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Mechanism and Regulation of Centriole and Cilium Biogenesis

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Abstract

The centriole is an ancient microtubule-based organelle with a conserved nine-fold symmetry. Centrioles form the core of centrosomes, which organize the interphase microtubule cytoskeleton of most animal cells and form the poles of the mitotic spindle. Centrioles can also be modified to form basal bodies, which template the formation of cilia and play central roles in cellular signaling, fluid movement, and locomotion. In this review, we discuss developments in our understanding of the biogenesis of centrioles and cilia and the regulatory controls that govern their structure and number. We also discuss how defects in these processes contribute to a spectrum of human diseases and how new technologies have expanded our understanding of centriole and cilium biology, revealing exciting avenues for future exploration.

Contents

INTRODUCTION

Pericentriolar material (PCM): the electron-dense material that surrounds the centrioles and makes up part of the centrosome

Centrosome: a cellular structure comprising a pair of centrioles embedded in pericentriolar material; often forms the major microtubule-organizing center of the cell

Basal body: a mature centriole that docks at the plasma membrane to nucleate the formation of a cilium

Centrioles are evolutionarily conserved microtubule-based structures that have diverse functions in controlling cell polarity, proliferation, division, motility, and signaling. Centrioles recruit a surrounding pericentriolar material (PCM) to form centrosomes, which serve as the major microtubule-organizing center in most animal cells (**Figure 1*a***). Centrosomes nucleate the formation of the microtubule cytoskeleton in interphase cells and form the poles of the bipolar microtubule spindle during mitosis. In quiescent cells, a fully mature centriole can dock at the plasma membrane and act as a basal body that anchors a cilium. The cilium comprises axonemal microtubules that elongate from the distal end of the basal body and a ciliary membrane that surrounds the axoneme. Phylogenetic studies indicate that centrioles were present in the last eukaryotic common ancestor but were lost in some branches of the tree of life, such as some yeasts and higher plants (1). The presence of centrioles specifically correlates with the presence of cilia, not centrosomes, suggesting that the ancestral role of centrioles was to direct formation of cilia (2).

Recent work has begun to elucidate the molecular framework that underlies centriole and cilium assembly as well as how dysregulation in these organelles contributes to human disease. In this review, we explain recent advances in the centrosome and cilia field, with a focus on vertebrate centrosomes and cilia but referring to other systems when necessary. We begin by discussing centriole architecture and the centriole duplication cycle. We then deal with how centrosome defects contribute to human disease before discussing how cilia are assembled and disassembled in a cell cycle–dependent manner. We briefly explain the roles of cilia in cell signaling and how cilium dysfunction contributes to disease. Finally, we end by reviewing new technologies for studying

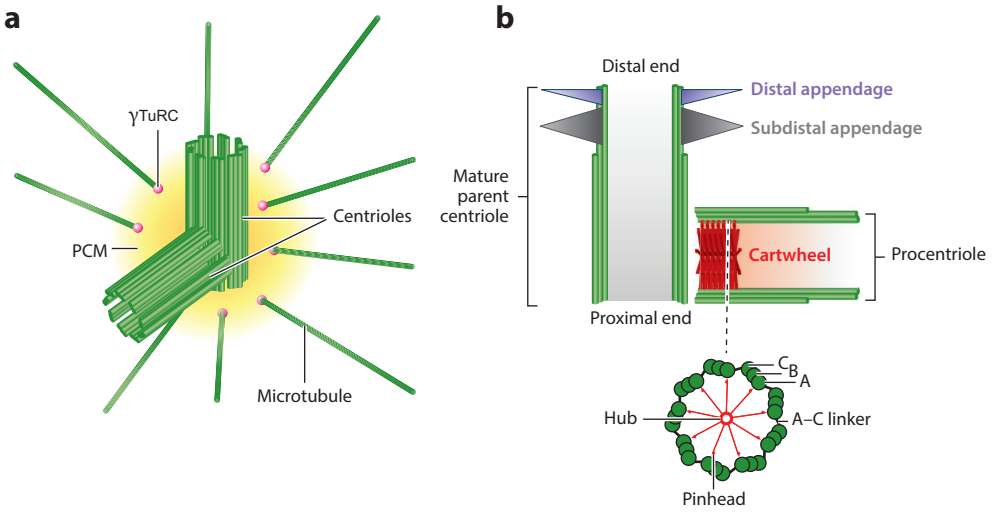

Figure 1

Centriole and centrosome structure. (*a*) Architecture of the mammalian centrosome. The centrosome comprises a pair of orthogonally oriented centrioles surrounded by a proteinaceous pericentriolar material (PCM). The PCM contains proteins required for microtubule nucleation and anchoring, such as the γ-tubulin ring complex (γTuRC) (*pink spheres*). (*b*) Schematic illustration of a mature parent centriole and associated procentriole. Centrioles are cylindrical structures comprising nine triplet microtubules, each of which contains a complete A-tubule and incomplete B- and C-tubules. The cartwheel is present in the proximal lumen of the procentriole and is formed by a central hub from which nine spokes emanate. Each spoke terminates in a pinhead structure that binds to the A-tubule of the microtubule triplet. The A-tubule of one triplet is linked to the C-tubule of the adjacent triplet via an A–C linker. Mature parent centrioles are decorated at their distal end with nine-fold symmetric distal and subdistal appendages.

centrosomes and cilia and highlight some important open questions and future avenues for exploration.

CENTRIOLE STRUCTURE

Centrioles are cylindrical in shape, with walls formed by a conserved nine-fold symmetrical array of microtubules (3). Although the radial symmetry of centrioles is invariant across life, centrioles can vary in size and diameter in different organisms and cell types. In mammalian cells, centrioles are ~230 nm in diameter and ~420 nm in length (4). Most centrioles have nine sets of interconnected triplet microtubule blades, although in some organisms the centriole wall is comprised of singlet or doublet microtubules (**Figure 1*b***). Triplet microtubules contain a 13-protofilament A-tubule and 10-protofilament B- and C-tubules, with the A-tubule from one triplet connected to the C-tubule of the neighboring triplet through an A–C linker (5). A recent cryo–electron microscopy study has shown that mammalian centrioles are organized into two structurally distinct regions along the proximal–distal axis (4). The proximal domain is ~200 nm in length and shares a common core architecture with the shorter *Drosophila* centriole, whereas the distal region of the mammalian centriole has a distinct A–C linker, an incomplete C-tubule, and a narrower diameter. Given that fly centrioles are structurally similar to the proximal region of mammalian centrioles and do not generate motile cilia, it is plausible that the distinct architecture of the distal portion of the mammalian centriole provides this expanded functionality (4).

Axoneme: a ring of nine doublet microtubules and associated proteins that form the cilium core; can also contain a central microtubule pair

The centriole is polarized along the proximal–distal axis for distinct functions. The proximal end recruits and organizes PCM required for the centrosome to nucleate microtubules. In some

vertebrate cell types, small aggregates of proteinaceous material termed centriolar satellites are also observed in the vicinity of centrosomes (6). Centriolar satellites traffic PCM components to the centrosome and act as assembly points for proteins required for cilia assembly (7). The distal end of a fully mature centriole carries nine distal appendages and a variable number of subdistal appendages. Distal appendages are required for docking of centrioles at the plasma membrane during the process of ciliogenesis (8), whereas subdistal appendages are involved in anchoring microtubules in interphase centrosomes and contribute to centriole cohesion (9). The cell cycle controls assembly of subdistal appendages, as they are lost from centrioles during mitosis and reassembled in the following G_1 phase; the timing of distal appendage assembly and their modification during the cell cycle is less well understood (**Figure 2**).

Centriolar satellites: electron-dense cytoplasmic granules occurring around the centrosome

Distal appendages: structures that radiate from the distal end of a fully mature parent centriole and mediate membrane docking during ciliogenesis

Subdistal appendages: structures projecting from the subdistal end of a fully mature parent centriole that anchor the minus-ends of microtubules in interphase cells

Parent centriole: a centriole that is able to duplicate but is not fully mature and lacks appendages; sometimes referred to as a daughter centriole

Mature parent centriole: a mature centriole that is able to duplicate and is decorated with appendages that enable ciliogenesis; sometimes referred to as a mother centriole

Procentriole: a newly formed centriole that is not competent for duplication

Cartwheel: a scaffolding structure located at the proximal end of the procentriole comprising a hub and nine radially arranged spokes

CENTRIOLE BIOGENESIS

In cycling cells, centriole number is maintained through a duplication cycle that is tightly coordinated with cell cycle progression (10) (**Figure 2**). At the start of the cycle, cells contain two centrioles connected by a flexible linker at their base. The younger of the two centrioles was assembled in the previous cell cycle and is referred to here as a parent centriole, whereas the older centriole is referred to here as the mature parent centriole. Centriole duplication begins at the G_1–S phase transition, when a new procentriole grows perpendicularly from a single site at the proximal end of each existing centriole. Each procentriole remains engaged in this orthogonal orientation during S and G2 phases, during which time it elongates, reaching ~80% of the length of a parent centriole prior to mitotic entry. In late G2, the flexible linker that connects the proximal end of the two parent centrioles is lost, allowing them to separate and guide the formation of the mitotic spindle. During mitosis, the procentriole disengages from the parent centriole so that the two newly created daughter cells each inherit a pair of parent centrioles that are competent for duplication in the next cell cycle. Importantly, the parent centriole that was formed 1.5 cell cycles ago reaches its full length in the following G_1 phase and acquires subdistal and distal appendages that allow it to function as a basal body.

