The perennial organelle: assembly and disassembly of the primary cilium

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Summary

Primary cilia contain signaling receptors of diverse classes, and ciliary dysfunction results in a variety of developmental defects. Thus, primary cilia are thought to have an important role in sensing and transducing cellular signals. Although there is clear evidence demonstrating that these organelles are assembled and disassembled dynamically as cells progress through the cell cycle, the mechanisms by which the cell cycle controls the assembly and disassembly of the primary cilium remain poorly understood. In this Commentary, we review the basic cellular mechanisms that underlie the early stages of cilium assembly and discuss how the cell cycle communicates with the ciliation program. A commonly held view is that ciliation occurs exclusively in cells that have exited the cell cycle and entered quiescence or differentiation. However, this concept is at odds with the finding that, during development, many actively proliferating cells require cilia-mediated signaling pathways to instruct their developmental fate. Here, we reassess the quiescence-centric view of ciliation by reviewing historic and current literature. We discuss ample evidence that cilia are in fact present on many proliferating cells, and that a transient peak of ciliation before the G1-S transition might be tightly coupled to entry into the DNA replication phase. Finally, we touch on the relationship between the ciliation and cell-division cycles and the tissue distribution of primary cilia in order to highlight potential roles for the primary cilium in restraining cells from the hyperproliferative state that contributes to cancer.

This article is part of a Minifocus on cilia and flagella. For further reading, please see related articles: 'The primary cilium at a glance' by Peter Satir et al. (*J. Cell Sci.* **123**, 499-503), 'Sensory reception is an attribute of both primary cilia and motile cilia' by Robert A. Bloodgood (*J. Cell Sci.* **123**, 505-509), 'Flagellar and ciliary beating: the proven and the possible' by Charles B. Lindemann and Kathleen A. Lesich (*J. Cell Sci.* **123**, 519-528) and 'Molecular mechanisms of protein and lipid targeting to ciliary membranes' by Brian T. Emmer et al. (*J. Cell Sci.* **123**, 529-536).

Key words: Cilia, Tubulin acetylation, Centrosome

Introduction

Primary cilia are sensory organelles that are present on most human cell types (http://www.bowserlab.org/primarycilia/cilialist.html). Primary cilia have been long suspected to possess important sensory functionalities, but recent data suggest that they also play important roles in the control of cell proliferation and in several developmental signaling pathways. Indeed, the Hedgehog, Wnt and platelet-derived growth factor (PDGF) signaling pathways seem to rely on the highly organized confines of the ciliary membrane to coordinate the initiation and transmission of extracellular signals to the interior of the cell (Morgan et al., 2002; Cano et al., 2004; Haycraft et al., 2005; Schneider et al., 2005; Corbit et al., 2005; Corbit et al., 2008). Although it is now clear that primary cilia sample the extracellular environment and are the sites of organization of multiple signal transduction pathways, basic questions regarding the regulated assembly and disassembly of these organelles remain. Most notably, exit from the cell cycle and entry into quiescence is the most permissive condition for building a primary cilium (Dingemans, 1969; Fonte et al., 1971; Uetake et al., 2007) and it is therefore often believed that proliferating cells are unable to ciliate (Quarmby and Parker, 2005). However, proliferating cells in several developmental contexts rely on the primary cilium for the transduction of developmental signaling pathways. For example, during embryogenesis, cells of the neuroepithelium divide rapidly but at the same time sense a Hedgehog morphogen gradient to adopt a given neuronal fate. Thus, the relationships between cilium assembly, disassembly and the cell cycle remain unclear.

In the remainder of this Commentary, we review several examples of ciliated yet proliferating cell types; the potential relationships between cilium assembly, disassembly and the cell cycle; and the known molecular players and pathways that regulate cilium disassembly. Finally, we provide examples of ciliated and cilia-less cell types as they are found in situ and discuss possible links between primary cilia, tissue repair and neoplasia.

Structural signatures of basal-body differentiation

Primary cilia are assembled atop mother centrioles, are present at a copy number of one per cell, and are ensheathed within a membrane that is continuous with, but molecularly distinct from, the plasma membrane (Fig. 1). The 9+0 ciliary axoneme includes a radial array of nine doublet microtubules but, unlike typical motile cilia and flagella, lacks a central pair of microtubules. The commitment of a cell to primary cilium assembly is first reflected by the differentiation of the mother centriole into a basal body (Fig. 1). Centrioles are short (0.4 μm length) barrels of nine triplet microtubules, and each cell in the G1 phase contains one centriole that was assembled during the previous division (daughter centriole) and one older centriole that acted as a template for the assembly of the daughter centriole (mother centriole). Although both centrioles at first appear structurally similar, the mother centriole is the determining player in centrosome and basal-body function. First, although both mother and daughter centrioles can nucleate microtubules, only the mother centriole can anchor microtubules

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and therefore serve as a microtubule-organizing center (Piel et al., 2000). Second – and by definition – the mother centriole nucleates the assembly of new daughter centrioles during each passage through S phase. Finally, only the mother centriole differentiates into a basal body from which axoneme elongation takes place. Mother and daughter centrioles are embedded within the centrosome near the nucleus in interphase, whereas basal bodies are tightly apposed to the plasma membrane and possess three types of appendages that serve as plasma-membrane anchors. These appendages might regulate cargo entry and exit into and out of the cilium, and might have additional functions related to primary cilium integrity or function (Fig. 1).

