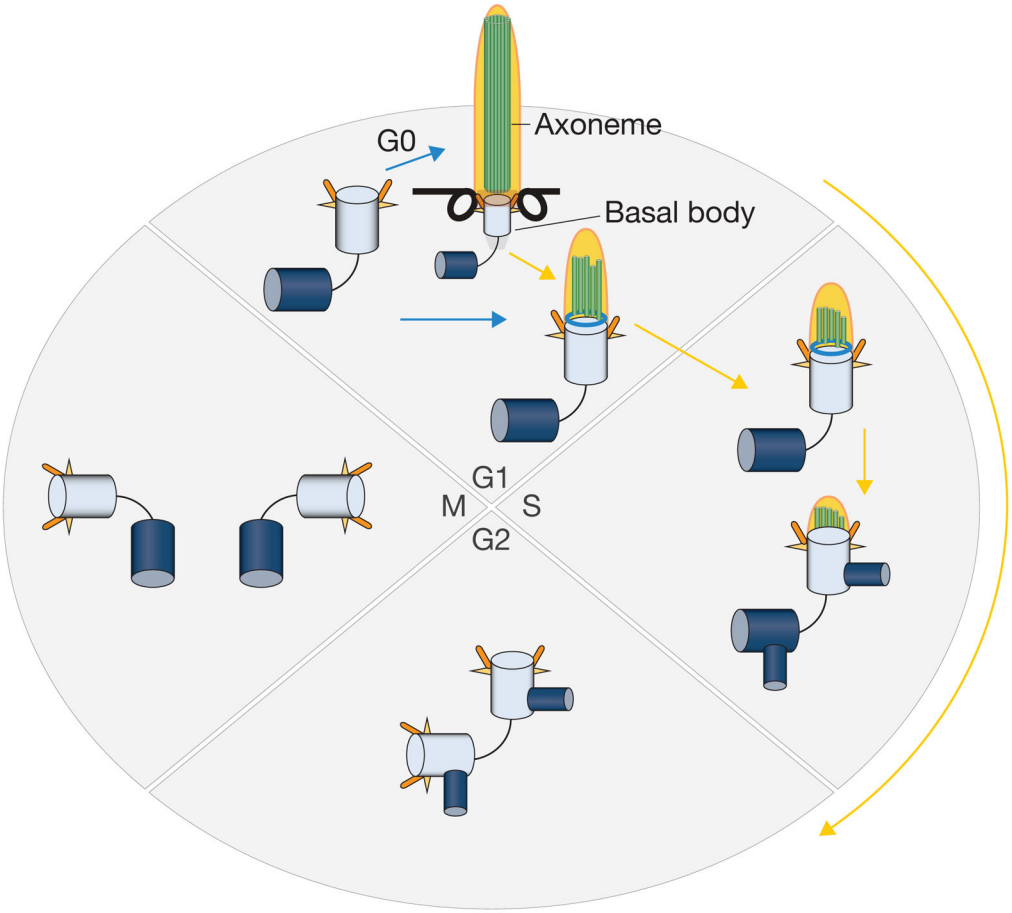


**Figure 1. Linkage of the centrosome–cilium cycle to the cell cycle**

Primary cilia assemble specifically when cells exit the cell cycle and become quiescent or differentiate. Cells are also competent to form cilia in G1. Phases of the cell cycle are indicated, and blue and yellow arrows indicate cilium assembly and disassembly, respectively. Only the mother centriole (light blue) can initiate ciliogenesis. The daughter centrioles are shown in dark blue. During the process of ciliogenesis, an axoneme is assembled. This microtubular structure (indicated with parallel green rods) is disassembled as cells progress towards S phase, concomitant with remodelling of the distal end of the basal body (aqua ring). During S phase, centrosomes commence duplication, at which point cilia have largely disassembled. After mitosis, centrosomes are again competent to assemble primary cilia, either in G0 or in early G1 phase.