Building a New Centriole

Pioneering work in *Caenorhabditis elegans* led to the identification of a highly conserved set of five core proteins that are required for the initiation of centriole duplication: PLK4 (ZYG-1 in *C. elegans*), CEP192 (SPD-2 in *C. elegans* and Spd-2 in *Drosophila*), CPAP (also known as CENPJ; SAS-4 in *C. elegans* and Sas-4 in *Drosophila*), STIL (SAS-5 in *C. elegans* and Ana-2 in *Drosophila*), and SAS6 (3). A list of key genes and corresponding orthologs is shown in **Table 1**. The kinase PLK4 is the master regulator of centriole duplication and is the earliest known marker for the site of procentriole assembly (11–13). In vertebrates, PLK4 is recruited to parent centrioles in G_1 phase by binding to the centriole receptors CEP152 (Asterless in *Drosophila*) and CEP192, which encircle the proximal end of the parent centriole (14–18). While PLK4's centriole receptors are localized as a ring throughout the cell cycle, PLK4 transitions from an initial ring-like localization around the parent centriole in G_1 phase to a single dot at the G_1–S phase transition (13, 16, 19, 20). This transition requires binding of PLK4 to STIL (19), which activates PLK4 dimers by inducing *trans*-autophosphorylation of the kinase's activation loop (21–23). Active PLK4 then phosphorylates STIL in a conserved STAN domain to trigger binding and recruitment of SAS6. SAS6 in turn initiates the assembly of the cartwheel, which forms a structural foundation for the procentriole (19, 21, 24, 25). PLK4 also phosphorylates STIL at additional sites that are required for the loading of STIL at the site of procentriole assembly (20, 26).

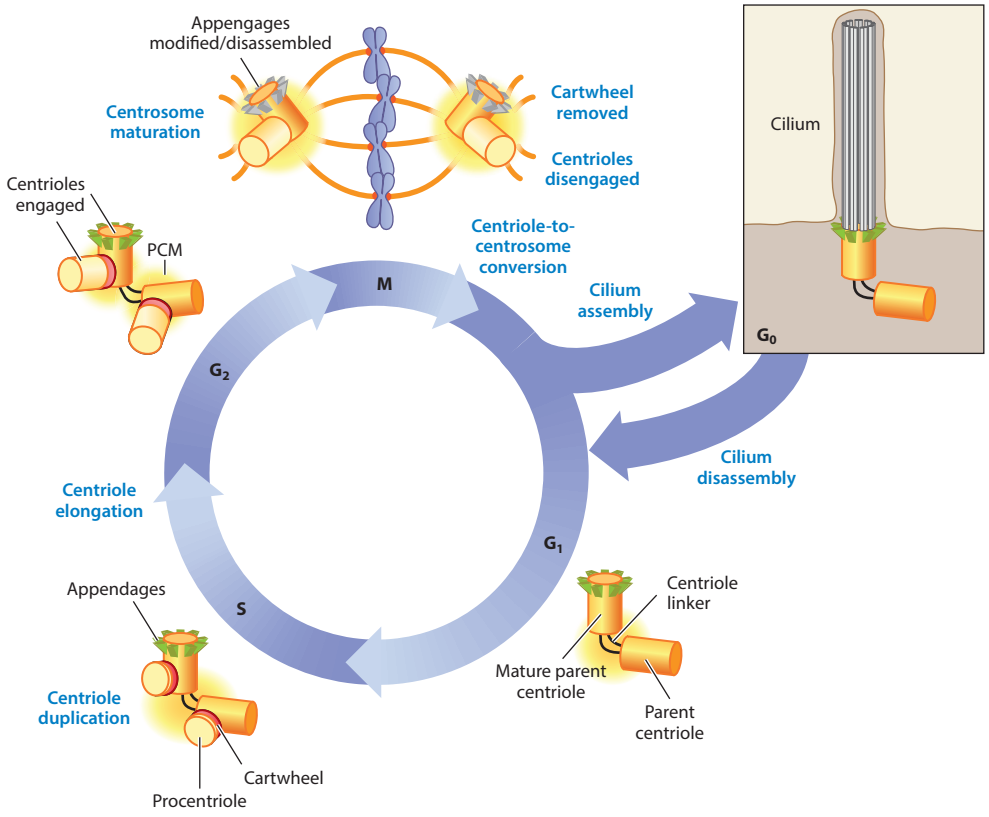

Figure 2

Regulation of centriole and cilium biogenesis during the cell cycle. G_1 cells contain two parent centrioles connected at their base by a flexible linker. At the beginning of S phase, each parent centriole assembles one new procentriole aligned orthogonal to its proximal end. This arrangement is termed engagement and acts to prevent the reduplication of the parent centriole. The procentrioles elongate as cells progress through the cycle, and in late G_2 phase, the flexible linker that holds the two parent centrioles together is dissolved to permit centrosome separation. In preparation for mitotic spindle formation, centrosome maturation occurs, resulting in pericentriolar material (PCM) expansion. In human cells, the cartwheel is removed from the lumen of the procentriole during mitosis. At the end of mitosis, the centriole pair disengages and loses its orthogonal arrangement. This step is required to relicense the parent centriole for duplication in the next cell cycle. At the same time, the procentriole is converted into a parent centriole. This centriole-to-centrosome conversion allows the procentriole to recruit PCM material and acquire competence for duplication. The distal and subdistal appendages are transiently modified/disassembled in mitosis. In G_1 phase, appendages form on the mature parent centriole that was created one-and-a-half cell cycles earlier. In nonmitotic cells, the mature parent centriole can migrate and initiate the formation of the axoneme of a cilium. Cell cycle progression is accompanied by disassembly of the cilium prior to mitosis.

The cartwheel comprises a stack of a ring-like assemblies that occupy the proximal ~100 nm of the human procentriole (27) (**Figure 1*b***). Each stack contains a central circular hub, from which nine spokes emanate and connect to the A-tubule of the microtubule triplets in the centriole wall. Elegant in vitro reconstitution has shown that recombinant SAS6 forms a homodimer that can oligomerize into a nine-fold symmetrical cartwheel structure in vitro (28–31), and this assembly is facilitated by binding to Bld10 (CEP135 in humans) (32). Growth of the cartwheel occurs through the addition of SAS6 molecules to the proximal end of the cartwheel stack, with the rate of growth set by PLK4 activity (33). Although the cartwheel plays an important role in establishing the centriole's nine-fold radial symmetry, cartwheel-independent mechanisms also contribute to symmetry, including potentially the structural constraint imposed by the A–C linker in the

Table 1 Key gene names

Homo sapiens	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Chlamydomonas reinhardtii*	Localization
PLK4	*Plk4* (*SAK*)	*zyg-1*		Cartwheel
SASS6 (*SAS6*)	*DSas-6*	*sas-6*	*BLD12*	Cartwheel
STIL	*Ana2*	*sas-5*		Cartwheel
CPAP (*CENPJ*)	*DSas-4*	*sas-4*		Centriole
CEP135	*DCep135*		*BLD10*	Centriole
CEP152	*Asl*			Centriole/PCM
CEP192	*DSpd-2*	*spd-2*		Centriole/PCM
CEP215 (*CDK5RAP2*)	*Cnn*	*spd-5*		PCM
CEP295	*Ana1*			Centriole
PCNT	*Plp*			PCM

Abbreviation: PCM, pericentriolar material.

microtubule wall (34, 35). Furthermore, in some species the cartwheel is a stable part of the centriole structure, but in human cells the cartwheel is removed during early mitosis and is therefore absent from mature centrioles.

Once the cartwheel has assembled, CPAP, in collaboration with its binding partners CEP135, CEP120, and SPICE1, facilitates the formation and stabilization of the procentriole's microtubule wall (36–41). Centriolar microtubules grow at a very slow rate and are exceptionally stable, in part because of extensive posttranslational modification by acetylation and polyglutamylation (42–44). In addition to canonical α- and β-tubulin, the assembly of centriolar microtubules likely requires the centriole-specific tubulin isoforms δ- and ε-tubulin. δ- and ε-tubulin form a biochemical complex with two proteins required for centriole stability named TEDC1 and TEDC2 (45). Cells lacking δ- and ε-tubulin form unstable centrioles with singlet microtubules, suggesting that these tubulin isoforms may provide critical interactions required for forming or stabilizing triplet microtubules (5, 46).