First, ciliary rootlets (Fig. 1) extend from the proximal end of the basal body deep into the cell interior, in some instances nearly contacting the Golgi (Tachi et al., 1974). It was long thought that the ciliary rootlet anchors the basal body or functions in the trafficking of proteins or membranes from the Golgi to the base of the cilium. However, although the deletion of rootletin, a major component of the ciliary rootlet, ablates the ciliary rootlet, it does not prevent cilium assembly or lead to developmental phenotypes that are commonly associated with defective cilia. Instead, rootletin-null mice show more subtle defects in ciliated cells: they exhibit fragility around the ciliary base of photoreceptor cells as well as insufficient mucociliary clearance (Yang et al., 2005). These findings suggest that ciliary rootlets are not essential to ciliary biogenesis or function but instead provide mechanical support to cilia in certain specialized settings.

Second, basal feet (Fig. 1) are modified centriolar subdistal appendages that project laterally from the sides of the triplet microtubule barrel (Anderson, 1972). Microtubule ends can be seen embedded in each basal foot, suggesting that basal feet might serve to firmly anchor cytoplasmic microtubules to the base of the cilium.

Third, transition fibers (Fig. 1) of the basal body are thought to originate from the distal appendages of the mother centriole and connect the outer surface of the triplet microtubule barrel to the plasma membrane (Anderson, 1972). Although they are referred to as transition ‘fibers’, they are actually sheet-like projections that appear as a pinwheel when viewed in cross section. The transition fibers might sufficiently obstruct the periciliary entry region so as to restrict vesicle and macromolecule exchange between the cytoplasm and the ciliary lumen, thereby providing a physical basis for the concept of a ciliary pore. Finally, transition fibers might serve as docking platforms for the building blocks of the cilium and their associated transport factors (Deane et al., 2001). Currently, the only protein that is thought to play a role in transition-fiber assembly is CEP164 (centrosomal protein of 164 kDa). Indeed, elegant immunoelectron microscopy studies have shown that CEP164 localizes very specifically to distal appendages, and that CEP164 depletion prevents cilium assembly (Graser et al., 2007). However, exactly where this protein localizes on basal bodies and whether CEP164 depletion reduces basal body attachments to the plasma membrane remain to be determined. Nevertheless, studies of CEP164 are clearly a crucial starting point for the understanding of distal appendages and transition fibers.

Molecular control of the ciliation cycle

Although the key structural features of the basal body have been known for decades, the molecular switches that activate conversion of the maternal centriole to the basal body, and in turn cilium assembly, remain nearly entirely unknown. Recent reports suggest