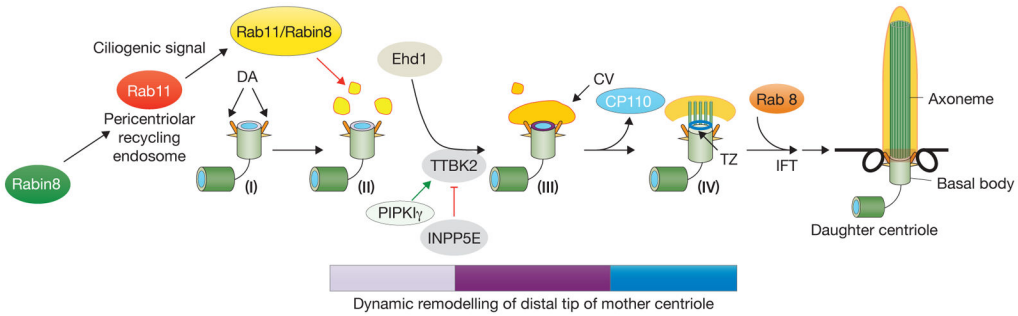


**Figure 2. Key players and events in cilium assembly**

Cilium assembly proceeds through a series of orchestrated and well-defined stages (labelled I–IV), resulting in the dramatic remodelling of the maternal centriole. Exit from the cell cycle initiates the recruitment of Rabin8, a guanine nucleotide exchange factor (GEF), to the pericentriolar recycling endosome, whereupon it is activated by Rab11, setting off a cascade that ultimately activates Rab8a vesicles for recruitment and docking to the distal appendages (DA, orange antennae) of mother centrioles (I–II). Shortly after quiescence is induced, small vesicles (distal appendage vesicles, yellow) associated with Rab11 assemble around the DA, docking at the appendages (II). Distal appendage vesicles fuse to form ciliary vesicles through the action of the Ehd1 protein (II–III). After ciliary vesicle (CV) formation, the distal ends of mother centrioles/basal bodies are remodelled (concentric rings) through the action of: the kinase TTBK2, the balance of PtdIns (modulated by INPP5E and PIPKIγ), and CP110 removal, which releases the inhibition of microtubule growth and axonemal extension (III–IV). A second kinase, MARK4, could collaborate with TTBK2 to remove CP110 from distal ends. Vesicular Rab11 probably activates Rab8 for subsequent elongation of the ciliary membrane. After removal of CP110, the transition zone (TZ), which functions as a ciliary gate, is assembled, and IFT helps transport proteins through this gate.

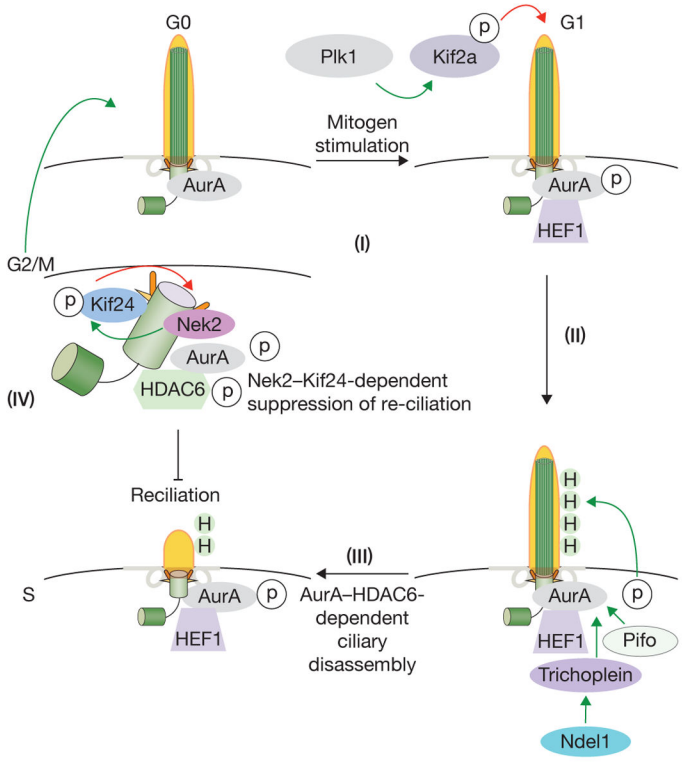


**Figure 3. Cilium disassembly**

Serum growth factors trigger cilium disassembly in G1 phase through the concerted actions of two kinesins (Kif2a and Kif24) and destabilization of acetylated tubulins in the ciliary axoneme. The initiation of ciliary disassembly in early G1 requires the inhibition of axoneme extension (Plk1/Kif2a) and recruitment and activation of AurA/HEF1, which activates HDAC6 (H, green circles) and tubulin de-acetylation; this, in turn, leads to disassembly (steps I–III). Pitchfork (Pifo) and trichoplein collaborate to activate AurA, and Ndel1 stabilizes trichoplein at basal bodies to suppress ciliogenesis. Plk1-mediated phosphorylation of Kif2a stimulates its ability to depolymerize axonemal microtubules shortly after mitogen stimulation. At a later point, Nek2 is expressed and associates with Kif24, phosphorylating and activating this second de-polymerizing kinesin. Activation of Kif24 provides a sustained block to re-ciliation throughout S/G2/M (step IV), thereby ensuring that centrioles remain competent to replicate in S phase and assemble a mitotic spindle in M phase.