Setting Centriole Length

Although centriole length varies among species and cell types, centrioles from a given species or cell type achieve a remarkably reproducible length. In *Drosophila* and *C. elegans*, the cartwheel extends through the entire length of the centriole, and thus cartwheel height seems to control centriole length (33). In vertebrates, the centriolar microtubule triplets extend ∼300 nm beyond the height of the cartwheel, suggesting that other mechanisms must determine the final length of centriolar microtubules and thus that of the organelle. A central player in setting centriole length is CPAP, which associates with centriole microtubules and controls their growth and stability (47, 48). Overexpression of CPAP, or its interacting proteins CEP120 and SPICE1, leads to hyperelongation of centriolar microtubules in mammalian cells (36–38, 40, 49). Other proteins such as POC1 (50), hPOC5 (51), Asterless (CEP152 in humans) (52), and CEP295 (Ana1 in *Drosophila*) (53, 54) have also been implicated in centriole length control. CP110 and CEP97 cap the distal end of the centriole and restrict centriolar microtubule growth in mammals (49, 55); as such, the removal of CP110 is necessary for formation of the ciliary axoneme (see the section titled Cilium Growth and Maintenance) (49, 56, 57).

CENTRIOLE COPY NUMBER CONTROL

In proliferating cells, centrioles duplicate every cell cycle by forming one new procentriole adjacent to each existing parent centriole. Recent years have seen an explosion of interest in understanding how cells maintain centriole copy number through successive cell cycles. In the sections that follow, we discuss three conceptually distinct levels of control that are required to maintain centriole homeostasis.

Spatial Control: Build Locally

During canonical centriole biogenesis, centriole formation is spatially restricted to a site close to existing centrioles. This spatial control is dictated by the preferential recruitment of PLK4 to the wall of the parent centriole by its centriole receptors CEP152 and CEP192. PCM at the proximal end of the parent centriole also provides a favorable environment for centriole assembly (58). Centrioles thus catalyze their own assembly by recruiting and locally regulating key factors required for centriole duplication.

In addition to canonical centriole biogenesis, centrioles can also form in the absence of preexisting centrioles in a process known as de novo centriole biogenesis. One example in which this occurs is mouse embryos, where cell divisions are initially acentriolar, and centrioles are created de novo at the 64 cell stage (59). Importantly, de novo centriole formation is suppressed by the presence of even a single preexisting centriole, ensuring that canonical biogenesis takes precedence over de novo formation (60). In mammalian somatic cells, de novo formation of centrioles occurs if centrioles are experimentally depleted, but the process is error-prone, resulting in the generation of a variable number of centrioles that often have an abnormal geometry (34, 60–63). Thus, spatially restricting centriole duplication to the parent centrioles helps ensure the structural integrity and numerical control of procentriole formation.

Numerical Control: Build Only One

A central feature of centriole copy number control in cycling cells is that each parent centriole forms exactly one new procentriole. This regulation depends upon finely tuned levels of the centriole duplication proteins PLK4, STIL, and SAS6. Overexpression of any of these three initiator proteins induces the simultaneous production of multiple procentrioles around one parent centriole (11, 64). Centriole duplication is particularly sensitive to alterations in the level of PLK4, and accordingly, PLK4 abundance is controlled by feedback regulation. PLK4 dimerizes through a cryptic polo box domain (17, 65, 66), and the dimeric kinase phosphorylates itself in *trans* within a phosphodegron (67–70). This autophosphorylation creates a binding site for the $SCF^{\beta\text{-}TrCP}$ ubiquitin ligase, which ubiquitinates and targets active PLK4 for proteasomal destruction, thus placing the stability of PLK4 under the control of its own activity (68).

The relocalization of PLK4 to a discrete locus on the wall of the parent centriole is thought to be critical for selecting a single site for procentriole assembly (13, 16, 19, 20). However, it remains unclear how PLK4 achieves this asymmetric localization and how the kinase escapes its own degradation when concentrated at this site. In one model, PLK4 is degraded en masse around the parent centriole but is stabilized at a single site through binding to its activator STIL (19). Because PLK4 can self-organize into supramolecular assemblies, it is also possible that these assemblies protect PLK4 from proteolysis at the site of procentriole formation (71, 72).

Ultimately, the transition of PLK4 from an initially symmetric localization on the parent centriole to a discrete site is a symmetry-breaking reaction that bears striking similarity to that

observed for budding yeast Cdc42 GTPase during bud site selection (73, 74). A recent biophysical model of the PLK4 symmetry-breaking reaction has shown that two cooperating feedback loops are required to establish a single PLK4 focus from an initially symmetric state (75). A future challenge, therefore, will be to identify the feedback loops that control PLK4 localization and/or activity. Importantly, the symmetry-breaking model assumes that the site of procentriole assembly is randomly selected on the parent centriole. However, it is worth noting that in algae and ciliates, in which basal bodies are anchored and each triplet microtubule blade can be readily distinguished, new basal body assembly occurs at a defined location on the parent (76–79). The molecular basis for this preferential assembly site remains unclear.

Temporal Control: Build Once Per Cell Cycle

In addition to spatial and numerical control, centriole biogenesis must also be licensed to ensure that duplication initiates only once per cell cycle. This is achieved through a centrosome-intrinsic block to reduplication, in which duplication of the parent centriole is prevented as long as the parent and procentriole remain tightly associated or engaged with each another (58, 80). The dissolution of this linkage following passage through mitosis is known as disengagement and licenses centrioles for a new round of duplication in the next cell cycle (**Figure 2**). The identity of the linker connecting the parent and procentriole and the molecular mechanism by which centriole duplication is inhibited remain unclear. One intriguing model postulates that the cartwheel of the procentriole acts to prevent the reduplication of the parent (81), although it remains to be determined how the parent centriole would read out the proximity of the cartwheel complex in the procentriole.

Centriole disengagement requires the activity of the kinase PLK1 and the protease separase (82, 83). Although separase is well known for cleaving the cohesin complex to initiate sister chromatid separation at anaphase, it has also been shown to cleave the PCM protein PCNT during mitosis. PCNT cleavage is required to license centrioles for duplication in the subsequent cell cycle (84, 85). One possibility is that PCNT cleavage alters PCM structure, allowing the procentriole to separate from its parent centriole and recruit its own PCM material in the following G_1 phase. Although separase activation is not essential for centriole disengagement, PLK1 activity is critical for this process (83). High levels of active PLK1 are sufficient to promote centriole disengagement and reduplication in interphase without passage through mitosis (86, 87). One likely target of PLK1 is PCNT, with phosphorylation of PCNT by PLK1 facilitating its separase-mediated cleavage and centriole separation (88). Additional PLK1 targets required for centriole disengagement await identification.

Once disengagement has occurred, the parent centriole is competent to reduplicate in the next cell cycle. However, a disengaged procentriole needs to acquire the ability to recruit PCM before duplication can be initiated, a process termed the centriole-to-centrosome conversion (89). In *Drosophila* embryos, centriole-to-centrosome conversion requires phosphorylation of SAS4 by CDK1 in mitosis; this phosphorylation generates a binding site for Plk1 that in turn recruits CEP152/Asl to license the procentriole for duplication in the next cell cycle (90, 91). Centriole-to-centrosome conversion also requires a conserved set of scaffolding proteins including CEP295 (Ana1 in *Drosophila*) (89, 92). CEP295/Ana1 is required to stabilize the new centriole after cartwheel removal in mitosis and is responsible for recruiting factors required for centriole duplication and PCM assembly, such as the PLK4 interacting proteins CEP152 and CEP192 (93, 94). *C. elegans* lacks a clear CEP295/Ana1 homolog, but the SAS-7 protein may play an analogous role by recruiting CEP192/SPD-2 and endowing new centrioles with competence for duplication (95).

A final aspect of temporal control is to ensure that centriole duplication, like DNA replication, is coordinated with cell cycle progression. Both centriole duplication and DNA replication initiate at the G_1–S phase transition and rely on the activation of cyclin-dependent kinases (CDKs) that drive cell cycle transitions. CDK2 is activated at the G_1–S phase transition and is required for centriole duplication in both *Xenopus* (96, 97) and mammalian cells (98, 99). Nevertheless, CDK2 knockout cells have normal centriole numbers, likely because CDK1 is able to compensate for loss of CDK2 activity (100). While CDK2 activity promotes centriole duplication at the G_1–S phase transition, CDK1 activity suppresses centriole duplication in mitosis by inhibiting the interaction of PLK4 with STIL (101). Several additional proteins with roles in DNA replication and chromosome segregation have also been proposed to play roles in centriole duplication, but indirect effects remain difficult to exclude.

Deuterosome: a protein structure formed in the cytoplasm of multiciliated cells that templates the formation of multiple procentrioles

Multiciliated Cells: Breaking the Rules

Although cycling cells construct exactly two new centrioles per cell cycle, centriole number can be modified in specialized cell types. For example, multiciliated epithelial cells that coat the airways, ventricles, and oviducts of vertebrates contain hundreds of motile cilia that drive extracellular fluid flow (102). In contrast to the strict control of centriole number observed in cycling cells, multiciliogenesis relies on the production of large numbers of centrioles that are converted into basal bodies and produce motile cilia. To achieve this feat, postmitotic multiciliated cells use specialized structures termed deuterosomes to rapidly amplify centriole content (103, 104). Deuterosomes comprise several proteins required for centriole duplication and can be nucleated by an existing centriole (105) or form spontaneously in the cytoplasm (106). Centrioles grow on the surface of deuterosomes until they reach their correct length, when they are released into the cytoplasm and dock at the plasma membrane. To promote the distinct stages of centriole biogenesis, multiciliated cells undergo biochemical changes that are similar to those that promote cell cycle transitions in proliferating cells but without DNA replication and commitment to mitosis (107, 108). Therefore, centriole amplification in multiciliated cells is controlled in a specialized cycle that bypasses the tight spatial and temporal controls on centriole biogenesis that operate in cycling cells.