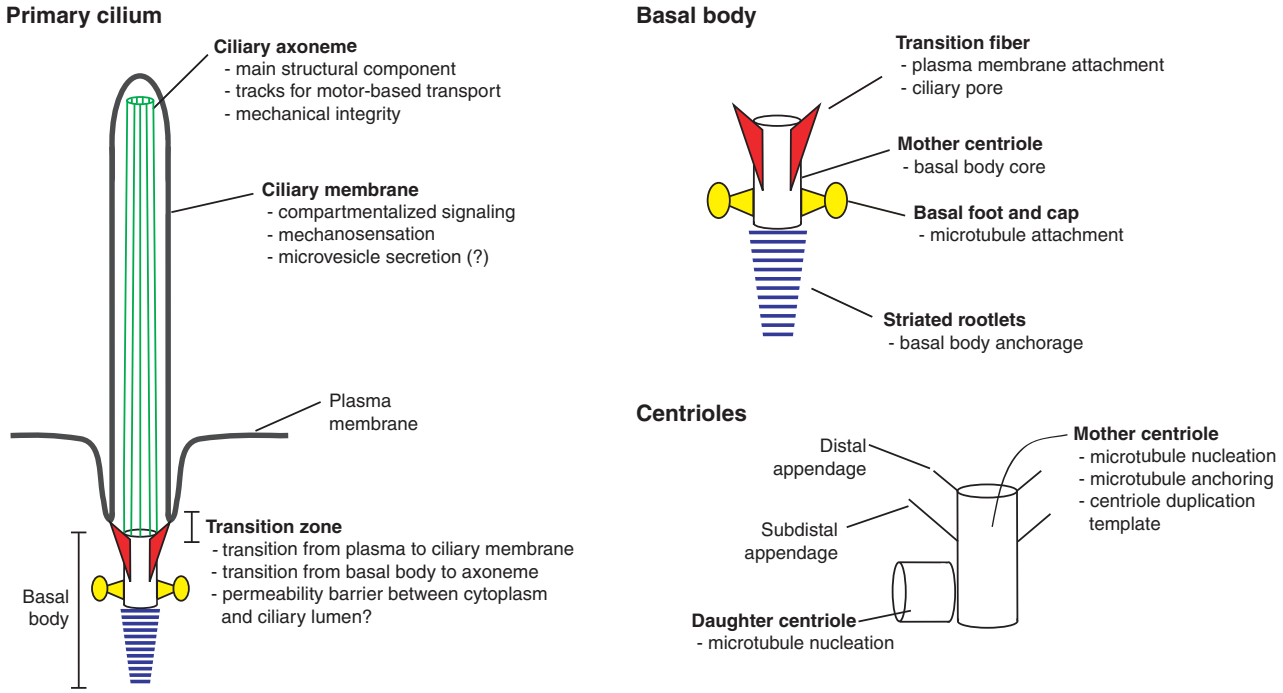

Fig. 1. Major structural features and functions of primary cilia, basal bodies and centrioles. The primary cilium is comprised of a basal body, an axoneme and the ciliary membrane. Basal bodies are mother centrioles that have been modified by the addition of defining accessory structures including transition fibers, basal feet and caps, and striated rootlets. The triplet microtubules of the basal body centriole give rise to the doublet microtubules of the ciliary axoneme at the region of the transition zone. Centrioles are typically found as orthogonal pairs comprising a mother and a daugher, the former being associated with specialized functions.

that two centrosomal proteins, CEP97 and CP110 (of 97 and 110 kDa, respectively), coordinately suppress the assembly of the ciliary axoneme (Fig. 2), CEP97 by recruiting CP110 to the centriole and CP110 by capping the ends of the distal centriolar microtubules (Spektor et al., 2007). In accordance with the role of CEP97 and CP110 in preventing aberrant cilium assembly during interphase, Spektor and colleagues found that the levels of both proteins were markedly decreased during quiescence compared with a state of proliferative cell growth. In NIH3T3 fibroblasts, overexpression of CP110 was found to block cilium formation during serum starvation, whereas depletion of CP110 from U2OS osteosarcoma cells led to the assembly of elongated structures that resembled aberrant primary cilia (Spektor et al., 2007). In addition to containing acetylated tubulin, these cilia-like structures contained centrin, a centriolar protein that is typically absent from primary cilia. Further analysis by three other groups demonstrated that these structures were not ensheathed by a membrane, were negative for intraflagellar transport protein of 88 kD (IFT88; a canonical marker of the cilium) and were most probably abnormally elongated centrioles (Tang et al., 2009; Kohlmaier et al., 2009; Schmidt et al., 2009). Together, these findings suggest that CP110 functions to limit the length of centriolar microtubule triplets in proliferating cells and that CP110 removal from the mother centriole is a prerequisite for axoneme elongation.

If CP110 does prevent centriole-to-basal-body conversion in 3T3 and RPE cells, how might it be removed from the centriole? Because CP110 was originally identified as a target of S phase cyclin-dependent kinase by the Dynlacht group (Chen et al., 2002), it is tempting to speculate that phosphorylation regulates the association of CP110 with the centriole and/or the stability of the CP110 protein (Fig. 2).

Further exploration of the mechanism by which CP110 inhibits axoneme assembly and centriole elongation is likely to reveal novel insights into the assembly of the triplet microtubules of the centriole and basal body. Similarly to CP110 depletion, the overexpression of CPAP (also known as CENPJ, centromere protein J) leads to a marked elongation of the centriolar microtubule triplet barrel (Tang et al., 2009; Kohlmaier et al., 2009; Schmidt et al., 2009). However, it does not prevent CP110 from capping the distal ends of centriolar microtubules (Schmidt et al., 2009), suggesting that CP110 does not prevent centriole elongation by simply capping the tip of triplet microtubules.

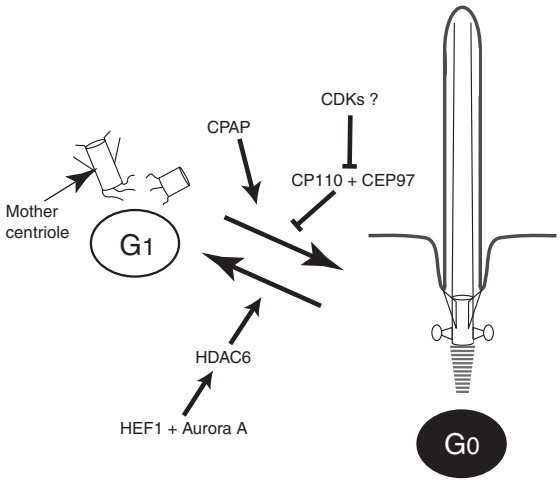

Fig. 2. Molecular control of the ciliation cycle. A growing number of proteins are now recognized to regulate primary cilium assembly at the centriole. CP110 caps the distal ends of the mother centriole. The release of CP110 and CEP97 from the mother centriole, coupled to the activities of CDKs, permits microtubule elongation and axoneme elongation. CPAP promotes centriole elongation. However, the relationship between CPAP and axoneme assembly remains uncharacterized. Cilium disassembly as cells re-enter the cell cycle can be mediated by HEF1- and Aurora-A-mediated activation of HDAC6, which in turn, might promote cilium disassembly by deacetylating axonemal microtubules.