Another example in which centriole number is modulated is the asymmetric inheritance of centrioles during fertilization. During sexual reproduction in most mammals, centrioles are eliminated from oocytes and contributed to the zygote by the sperm. The sperm-derived centriole pair then duplicates during zygotic S phase to provide the two centrosomes required for successful mitotic divisions. Centriole elimination in oocytes is thus critical to balance centriole number following fertilization. The elimination of centrioles in *Drosophila* oocytes is triggered by the downregulation of Polo kinase, which leads to PCM loss and subsequent centriole elimination (109, 110). Centrioles are also lost during muscle development, but in this case the mechanism and functional significance of centriole loss remain unclear (111, 112).

BUILDING THE CENTROSOME

To form a centrosome, the parent centriole recruits a matrix of PCM comprising several hundred proteins, including many that are required to nucleate or anchor microtubules (113, 114). Unlike many cellular organelles, the centrosome lacks a delimiting membrane, raising the question of how PCM assembly and size are controlled. The PCM was initially described by electron microscopy (EM) studies as an electron-dense and amorphous cloud. However, more recently, super-resolution imaging revealed that interphase PCM has an ordered structure with many proteins localizing to distinct toroidal layers that surround the proximal end of the parent centriole

(115–119). In addition, two proteins, CEP152 and PCNT, form elongated filaments with one terminus located close to the centriole wall and the other extending into the PCM (117–119). Such filaments may act as a scaffold that organizes the interphase PCM. It is worth noting that in some terminally differentiated cell types such as muscle, neurons, and epithelial cells, centrosomes no longer function as the dominant microtubule-organizing centers, and centrioles organize very little PCM material.

Following mitotic entry, the interphase PCM rapidly increases in size to support the robust microtubule nucleation needed for mitotic spindle assembly. This PCM expansion, or centrosome maturation, is dependent on the activity of PLK1 (Polo in *Drosophila*). PLK1 phosphorylates multiple proteins including the PCM components PCNT, CDK5RAP2 (Cnn in *Drosophila*), and CEP192 (Spd-2 in *Drosophila*), which are thought to form an underlying mitotic PCM scaffold (120–124). Importantly, PCNT and CDK5RAP2 also directly bind and recruit γ-tubulin ring complexes (γTuRCs) that nucleate centrosomal microtubules, whereas CEP192 recruits γTuRCs through the adapter NEDD1 (125–127) (**Figure 1*a***).

In contrast to the ordered interphase PCM, the mitotic PCM appears to form a more disordered, gel-like scaffold. Mitotic PCM assembly is best understood in *Drosophila* and *C. elegans*. In *Drosophila* embryos, Cnn is recruited in a Spd-2–dependent manner to the parent centriole, where it is phosphorylated by Polo to promote multimerization and scaffold assembly (115, 128). Phosphorylated Cnn fluxes outward from the parent centriole along centrosomal microtubules (122, 129). The outward spread of Cnn separates it from the source of Polo/PLK1 activity at the parent centriole, favoring dephosphorylation and thereby limiting scaffold assembly. Whether the flux of PCM scaffolding proteins is a general pathway to control PCM size remains unclear: Outward flux of Cnn is not observed in *Drosophila* somatic cells, and in *C. elegans*, SPD-5 (130), the functional ortholog of Cnn, incorporates isotropically into the PCM (131).

Similar to *Drosophila*, the assembly of the mitotic PCM in *C. elegans* requires SPD-5 phosphorylation by PLK-1 (123). Like Cnn, SPD-5 can assemble into supramolecular assemblies in vitro that are enhanced by the presence of SPD-2 and PLK-1 (123). Macromolecular crowding agents drive recombinant SPD-5 to phase separate into spherical, liquid-like condensates that rapidly harden into solid-like structures (132). The microtubule polymerase XMAP215 and the microtubule-stabilizing protein TPX2 can partition into SPD-5 condensates, where they concentrate tubulin and promote microtubule nucleation. These studies raise the question of whether the mitotic PCM forms through phase separation of components into condensates with liquid-like properties or, alternatively, assembles from well-ordered protein–protein interactions that form a gel-like or solid phase. One possibility is that the mitotic PCM starts as a liquid-like droplet around the parent centriole that then solidifies into a porous gel-like matrix (114).

CENTROSOMES IN CELL PROLIFERATION

In most mammalian cells, centrosomes nucleate the majority of the spindle microtubules during mitosis and increase the speed and efficiency of spindle assembly. However, additional microtubule-nucleation pathways also contribute to spindle formation and allow for cell division in the absence of centrosomes (133). Thus, centrosomes are not required for cell division per se, but they nonetheless are required for the continued proliferation of many mammalian cells. Cells lacking centrosomes activate a USP28–53BP1–P53 signaling axis that leads to either cell death or a cell cycle arrest (134–137). 53BP1 is a key regulator of DNA double-strand break repair that binds P53, whereas USP28 is a deubiquitinase that interacts with 53BP1. Although 53BP1 and USP28 have both been reported to play roles in DNA damage signaling, multiple lines of evidence have shown that growth arrest in response to centrosome loss is

mechanistically distinct from the DNA damage response (138). How centrosome loss is sensed by USP28 and 53BP1 remains to be determined. As USP28, 53BP1 and P53 are also required to arrest the cell cycle following a prolonged mitosis, one attractive possibility is that centrosome loss indirectly activates a USP28–53BP1–P53-mediated mitotic surveillance pathway by delaying mitosis (134–136).

Mitotic surveillance pathway: a USP28–53BP1–P53 signaling pathway that prevents the proliferation of unfit cells that undergo centrosome loss and/or delay in mitosis

There are likely to be tissue- and organism-specific differences in the function of the mitotic surveillance pathway. For instance, the mitotic surveillance pathway must be inactive in early mouse embryos, which proliferate in the absence of centrosomes until the 64-cell stage. Additionally, the mitotic surveillance pathway is not present in flies, in which centrosomes are only required for the rapid divisions of the syncytial embryo but are dispensable for cell divisions thereafter (139, 140). Future work is required to understand how the mitotic surveillance pathway is triggered and to establish its role in normal physiology and disease.

CENTROSOME DEFECTS IN DISEASE

Given the central role of centrosomes in diverse cellular processes, it is unsurprising that centrosome dysfunction has been linked to several human diseases. A wealth of data have shown that centrosome aberrations are commonly observed in human tumors and are often correlated with clinical aggressiveness (10, 141). Centrosome defects in tumors take the form of either numerical or structural alterations. Numerical alterations reflect increases in the number of centrosomes (known as centrosome amplification), whereas structural alterations encompass alterations in the shape and size of centrosomes. Although conceptually distinct, numerical and structural alterations in centrosomes frequently coexist in human cancers. A causal link between centrosome amplification and cancer recently emerged with the demonstration that extra centrosomes can trigger and/or accelerate tumorigenesis in mice (142–144). Exactly how centrosome aberrations contribute to tumorigenesis remains to be clarified. Supernumerary centrosomes can promote genomic instability by increasing the rates of chromosome missegregation and micronucleus formation (145–148), and consistently, the tumors that form in mice with extra centrosomes show dramatically altered karyotypes (143). In addition, the presence of supernumerary centrosomes can alter the interphase microtubule cytoskeleton to increase tumor cell migration and invasion (149). Similarly, structural defects in centrosomes have also been shown to increase the extrusion of individual mitotic cells from an epithelial layer, possibly providing a route for metastasis (150). Importantly, cellular extrusion is a non-cell-autonomous process that relies on the cooperation of cells within the epithelium. Thus, centrosome aberrations could contribute to metastasis without the disseminating cells themselves harboring centrosomal alterations.

In addition to a role in tumorigenesis, links between congenital centrosome defects and developmental disorders have been intensively studied. Primary autosomal microcephaly (MCPH) is a rare condition in which individuals are born with a brain that is considerably smaller than normal (151). MCPH is caused by a depletion of the neural progenitor cell (NPC) pool during embryonic development, resulting in the production of fewer mature neurons. Surprisingly, more than half of the known MCPH genes encode proteins that localize to the centrosome and play important roles in centriole biogenesis. It remains to be understood why mutations in ubiquitously expressed centrosome proteins specifically impair brain development. One intriguing possibility is that centrosome defects delay mitosis and lead to pathological activation of the mitotic surveillance pathway in NPCs (10). Indeed, an increase in the length of NPC mitosis has been observed in several mouse models of centrosome-associated microcephaly, and P53 deletion is able to rescue cell death and reduced brain size in these mice (152–154). In this model, the tissue specificity could be explained if NPCs have a lower threshold for activation of the mitotic surveillance pathway

compared with other cell types (155). How cells measure mitotic duration and what sets the sensitivity of this response are important questions for future exploration.

THE CILIUM: A CENTRIOLE-DEPENDENT ORGANELLE

In most mammalian cells, the mature parent centriole templates the formation of a cilium that protrudes from the cell surface. Cilia are typically ~350 nm in diameter and 1–10 μm in length, and like centrosomes, they lack a delimiting membrane. As a result, the ciliary lumen is continuous with the cytosol, and the ciliary membrane is continuous with the plasma membrane (**Figure 3**). Nonetheless, cilia maintain a unique complement of biomolecules through the combined action of dedicated trafficking machineries and diffusional barriers at the cilium base (156–158). A region

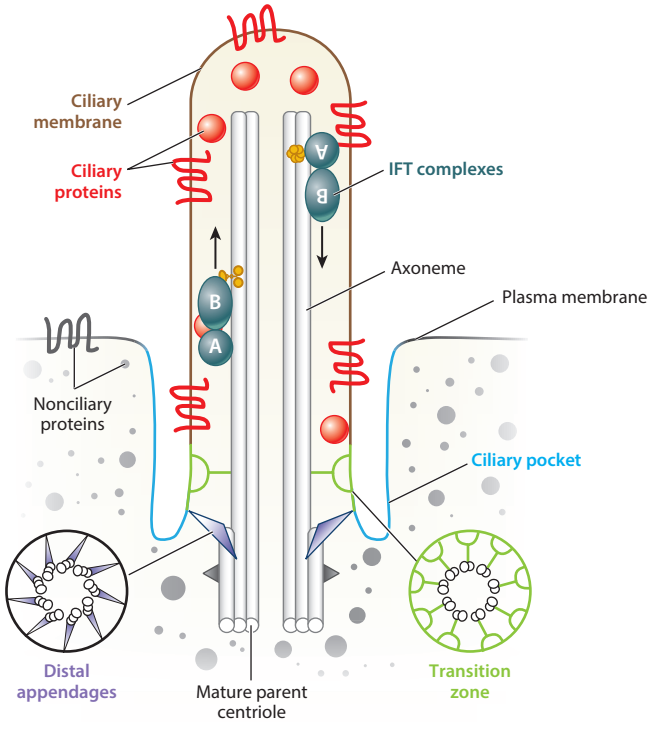

Figure 3

Primary cilium structure. Architecture of a mammalian primary cilium, highlighting key structural features. The axonemal microtubules form the core of the cilium and extend from the mature parent centriole, which is docked at the plasma membrane. This docking is mediated by the mature parent centriole's distal appendages and often occurs at a site on the cell surface where the plasma membrane is invaginated. This invaginated region of the plasma membrane adjacent to the cilium is known as the ciliary pocket and is a key site for exo/endocytosis of ciliary materials. Although the cilium lacks a delimiting membrane, it contains a distinct complement of soluble and membrane proteins. This compartmentalization is enabled by diffusion barriers near the base of the cilium at a region known as the transition zone. The transition zone is made up of several functional and physical modules, including MKS and NPHP proteins, which are mutated in Meckel syndrome and nephronophthisis, respectively. Selective trafficking of proteins to cilia across the transition zone is mediated by trafficking machineries, such as intraflagellar transport complexes A and B (IFT-A and IFT-B), that cooperate with ciliary kinesin and dynein motors. Additionally, IFT-A and IFT-B mediate protein transport within cilia along the axonemal microtubules and are required for ciliogenesis.

of particular importance for compartmentalization of the cilium is the transition zone, a proximal domain of the cilium where linkers tether the axonemal microtubules to the surrounding ciliary membrane (157, 159) (**Figure 3**). Additionally, the cilium often lies within a ciliary pocket formed by invagination of the plasma membrane adjacent to the ciliary membrane (160).

Cilia were likely present in the last eukaryotic common ancestor and are found today in a diverse array of organisms ranging from single-celled protists to vertebrates (161). Many of the structural features of cilia and the genes required for their function are highly conserved. Despite these commonalities, cilia in different cell types and organisms exhibit considerable diversity in their axonemal structure, length, morphology, and function. Here, we focus on nonmotile primary cilia, as they are widespread in vertebrates and exhibit many of the essential features of cilia. For those interested in the specialized features and functions of motile cilia, we refer the reader to recent review articles (102, 162, 163).

The assembly of primary cilia is a tightly regulated, multistep process that is strictly dependent on the mature parent centriole. The nine doublet microtubules of the ciliary axoneme are formed through elongation of the A and B tubules of the parent centriole (**Figure 3**). Additionally, the centriolar distal appendages form the interface that connects the centriole to the nascent ciliary membrane and anchors it to the cell surface when a mature cilium has formed (157, 159). These essential roles of the mature parent centriole have several critical consequences. First, because each cell has only one mature parent centriole, cells are limited to generating a single cilium (except in specialized cases, e.g., multiciliated cells). Second, the position within the cell of the mature parent centriole and the base of the cilium are by necessity coupled. Finally, centrioles must live dual lives, acting both as basal bodies that anchor primary cilia and as key components of centrosomal microtubule-organizing centers. A dichotomy between these functions is evident in the regulation of cilia: Across many species, the cilium must be disassembled prior to mitosis so that the mature centriole can help organize the mitotic spindle (164–166). Indeed, mammalian primary cilia are predominantly found on cells in the G_0 or G_1 phases of the cell cycle, and ciliogenesis is commonly initiated for cells in culture by withdrawal of serum growth factors (**Figure 2**). Thus, the cell cycle and coordinately regulated events in centriole duplication and maturation are intimately linked to the cilium assembly and disassembly programs.

Transition zone: a domain at the base of the cilium that links the axoneme to the ciliary membrane and that controls protein entry and exit from cilia

Ciliary pocket: an invaginated plasma membrane domain found adjacent to some cilia that may participate in membrane trafficking to and from the cilium

Ciliary vesicle: a vesicle associated with the mature parent centriole during ciliogenesis; it is the precursor to the ciliary membrane

PATHWAYS FOR INITIATING CILIOGENESIS

Foundational studies on ciliogenesis have revealed two principal pathways for cilium assembly in vertebrate cells (167, 168). One, a so-called extracellular pathway, is characterized by the migration and docking of the mature parent centriole to the plasma membrane via the centriolar distal appendages (**Figure 4**). After centriole docking, the axonemal microtubules extend, the transition zone forms, and ciliary trafficking machineries such as intraflagellar transport (IFT) complexes A and B deliver material to the growing cilium. The second, an intracellular pathway, shares many commonalities with the extracellular pathway but instead begins with the recruitment, docking, and fusion of vesicles at the distal appendages of the mature parent centriole (**Figure 4**). The resulting ciliary vesicle is then deformed as the axonemal microtubules extend and the transition zone forms, giving rise to a nascent ciliary structure that is entirely inside the cell. Finally, fusion of the ciliary vesicle with the plasma membrane leads to external exposure of a mature primary cilium. In this pathway, the outer region of the ciliary vesicle gives rise to the ciliary pocket adjacent to the ciliary membrane.

Recent studies have provided an increasingly detailed view of the sequence of events and proteins needed for ciliogenesis. In the intracellular pathway, cilium formation begins with the trafficking and capture of vesicles at the mature parent centriole. Vesicles are first transported to the

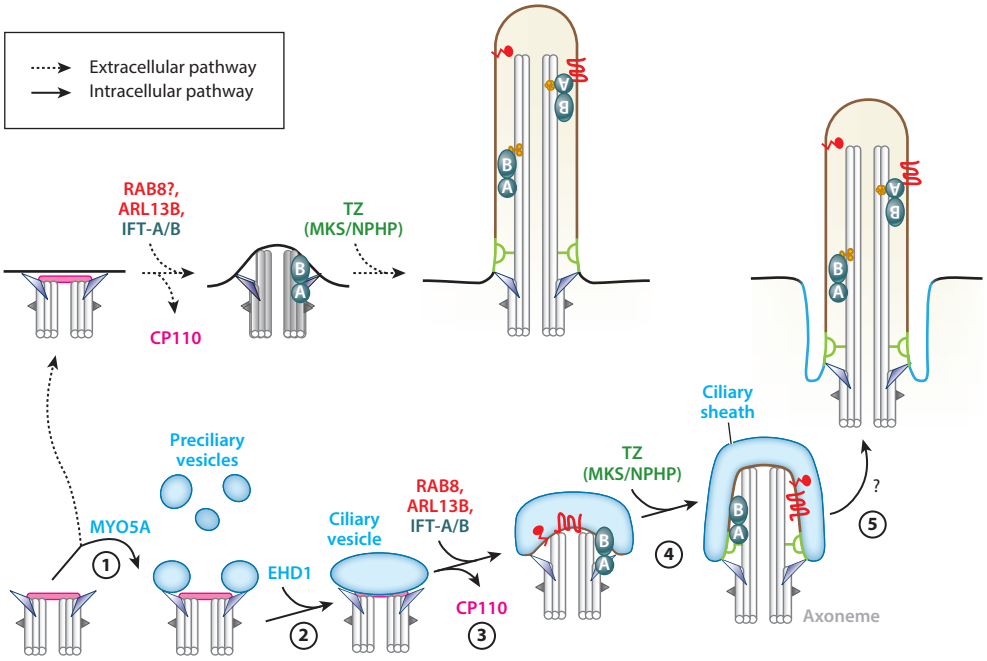

Figure 4

Pathways for primary cilium assembly. The mature parent centriole (*bottom left*) serves as the foundation for primary cilium assembly via either an extracellular pathway (*top*, *dashed arrows*) or an intracellular pathway (*bottom*, *solid arrows*). In the intracellular pathway, key steps include (①) MYO5A-dependent recruitment of preciliary vesicles to the distal appendages; (②) EHD1-mediated fusion of these vesicles to form an enlarged ciliary vesicle; (③) the growth of the ciliary vesicle via the joint action of RAB8, ARL13B, and the intraflagellar transport (IFT) complexes, a process that occurs in conjunction with removal of the CP110 cap from the distal end of the fully mature centriole; (④) the growth of the axoneme, formation of the transition zone (TZ), and maturation of the ciliary vesicle into distinct domains corresponding to the ciliary sheath and the nascent ciliary membrane; and (⑤) the fusion of the ciliary sheath with the ciliary membrane, which exposes the cilium to the external environment. In the extracellular pathway, a key distinction is that the mature parent centriole initially migrates to the cell surface and docks to the plasma membrane via its distal appendages. Subsequent steps appear to occur in a similar fashion as the intracellular pathway, although the precise sequence of events and molecular requirements are not fully known.