The mechanisms that mediate cilium disassembly are perhaps better studied than those that mediate cilium assembly, but many questions in this area also remain wide open. Primary cilia are disassembled as cells re-enter the cell cycle, prior to mitosis and, in some cases, prior to S phase (see below). What is the trigger for primary cilium disassembly? Studies from the Golemis laboratory suggest that cilium disassembly can be initiated by the Aurora A centrosomal kinase, whose activity also regulates entry into mitosis through the activation of cyclin-dependent kinase 1 (CDK1)-cyclin B (Pugacheva et al., 2007).

It has been shown that Aurora A localizes to centrosomes, that its kinase activity is activated following serum stimulation coincident with cilium disassembly, that it triggers rapid cilium disassembly when microinjected into cells, and that it interacts with and phosphorylates histone deacetylase 6 (HDAC6), which can act on tubulin (Pugacheva et al., 2007). Furthermore, depletion of Aurora A or its activator, enhancer of filamentation 1 (HEF1), or administration of Aurora A inhibitors prevents cilium disassembly (Pugacheva et al., 2007). On the basis of these data, a model of cilium disassembly was proposed whereby Aurora A activates ciliary HDAC6, which in turn deacetylates axonemal microtubules, leading to the rapid collapse of the primary cilium (Fig. 2). However, the finding that tubulin acetylation does not cause microtubule stabilization and is in fact thought to be a mark of long-lived microtubules is problematic in consideration of this model (Palazzo et al., 2003). Furthermore, knocking out the *Hdac6* gene in mice produces only subtle immunological phenotypes rather than the gross abnormalities and embryonic lethality (Zhang et al., 2008) that would be expected if the lack of this protein caused hyperstable microtubules or persistent cilia. Although the data from Pugacheva and colleagues (Pugacheva et al., 2007) are intriguing, further studies will be required to clarify the mechanism by which Aurora A mediates the disassembly of the primary cilium and what role, if any, tubulin acetylation has in antagonizing this process.

Finally, intraflagellar transport (IFT) – the process that transports structural building blocks between the bases and tips of cilia (and vice-versa) – has also been implicated in cilium shortening. IFT particles are mutliprotein complexes that associate with ciliary proteins and participate in their transport from the cell body into the primary cilium. In this manner, axoneme subunits are transported to the cilium tip via anteograte movement, and cargo-less IFT particles, or particles carrying turnover products, return to the cell body via retrograde movement. Flagellar disassembly in *Chlamydomonas* is accompanied by an increase in the rate at which cargo-less IFT particles enter the primary cilium (Pan and Snell, 2005). Thus, it is probable that cilium disassembly involves a decrease in the rate of delivery of axoneme subunits to the tip of the cilium and an increase in the rate of retrograde trafficking of disassembled cilium components (Pan and Snell, 2005). IFT-mediated cilium disassembly is a conserved mechanism because IFT proteins are required for the disassembly of mammalian cilia in RPE cells (Pugacheva et al., 2007). Nonetheless, alternative IFT-

independent mechanisms for cilium disassembly might also exist because Ptk1 (mammalian) cells appear to release the entire axoneme into the cytoplasm before shortening to the length of a centriole (Rieder et al., 1979). IFT-independent cilium disassembly might provide a fail-safe mechanism to release centrioles before mitotic-spindle assembly (see below).

Cilia and the cell cycle

Ever since researchers reported the first descriptions of primary cilia, an intriguing pattern of appearance and disappearance of these organelles has been noted and linked to cellular proliferation. The current consensus is that obligate primary cilium disassembly occurs prior to the appearance of the mitotic spindle, that assembly occurs during cell-cycle exit and that disassembly occurs during cell-cycle re-entry. It is presumed that ciliated centrioles (i.e. basal bodies) have exited the centriole duplication cycle, and that cilium disassembly frees centrioles to undergo duplication and segregation to the poles of the mitotic spindle.

Cilia in situ: 1969-1974

The earliest quantitative studies of the occurrence of primary cilia were undertaken utilizing whole-organ serial sectioning and electron microscopy and provided an immediate glimpse of the potential diversity of the relationship between ciliation and cell-cycle status. Dingemans' 1969 study provided perhaps the first quantitative assessment of the relationship between primary cilia and cell multiplication (Dingemans, 1969). Here, the presence of primary cilia and nuclei was assessed following chemical treatments that induced the massive proliferation of two cell types in the pituitary gland. It was found that the ratio of cilia to nuclei decreased after induction of cell proliferation, indicating that primary cilia are less likely to be found in actively proliferating cells.

These conclusions are supported by studies of the lining epithelium of the uterus (Tachi et al., 1974). This epithelium is not normally ciliated due to constant turnover; however, ovariectomy leads to quiescence and uniform apical ciliation. Following estradiol administration, the uterine epithelial cells re-enter the cell cycle in a highly synchronous manner and a wave of cilium disassembly occurs 12-24 hours later that is accompanied by the loss of ciliary rootlets, basal feet and transition fibers from the basal body (Tachi et al., 1974). Then, at 24 hours, most of the cells enter mitosis. These findings clearly demonstrated that primary cilia are disassembled during entry into the cell cycle, and that cilia are absent from mitotic cells (Archer and Wheatley, 1971).