centriole through the sequential action of dynein and myosin MYO5A (169). These preciliary vesicles associate with the distal appendages and then fuse to form a larger ciliary vesicle in a manner that depends on the EHD family of membrane-tubulating proteins (169, 170). MYO5A and EHDs are among the first factors to be recruited to the mature parent centriole during ciliogenesis and localize to the subdomain of the growing ciliary vesicle that gives rise to the ciliary pocket. Soon after MYO5A and EHDs are recruited, several other proteins that are enriched in the ciliary membrane, including the small GTPase ARL13B and components of the RAB8–RAB11 GTPase cascade, can be detected at the ciliary vesicle (169, 170). In this cascade, RAB11 recruits its effector RABIN8, which then serves as the guanine nucleotide exchange factor (GEF) that activates RAB8 (171). RAB8 and ARL13B in turn promote the growth of the ciliary membrane and the selective trafficking of ciliary proteins to the cilium (172). Following growth of the nascent cilium, the ciliary vesicle fuses with the plasma membrane to give rise to a surface-exposed cilium. At present, the proteins required for this fusion event are not known.

These initial events differ significantly in the extracellular ciliogenesis pathway. In this case, the mature parent centriole does not capture vesicles in the cytoplasm but instead migrates to

the cell surface and docks to the plasma membrane. This migration is oriented toward the apical side of polarized epithelial cells and is promoted by the distal appendage protein CEP164 and by the microtubule and actin cytoskeletons (173, 174). In particular, actin is cleared from the region of the apical membrane where the centriole docks (175), perhaps explaining why actin inhibitors can promote ciliogenesis (176). After plasma membrane docking, the axoneme extends and the transition zone forms (see the section titled Cilium Growth and Maintenance). These latter processes appear to occur in a similar fashion for the intracellular and extracellular pathways.

What determines whether specific cell types utilize the intracellular or extracellular pathway for ciliogenesis, and does the choice of pathway dictate different molecular requirements for ciliogenesis or different functional properties for the mature cilia? The answers to these questions are not well understood, but the mode of ciliogenesis appears to be a characteristic feature of particular cell and tissue types. For example, fibroblasts and retinal pigment epithelium (RPE1) cells predominantly use the intracellular pathway, whereas polarized epithelial cells typically use the extracellular pathway (167, 168, 177). Moreover, because the process of ciliogenesis is often linked to the ultimate position of the cilium, there may well be functional implications for the mature cilia. For example, in epithelial cells that use the extracellular pathway, the cilium is typically positioned apically, with almost the entire length of the cilium protruding from the cell (168). These cilia are therefore ideally positioned to sense extracellular fluid flow. By contrast, cells that use the intracellular ciliogenesis pathway typically maintain the basal body near the nucleus and Golgi apparatus, deep within the cell (9, 167, 177). These submerged cilia are often associated with a pronounced ciliary pocket and may barely protrude into the extracellular environment, making them poor sensors of fluid flow (160). A recent study tested this idea, finding that conversion of submerged cilia into surfaced cilia promotes flow sensing but dysregulates ciliary Hedgehog (Hh) signaling (9). It will be interesting to further examine how cilium positioning is regulated by factors including cell shape and contractility (173) and how this feature of cilia influences their functional properties.

A second area for future study is how the composition of the ciliary vesicle is specified during intracellular ciliogenesis. It is noteworthy that RAB8, ARL13B, and SMO-RFP are present in the ciliary vesicle soon after its formation (169, 170). The rapid enrichment of cilium-specific proteins in the ciliary vesicle highlights the need to identify the origin of the vesicles that give rise to the ciliary membrane and how their cargos are specified. Further work is also required to understand how the association of proteins with the ciliary membrane is dynamically regulated during ciliogenesis. For example, although some early ciliary markers such as ARL13B remain at the ciliary membrane through the completion of ciliogenesis, others such as RAB8 and MYO5A are typically absent from mature cilia (169, 178). An additional feature of *RAB8* and *MYO5A* that warrants further study is the discrepancy between the phenotypes associated with their disruption in vitro versus in vivo: Knockdown or knockout of these genes in cultured cells blocks ciliogenesis at an early stage (169, 170), whereas mouse mutants do not exhibit overt ciliary defects (179, 180).

CILIUM GROWTH AND MAINTENANCE

After the initial membrane association of the mature parent centriole, the axoneme elongates, the ciliary membrane grows, and the transition zone forms. These events begin with the removal of CP110 from the distal end of the mature parent centriole, which allows the centriolar microtubules to extend and form the axoneme (55). CP110 removal is driven by TTBK2, a kinase which binds to distal appendage protein CEP164 and phosphorylates substrates including CEP164 and the kinesin KIF2A (181–183). At approximately the same time as CP110 removal, the IFT machinery is recruited to the distal appendages and mediates trafficking of ciliary axonemal precursors such as tubulin in conjunction with the ciliary motors kinesin-II and dynein-2 (170). IFT proteins

are organized into two large protein complexes, IFT-A and IFT-B, that have conserved roles in ciliary trafficking and are universally required for cilium assembly (158, 184) (**Figure 3**). IFT-B is thought to primarily associate with kinesin and mediate anterograde (base-to-tip) movement along axonemal microtubules, whereas IFT-A may primarily associate with dynein and mediate retrograde (tip-to-base) movement. However, recent studies have revealed additional complexities in ciliary trafficking, with IFT-A also promoting ciliary entry of some membrane proteins (172, 185). Additional information on how ciliary trafficking is mediated by IFT-A, IFT-B, and a protein complex known as the BBSome was provided in recent reviews (158, 172, 184, 186).

Shortly after IFT complex recruitment to the mature parent centriole, the transition zone that partitions the cilium from the cell body begins to form. The transition zone contains Y-shaped linkers that tether the axonemal microtubules to the ciliary membrane. The outer face of the ciliary membrane also exhibits a periodic series of particles termed the ciliary necklace (187). Although the precise protein components that correspond to these structures are unknown, many proteins localize to the transition zone and are organized into physical and functional modules (159, 188, 189). Together, these components are required to form a barrier that limits diffusional exchange of proteins between the cilium and the cell body, and transition zone defects impair ciliogenesis (190, 191).

After these early events in ciliogenesis, the cilium grows to a steady-state length and is maintained by the ongoing trafficking of components to and from cilia. How the length of cilia is determined after ciliogenesis and homeostatically maintained in mature cilia is an area of ongoing investigation (110, 192, 193). One area of the cilium that is likely to harbor structural elements and regulatory factors that control cilium length is the distal tip of the cilium. In particular, delivery of IFT cargos, incorporation/turnover of axonemal building blocks, and ectocytosis from the ciliary membrane are all processes occurring at the cilium tip that influence cilium length (194–198). A dynamic balance of these events is likely required for cilium homeostasis.

MECHANISMS OF CILIUM DISASSEMBLY

It has long been recognized that cilia are disassembled when cells progress through the cell cycle or upon differentiation of certain cell types. A number of different models have been proposed for how this disassembly is achieved, including excision of all or some of the protruding cilium, retraction of the axoneme into the cell body, and progressive shortening of the cilium followed either by undocking of the basal body from the plasma membrane or by endocytosis-like resorption of the final ciliary remnant. Critically, each of these models implies a different sequence of events and associated set of enzymatic activities needed for disassembly. Work in different experimental systems has generated support for several of these disparate models. For example, *Chlamydomonas reinhardtii* cilia appear to undergo excision at the base, promoted in part by the microtubule severing activity of katanin (166, 199), whereas chytrid fungi retract the axoneme into the cell through a reeling-type mechanism while discarding the ciliary membrane (200). In mammalian cells, different modes of disassembly have been observed in different experimental systems. In cultured mouse cells, a decapitation event near the tip or excision near the base may be a key step in cilium disassembly (197, 201). During chick neurogenesis, a similar disassembly process has been observed in which the apical, cilium-containing portion of the cell undergoes actomyosin-dependent abscission. However, in this case cilium shortening and basal body dissociation from the membrane precede abscission (202, 203). In another variation on these events, others have observed progressive shortening of cilia followed by endocytosis of the ciliary membrane remnant (204, 205). Notably, this membrane remnant can remain associated with the mature parent centriole throughout mitosis, and the daughter cell inheriting this centriole is able to more rapidly

reassemble a signaling-competent cilium after mitosis (204). Thus, by controlling the timing of ciliary signaling, the mechanism of cilium disassembly may contribute to asymmetric cell fates after cell division.