Notably, an earlier study of chicken embryonic limb buds explored the timing of cilium disassembly during cell-cycle progression (Fonte et al., 1971). Using radioactive thymidine labeling, serial sectioning and electron microscopy, it was shown that cells of the limb-bud mesenchyme are nearly always ciliated except when they are undergoing mitosis (Fonte et al., 1971). By taking into account a post-1-hour labeling efficiency of 50% and a doubling time of 10 hours, it was reasonably concluded that approximately half of all limb-bud mesenchyme cells are both ciliated and in S phase at any given time (Fonte et al., 1971). These results were the first to suggest that primary cilia can be present outside of quiescence in certain cell types and in proliferating cells.

Cilia in vitro

Although electron microscopy studies of primary cilia in tissues were sufficient to uncover the negative correlation between the presence of cilia and mitotic activity, a precise analysis of the ciliation status during each cell-cycle phase required the use of immunofluorescence microscopy and highly synchronous cell culture systems. As expected, immortalized mouse embryonic fibroblasts that had been rendered quiescent by serum starvation or contact inhibition were found to assemble primary cilia, and serum stimulation of quiescent cells led to cell-cycle entry and rapid (within less than 2 hours) disassembly of the primary cilium (Uetake et al., 2007; Tucker et al., 1979). Unexpectedly, however, primary cilia were seen to reappear 13 hours after serum addition (Fig. 3). Finally, starting at around 20 hours, primary cilia fully disassembled, coincident with the initiation of DNA synthesis. This biphasic cilium disassembly has recently been reported in RPE cells (as well as in IMCD3 and Caki-1 cells), although the wave of re-ciliation during late G1 phase was not observed, possibly owing to a lower resolution of the time course in this study (Pugacheva et al., 2007). Together, these data suggest that the presence of a cilium in late G1 is a characteristic of actively proliferating cells.

To characterize the two waves of cilium disassembly and their relationships to S-phase entry, Tucker and coworkers attempted to uncouple cilium disassembly from DNA synthesis by isolating activities that promoted cilium disassembly and activities that promoted DNA synthesis (Tucker et al., 1979). Serum can be fractionated into PDGF and platelet-free plasma. A combination of both fractions is sufficient to initiate both waves of cilium disassembly and DNA synthesis with normal kinetics (Fig. 3). Strikingly, PDGF alone triggered the initial wave of cilium disassembly, but failed to initiate either the second wave of ciliation and de-ciliation or DNA replication. By contrast, plasma alone induced neither cilium disassembly nor DNA synthesis (Fig. 3). A variety of treatments (such as Ca^{2+} or fibroblast growth factor) were found to substitute for PDGF in triggering cilium disassembly but, remarkably, a treatment that triggered the second wave of ciliation and de-ciliation without initiating DNA synthesis was not identified. The striking conclusion from these experiments – that the second

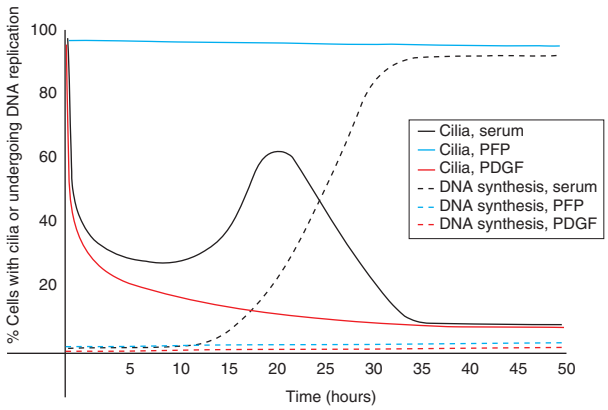

Fig. 3. Relationship between cilium disassembly and DNA synthesis. Isolated serum components exhibit separable effects on ciliation and the DNA replication machinery. Biphasic primary cilium disassembly occurs as cells enter the cell cycle and begin to synthesize DNA following treatment with serum, which contains both platelet-poor plasma and PDGF. The second round of cilium disassembly occurs coincident with initiation of DNA replication. PDGF induces one round of cilium disassembly but reassembly and DNA synthesis do not take place. By contrast, platelet-free plasma is capable of inducing neither cilium disassembly nor DNA synthesis. PFP, platelet-free plasma. Reproduced from Tucker and Pardee (Tucker and Pardee, 1979) with permission.

wave of ciliation and de-ciliation cannot be uncoupled from the initiation of DNA synthesis – suggests that these two processes are in fact tightly coupled. This prompted questions regarding the causal relationship(s) between these two events. Given that DNA synthesis proceeds with normal kinetics in the presence of the microtubule poison colchicine (which prevents cilium assembly) (Pardee, 1974), or in cells that are unable to ciliate due to genetic alteration (Ishikawa et al., 2005), it can be concluded that the second wave of ciliation and de-ciliation is not required for DNA synthesis. Thus, it would appear that entry into S phase (promoted by the accumulation of cyclin E and the resulting activation of CDK2) is the trigger for both de-ciliation and DNA replication. As cyclin-E–CDK2 activation is also known to be the trigger for centrosome duplication, it would not be surprising if cyclin-E–CDK2 also influences the ciliation status of the mother centriole.

Although cilia are not required for S-phase entry in the presence of high concentrations of serum, it remains possible that the cilium delivers S-phase-promoting signals to the cell in physiological contexts. A direct requirement for the cilium during S-phase entry is suggested by the work of Christensen and colleagues. Their findings suggest that cilia might deliver growth-factor-activated signals to the cytoplasm, although whether this would occur via cilium disassembly, retrograde trafficking or a combination of both remains unclear (Schneider et al., 2005).

If the observation that cilia are present during late G1 phase in proliferating cells seems surprising, the ciliation behavior of Ptk1 cells lies even further from the ‘quiescence-centric’ view. Using thick-section electron microscopy, Conly Rieder and colleagues conducted a careful quantification of cilia and found that cilia disassembly takes place at a time in early mitosis between chromosome condensation (prophase) and nuclear-envelope breakdown (prometaphase) (Rieder et al., 1979). This remarkable timing of cilium disassembly correlates with another oddity of Ptk1 cells – the absence of acetylated microtubules (Piperno et al., 1987).

Following the recent findings of Pugacheva, Golemis and colleagues that tubulin deacetylation triggers cilium disassembly (Pugacheva et al., 2007), it is tempting to speculate that, in the absence of microtubule acetylation, the cilium cycle becomes largely uncoupled from the cell division cycle, and that a fail-safe mechanism is responsible for disassembling the cilium to liberate the mother centriole in time for mitotic-spindle assembly to take place. In this context, it would be interesting to test whether re-introducing tubulin acetylation into Ptk1 cells would be sufficient to trigger a normal pattern of IFT-dependent cilium disassembly in late G1. In addition, the findings of the Reider group (Rieder et al., 1979) strongly suggest that centriole duplication can take place while the mother centriole is still differentiated into a basal body and firmly anchored at the base of the cilium.

Although these results are surprising, the concept that basal bodies can serve as templates for centriole duplication is not without precedent. Nearly all ciliated protozoans carry out centriole duplication without disassembling their cilia or flagella (Dippell, 1968). Overall, these findings serve to highlight the remarkable plasticity of the relationships between the cell division cycle and the cilium cycle.

Terminal differentiation without ciliation: a paradox

Although it is true that all tissues contain populations of cells bearing primary cilia, there are certainly examples of major cell types that are not ciliated in situ. Rather than providing a comprehensive review of mammalian cell types that do and do not assemble primary cilia (see http://www.bowserlab.org/primarycilia/cilialist.html), we will provide and discuss examples that are conceptually illustrative. Because cell culture can lead to differentiation and transdifferentiation, we will limit our discussion to primary cilia found in intact tissues.

There have been numerous electron microscopy studies of the primary cilia in the organs of the gastrointestinal system. The pancreas is comprised of four main cell types: acinar, centroacinar, islet and duct. Whereas the latter three form primary cilia, acinar cells, which account for nearly 80% of the volume of the pancreas, are not ciliated in the healthy pancreas (Aughsteen, 2001; Zhang et al., 2005; Cano et al., 2004). Similarly, ductal epithelial cells of the biliary tree are ciliated, whereas hepatocytes, which comprise almost 90% of the volume of the liver, do not assemble primary cilia (De La Iglesia and Porta, 1967; Wheatley, 1969). Minimally, these observations permit two separate conclusions to be drawn. First, the presence of a primary cilium does not equate with terminal differentiation; both acinar cells and hepatocytes are terminally differentiated and are highly specialized, yet in healthy pancreas and liver, neither cell type is found to be ciliated. Second, there are major cell types that do not form primary cilia under normal circumstances. Thus, one could wonder why different cell types might or might not grow a primary cilium in situ.

Several observations might shed light on this issue. The first relates to cell specialization and known aspects of cilium function. Hepatocytes and pancreatic acinar cells have in common a marked capacity for secretion and an extensive cytoplasm. It is not difficult to imagine that an apical primary cilium and the machinery associated with its basal body might interfere with high exocytic output at the apical plasma membrane (Fig. 4). This explanation might provide a physiological rationale for the absence of primary cilia on hepatocytes and pancreatic acinar cells. However, pancreatic islet cells, which have been well characterized for their capacity to synthesize and secrete insulin; the secretory cells of the human oviduct, which produce secretions important for sperm and ova