Mirroring the variety of pathways for cilium disassembly, a number of distinct disassembly factors have also been identified. These can be divided into proteins that serve as mediators of disassembly (discussed here) versus proteins that regulate initiation of disassembly or that suppress aberrant cilium assembly (discussed in the next section). Factors that directly participate in cilium disassembly include microtubule-modifying enzymes such as katanin, depolymerizing kinesins, and the HDAC6 tubulin deacetylase (56, 166, 206–209). These proteins likely contribute to axoneme disassembly, whereas regulators of the actin cytoskeleton such as phosphoinositide lipids, CDC42, and myosin may promote scission of the ciliary membrane (197, 202, 205, 210). These actin-associated proteins likely work in conjunction with clathrin, dynamin, and RAB5 to promote endocytosis of disassembling cilia (205). Given that dynamic remodeling of the cilium is needed for its disassembly, it is not surprising that ubiquitin–proteasome system components and ciliary trafficking regulators also participate in cilium disassembly (211, 212). Specifically, the IFT complexes and dynein regulators DYNLT1, NDE1, and NDEL1 have been shown to promote cilium disassembly (213–215). Lastly, centrosomal proteins such as TCHP, an Aurora A kinase regulator, and CPAP promote cilium disassembly by poorly defined means (216, 217).

REGULATION OF CILIUM ASSEMBLY AND DISASSEMBLY

Owing to the importance of ciliary signaling and the dual role of centrioles in the formation of basal bodies and centrosomes, the assembly and disassembly of cilia are tightly regulated processes. Longstanding observations that cilia are disassembled before mitosis and reassembled after mitotic exit or upon mitogen deprivation indicate that the cell cycle is a central regulator (**Figure 2**). But how do specific events in the cell cycle control the activity of cilium assembly/disassembly factors? And conversely, how does cilium assembly/disassembly regulate cell cycle progression? These are key questions for future research, but it appears that mitogens such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) both suppress ciliogenesis and activate disassembly (164, 218–220). These signals appear to converge on kinases, including PLK1, NEK2, and Aurora A, that stimulate depolymerizing kinesins, HDAC6, and other effectors (206, 209, 221, 222). Notably, these pathways appear to have conserved roles in cilium disassembly, with *C. reinhardtii* orthologs of AURKA and NEK2 also regulating cilium disassembly (223, 224). Furthermore, in both *C. reinhardtii* and mammalian cells, inhibition of cilium disassembly leads to a block in cell cycle progression. This block is specifically due to cilium maintenance, as it can be bypassed by disrupting ciliogenesis genes (166, 209, 213, 214, 216). Elucidating how cilium disassembly exerts this checkpoint-like regulation of the cell cycle is a key area for future study, as the mechanism appears to be distinct from that of other cell cycle checkpoints (213, 214, 216). It furthermore remains unknown how the timing of cilium disassembly is controlled, as disassembly has been reported to occur at cell cycle stages ranging from the G_0-G_1 transition to immediately before mitosis (164, 206, 225).

One promising strategy to understand regulation of cilium assembly/disassembly is to examine ciliated versus nonciliated tissues and cell types. For example, although most cells in the body are ciliated, it has long been recognized that some cell types, such as cells of the immune lineage and the intestinal epithelium, lack cilia. Recently, Bangs et al. (226) examined cilia during mouse embryonic development and found that nearly all epiblast cells are ciliated at E8.0 (except cells in mitosis). In contrast, the extraembryonic cells of the visceral endoderm and trophectoderm lack cilia, in part owing to activity of the Aurora A and HDAC6 disassembly factors. Given that the

ciliated epiblast cells give rise to all cell types found in the adult, certain nonciliated cell lineages must selectively lose the ability to ciliate during development, although the underlying mechanisms are not known. Interestingly, in the case of nonciliated immune cells, a latent capacity for cilium assembly is suggested by the ability of some cell lines derived from B cells and T cells to form cilia at low rates in culture (227). Moreover, primary T cells can successfully carry out some key initial steps in ciliogenesis. For example, in activated T cells the mature centriole migrates to the plasma membrane and undergoes CEP164-dependent docking at the immune synapse, although an axoneme is not extended (228, 229). Parallels between the immune synapse and cilium are further supported by findings that ciliary proteins such as Unc119, Arl13b, and IFT complexes localize to the immune synapse and modulate T cell signaling (230–232). Unraveling these similarities and differences in detail is an exciting area for further research and may help reveal how cilium biogenesis is developmentally regulated.

Ciliopathy: a disease that is part of a group of human developmental disorders that are caused by cilium dysfunction

CILIA IN PHYSIOLOGY: SIGNALING, CILIOPATHIES, AND CANCER

The importance of understanding cilium assembly and disassembly is underscored by the vital roles of cilia in signaling (for a detailed discussion of how cilia enable signaling, see References 233–238). It is clear that cilia contribute to a wide range of signaling processes that control embryonic development, tissue homeostasis, and sensory signaling. Specifically, cilia have been shown to be essential for Hh pathway signaling, left-right symmetry breaking, phototransduction, and olfaction; mounting evidence also shows that cilia modulate the PDGF, mTor, Notch, TGF-β, and Wnt pathways. Similarly, a host of signaling receptors and effectors localize to cilia, including components of the Hh pathway, PDGFRα, energy-sensing kinases LKB1 and AMPK, multiple adenylyl cyclase isoforms, the polycystin-2 ion channel, and G protein–coupled receptors such as SSTR3, D1R, 5HT6, MC4R, olfactory receptors, and rhodopsin (233, 235–240). This role for cilia in signaling is widely conserved, with many examples of cilium-dependent signaling seen in diverse organisms.

Understanding how cilia regulate signaling remains a central challenge in the field. Current obstacles include the fact that the functional outputs of some putative ciliary signaling pathways are not well characterized, and in other cases, cilia appear to modulate signaling but not be strictly required for signaling to occur. However, even in the case of vertebrate Hh signaling, in which cilia are absolutely required for transcriptional output and all core pathway components localize to cilia, the precise role of cilia is not yet known (233). A particularly perplexing feature is that *Drosophila* Hh signaling does not depend on cilia, thereby indicating that the same basic set of signaling components can require cilia for signal transduction in one species but not in another (241). How then do cilia enable signaling? One possibility is that cilia promote signaling by confining signaling components in a small compartment that may have unique features with respect to second messenger content, membrane lipid composition, and ratio of surface area to volume (234, 238, 242, 243). Alternatively (or additionally), some ciliary components such as the IFT machinery may directly participate in signal transduction (244).

The many contexts in which cilia promote signaling are illustrated by a group of pediatric disorders caused by inherited ciliary defects. These diseases are collectively known as ciliopathies and include Joubert syndrome, Bardet-Biedl syndrome, Meckel-Gruber syndrome, short rib thoracic dysplasia, polycystic kidney disease, retinitis pigmentosa, and nephronophthisis (190, 191). In brief, ciliopathies are characterized by intellectual disability, retinal degeneration, anosmia, kidney cysts, skeletal and craniofacial malformations, obesity, and congenital heart defects (191, 245). Ciliopathy gene products include ciliary motors and trafficking complexes, transition zone components, and a host of other proteins needed for the assembly and function of cilia (190, 191).

The many characteristic symptoms of ciliopathies reflect the diverse tissues and signaling pathways regulated by cilia. In some cases, particular symptoms can be ascribed to specific signaling pathways, such as polydactyly and Hh signaling and retinal degeneration and phototransduction by rhodopsin (233, 246). However, the molecular basis of other symptoms awaits further characterization. Additionally, although the developmental roles of ciliary signaling are highlighted by ciliopathies, cilia may also play important but incompletely characterized roles in adult tissue homeostasis and aging (247).

In addition to roles in development, several lines of evidence have linked ciliary aberrations to cancer. First, altered ciliary signaling (e.g., in the Hh pathway) can drive tumorigenesis in basal cell carcinoma and medulloblastoma (248–250). Second, the finding that cilium disassembly regulates cell cycle progression suggests that dysregulated cilium assembly/disassembly may contribute to uncontrolled cell growth in cancer. Consistent with this possibility, many tumors lack cilia despite arising from ciliated tissues (251–255). Moreover, a recent study observed progressive loss of cilia as breast cancer cells became more aggressive, and restoring ciliogenesis to these cells by inhibiting a depolymerizing kinesin reduced tumor cell proliferation (209). Thus, loss of cilia may bypass a brake on the cell cycle and promote tumorigenesis. Additionally, cilium absence may reprogram cellular signaling in a manner that promotes tumor growth or survival (255, 256). Further study of cilium loss during tumorigenesis may therefore provide opportunities not only to understand how cilium biogenesis is regulated but also to evaluate the potential therapeutic benefit of targeting pathways controlling cilium assembly or disassembly.