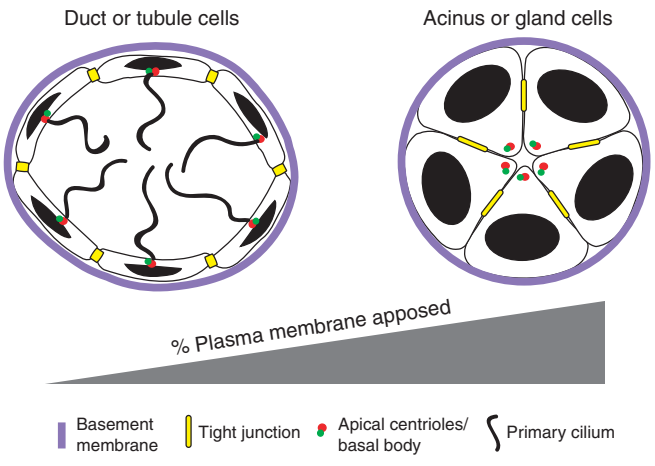

Fig. 4. Inverse relationship between stable plasma-membrane associations and ciliation. The epithelia of ducts and tubules, as found in the nephron, the pancreatic ducts and the hepatobiliary tree, exhibit substantial portions of lumen-exposed plasma membrane. By contrast, cells in which the plasma membranes are mostly apposed to those of neighboring cells or to basement membranes (e.g. pancreatic acini or liver hepatocytes) tend not to ciliate.

conductance; and the mucinous cells that form the glandular elements near the neck of the gall bladder are all ciliated and, at the same time, capable of high-output secretion (Hagiwara et al., 2002; Zhang et al., 2005; Cano et al., 2004; Hagiwara et al., 2008) (E.S.S., unpublished observations). As these secretory cell types have larger areas of lumen-facing plasma membrane than do pancreatic acinar cells or hepatocytes, they might be able to separate the sites of exocytosis from the area surrounding the base of the cilium. This implies that a cell might be able to sense the area of plasma membrane available for exocytosis and make a decision on whether or not to ciliate.

Second, the capacity for migration might also necessitate the presence of a primary cilium. Fibroblasts form interphase cilia and are known to migrate though tissues. In his 1977 study, Albrecht-Buehler found that, when fibroblasts were plated on a gold-particle-coated substrate and allowed to migrate, they left tracks of clearance in their wake (Albrecht-Buehler, 1977). Remarkably, their primary cilia were found to orient in the direction of cellular migration (Albrecht-Buehler, 1977). More recent findings from the Christensen laboratory support and extend the concept that primary cilia are required to orient fibroblast migration in a wounding assay (Schneider et al., 2009). By contrast, there have been no descriptions of ciliated lymphocytes or granulocytes, two populations of migrating blood cells. However, it is not known whether lymphocytes that are actively migrating through tissues are ciliated.

Third, primary cilia might function to fully enforce growth arrest in the presence of partial contact inhibition. Contact inhibition prevents the proliferation of cells that have developed stable physical associations with one another or with basement membranes. In the liver and pancreas, ciliated ductal epithelial cells and non-ciliated acinar cells and hepatocytes can proliferate in response to acute injury. Although approximately half of the surface area of the ductal epithelia is ‘naked’ and exposed to the fluids of the lumen, nearly all surfaces of acinar cells and hepatocytes are stably associated with neighboring cells or basement membranes (Fig. 4). Here, it is easy to appreciate how contact inhibition can suffice to suppress the growth of unciliated acinar cells and hepatocytes but not of ductal epithelial cells. Hence, the primary cilium could function as a complement to partial contact inhibition. In line with this concept, the loss of the primary cilium indeed leads to the development of hyperplastic epithelia in multiple organ systems, including liver, kidney and pancreas (Moyer et al., 1994). Moreover, when intercellular contacts are disturbed in the setting of acinar injury, regenerating acinar cells can be seen to ciliate during a duct-like stage of regeneration and subsequently de-ciliating after developing stable intercellular contacts.

A fourth possible explanation for the differential assembly of primary cilia among specific tissue cell types might relate to the regenerative potential of those cells. In the case of acute injury and cell loss, all cell types of the liver and pancreas are able to proliferate to replenish damaged areas (Sakaguchi et al., 2006; Strobel et al., 2007; Georgiev et al., 2008). However, with repeated injury, as seen in chronic pancreatitis and liver cirrhosis, the non-ciliated hepatocytes and acinar cells lose the ability to regenerate, and the injured area is replaced by fibrosis. In the fibrotic area, the sole remnants of the original populations are the ciliated ducts and islets in the pancreas, and the ciliated cholangiocytes of the biliary tree in the liver. Thus, primary cilia might guard cell types endowed with high regenerative potential against excessive or untimely proliferation.

Primary cilia and cancer

Indeed, we have found that primary cilia are lost specifically from the neoplastic ductal epithelium, but not the mesenchymal stroma, of human and mouse pancreatic cancer through a process that can be driven by oncogenic K-RAS and that occurs independently of the proliferative index (Seeley et al., 2009). Importantly, all putative precursor cell types of pancreatic ductal adenocarcinoma (PDAC), which now include duct, centroacinar, acinar-to-ductal metaplasia and islet cells, are known to assemble primary cilia. Although this loss seems to be a universal feature of pancreatic cancer, no causal relationship between the loss of the primary cilium and the development of pancreatic cancer has been established. Another question relates to the role of cilia in the pancreatic cancer stroma. Given that Hedgehog activity appears to be restricted to the pancreatic-cancer stroma, is important for stromagenesis and requires primary cilia, it is conceivable that stromal cilia might promote stroma formation. A subsequent report has shown that primary cilia are frequently depleted from the neoplastic epithelia of a subset of renal cancers, independently of the proliferation index (Schraml et al., 2009).

With regard to the relationship between primary cilia and Hedgehog-dependent cancers, two recent reports confirm that medulloblastoma and basal-cell carcinoma require cilia when growth is dependent on constitutively active forms of Smoothened but not when it is dependent on constitutively active forms of Gli (Wong et al., 2009; Han et al., 2009). These findings are well supported by several prior studies that demonstrated a requirement for the cilium in transduction of the Hedgehog signal from Patched through Smoothened to Gli (Haycraft et al., 2005; Corbit et al., 2005). However, the influence of the primary cilium on the development of more common epithelial malignancies remains unknown. Indeed, it has been suggested that a loss of primary cilia is a common feature of neoplastic cells; however, an article frequently cited as evidence of this phenomenon states: “it is already well known that a wide variety of tumours possess cells with primary cilia...several cases have been reported of malignant cell types apparently producing cilia after malignant transformation when their ‘normal counterparts’ were supposedly unciliated, as in the case of hepatocytes of the LI-IO line” and “...transformed variants of the otherwise well-behaved cell line, 3T3 still exhibit, however, the same high incidence of ciliation at confluency as the progenitor, untransformed 3T3 line” (Wheatley, 1995). Thus, at present, the fate of the primary cilium during oncogenesis is uncertain, and it is possible that it might vary widely between different types of cancer and their respective stromal populations. In addition, it should be noted that the presence or absence of cilia in cultured tumor cells might reflect differentiation, transdifferentiation or dedifferentiation, rather than what actually occurs in situ. Indeed, tumor cells generally proliferate much more rapidly in vitro than in situ.

The varied behavior of primary cilia throughout the cell cycle, and the non-uniform and dynamic distribution of these organelles in tissues, suggest that care should be taken in the design and interpretation of studies seeking to determine the relationship between primary cilia, the cell cycle and cancer. Early reports suggested a direct requirement for the von Hippel Lindau (VHL) tumor-suppressor protein (which binds to microtubules) in the assembly of primary cilia (Lutz et al., 2006; Esteban et al., 2006; Schermer et al., 2006) but how the loss of this protein influences cell-cycle activity was not assessed in these studies. Subsequent experiments have demonstrated that the loss of VHL blocks cilium

assembly only in combination with GSK3β (glycogen synthase kinase-3 β) or PTEN (phosphatase and tensin homolog) depletion (Thoma et al., 2007; Frew et al., 2008); however, because loss of either of these proteins also dramatically enhances rates of proliferation, it is difficult to interpret their relationships with the cilium cycle. In this setting, the loss of the organelle could be a physiological consequence of rapid cell division, rather than due to the absence of the microtubule-binding activity of VHL.

Overall, a role for epithelial primary cilia in the suppression of common epithelial malignancies is compatible with their preferential assembly by long-lived cell types and their loss during transformation in the pancreas. Although the cilium might in some instances be required to transduce oncogenic signals leading to cancer (such as Hedgehog), it is possible that in more common epithelial malignancies the cilium might function overall as a tumor suppressor. Here, the loss of the cilium might result in the decoupling of associated mitogenic signaling modules from sensory control and lead to their redistribution to other cellular locations, such as endosomes or the plasma membrane. Here, in closer proximity to their cytoplasmic effectors, it is not difficult to imagine a greater propensity for these effectors to activate downstream signaling because they would not need to translocate from the cilium to the cytoplasm. Alternatively, it is also possible that the primary cilium actively transduces cytostatic signals to the cell.

Perspectives

The reassessment of the specific patterns of distribution, formation and disassembly of primary cilia provides fresh perspectives on the physiological aspects of cilium biology and emphasizes some of the most interesting, meaningful and challenging questions regarding the function of these organelles. A particularly promising avenue of research to further our understanding of these aspects of cilium biology would combine the use of recently developed genetically encoded fluorescent markers of cilia (GFP-IFT88, GFP-Rab8) with biosensors of the cell cycle (Hahn et al., 2009). Such tools would allow for a rigorous description of the cilium assembly-disassembly cycle and its relationship with the cell cycle. Another interesting area of research concerns the impact of tubulin acetylation on cilium assembly and disassembly. Here, however, identification of the elusive tubulin acetyltransferase would be required to determine whether acetylation and deacetylation of the axonemal microtubules couples the ciliation cycle to the cell division cycle. In summary, the marked variability in cilium assembly and disassembly patterns observed during development and in different cell types in situ suggests that primary cilia have flexible and diverse functions in tissue development and adaptation. A fundamental understanding of this highly dynamic organelle, and identification of the consequences of its dysfunction, will ultimately require the study of cilia in a wide variety of settings.

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