NEW TOOLS FOR STUDYING CENTROSOMES AND CILIA

Several new technologies have recently emerged as powerful tools to study cilia and centrioles, led by prominent developments in the areas of functional genomics and proteomics. For example, the recent development of high-throughput screening using CRISPR-based gene disruption has made it possible to conduct genome-wide screens with unprecedented precision and sensitivity. A key success of initial CRISPR-based screens was the identification of genes that affect growth of cultured cells (257, 258). To do so, a pool of single-guide RNAs (sgRNAs) is introduced in bulk into a large number of cells (**Figure 5*a***). After a defined period of growth, essential genes are identified by the depletion of sgRNAs targeting these genes from the pool. Similarly, by applying a specific stress or perturbation, genes that participate in a biological process of interest can be identified. However, an initial challenge in applying these approaches to study cilia and centrioles was the need to identify conditions in which ciliary or centriolar functions specifically modulate growth (or otherwise confer an isolatable phenotype suitable for pooled screening). In the case of centriole biology, a key breakthrough was the elucidation of the mitotic surveillance pathway that stops proliferation in centriole-deficient cells (134–136). Here, functional screening for cells that escape growth arrest following centriole loss provided insight into a cellular process that had previously been poorly characterized.

To study ciliary signaling, a mouse fibroblast cell line was engineered in which cilium-dependent Hh signaling drives expression of a reporter gene that confers resistance to the antibiotic blasticidin (45). In this fashion, genes that affect ciliary Hh signaling were identified through their modulation of blasticidin resistance. Known ciliary proteins and ciliopathy genes were identified with high precision and sensitivity, while previously uncharacterized hits revealed new insights into cilia and ciliary disorders. Moreover, because the NIH-3T3 cell line used is deficient in the mitotic surveillance pathway, several centriolar genes were among the hits, as expected given the essential role of the basal body in ciliogenesis. Importantly, a similar screen used a GFP-based Hh reporter and fluorescence-activated cell sorting to isolate hits, illustrating the variety of means by

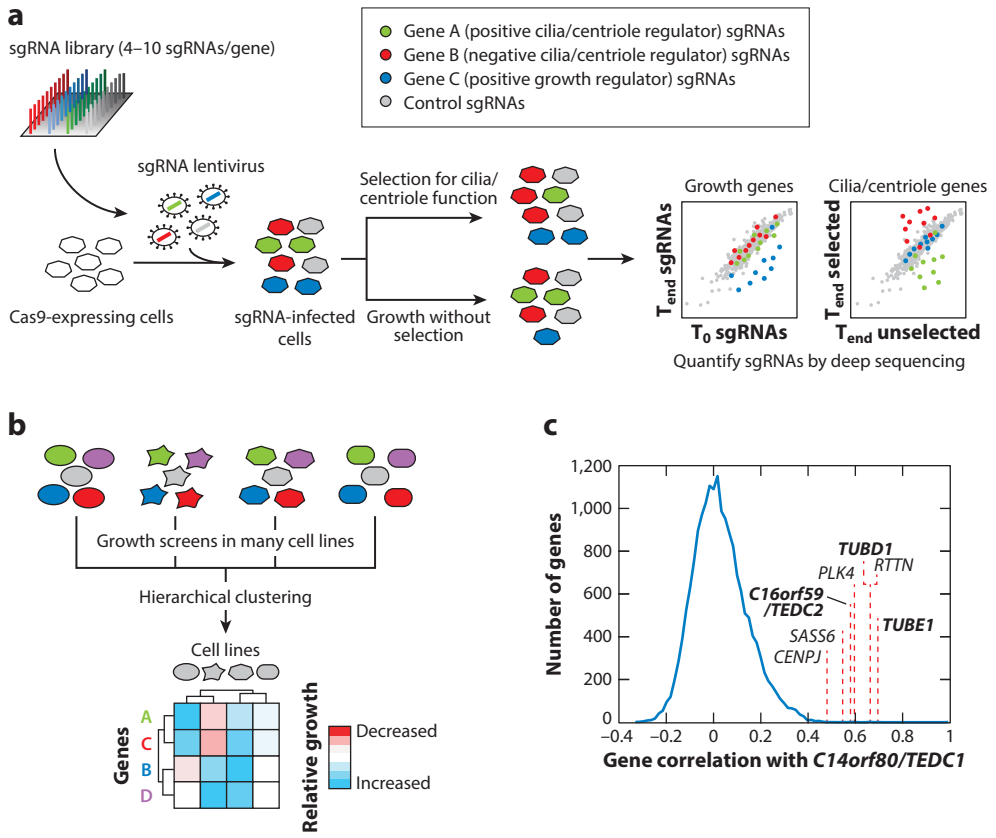

Figure 5

Application of functional screening to study cilia and centrioles. (*a*) Overview of pooled functional screening using CRISPR. A pool of single-guide RNAs (sgRNAs) is introduced into Cas9-expressing cells by lentiviral transduction. Transduced cells can then be grown under conditions that select for a functional property of centrioles or cilia or in the absence of such selection (note that the cells in question may need to be engineered such that centrioles/cilia control a selectable phenotype). Deep sequencing is then used to analyze the composition of sgRNAs present at the outset of the experiment (T_0 sgRNAs; e.g., the sgRNA library used to make lentiviral particles), in the unselected pool at the end of the experiment (T_{end} unselected), and in the selected pool at the end of the experiments (T_{end} selected). If sgRNAs targeting a particular gene are consistently depleted or enriched in the final selected sample relative to the final unselected sample, then the gene in question regulates centriole or cilium function. Similarly, changes in sgRNA abundance between the T_0 sample and the final unselected sample reveal genes that affect cell growth. (*b*) Schematic illustration of how growth phenotype screens conducted in different cell lines (indicated by cells of different shape) can be used to identify genes with shared function. Hierarchical clustering of growth phenotypes across all cell lines can identify genes having a shared function. (*c*) Several centriolar genes, including members of the TED complex, exhibit highly correlated patterns of growth phenotypes to that of *C14orf80/TEDC1* across 436 cell lines in the Achilles data set (Avana public 18Q2). The growth phenotypes for knockout of *C14orf80/TEDC1* were compared with those for all other genes in the data set, yielding the plotted distribution of correlation coefficients. Correlation values between *TEDC1* and other genes of interest are indicated, with TED complex components shown in bold font.

which screens can be tailored to investigate specific pathways or processes (259). Furthermore, by conducting screens under different conditions, it was possible to shift the focus of hit genes identified to specific functional categories, such as positive versus negative regulators of Hh signaling (259) or genes acting at a particular step in Hh signal transduction (45).

In contrast to these targeted screens, it is also possible to systematically probe diverse cellular processes through untargeted, growth-based screens. In particular, because such screens have now

been carried out in more than 400 cell lines that encompass diverse genetic and epigenetic states (260), it is commonly observed that a given gene's inactivation has variable effects on growth across cell lines (261–263). These context-dependent phenotypes are tightly linked to the gene's molecular function and thus can be viewed as a gene-specific functional signature. Systematic comparison of these signatures reveals genes with shared functions and can therefore be used to define the functions of uncharacterized genes (45, 261–263) (**Figure 5*b***). For example, in the Achilles Project collection of more than 400 CRISPR growth screens (260), many genes required for centriole duplication, such as *PLK4*, *SASS6*, *STIL*, *RTTN*, and *CENPJ*, exhibited highly correlated patterns of growth phenotypes. Unsupervised hierarchical clustering of these data revealed several clusters of functionally related centriole genes, including *CEP97* and *CCP110*; *CEP120*, *CEP44*, *HYLS1*, and *POC5*; and *CEP135* and *SPICE1* (45; D.K. Breslow & A.J. Holland, unpublished observations). Indeed, nearly all centriolar genes can be identified by analyzing CRISPR-based screens that were not focused on centriole biology, and functionally relevant subgroups can be defined. Further illustrating the value of these data, two uncharacterized hit genes from the Hh signaling screen, *C14orf80/TEDC1* and *C16orf59/TEDC2*, exhibited growth phenotype patterns that are highly correlated to each other as well as to those for genes encoding δ-tubulin, ε-tubulin, and several other centriolar proteins (45, 262) (**Figure 5*c***). This finding suggested a shared function for these genes, and indeed, TEDC1, TEDC2, δ-tubulin, and ε-tubulin form a protein complex required for centriole stability (45, 262).

In contrast to centriolar genes, most cilia-associated genes do not exhibit a distinct phenotypic signature, likely because their knockout had little effect on cell proliferation under the growth conditions and cell lines examined. Furthermore, this type of approach may miss proteins that have additional roles outside of cilium/centriole function that lead to distinct phenotypic signatures. Going forward, we anticipate that both highly targeted screens and large-scale growth data sets will provide complementary means to investigate the biology of cilia and centrioles.

In addition to these functional genomic approaches, new proteomic technologies have also been applied to cilia and centrioles. Shotgun proteomics of partially purified cilia and centrioles have provided important insights into the composition of these organelles (264–270). However, cilia and centrioles are not fully enclosed in the membrane, making it difficult to biochemically isolate them while ensuring that their contents remain stably associated (with the notable exception of organisms that can be induced to release their cilia intact, such as *C. reinhardtii*). This challenge is further compounded by the small size of cilia and centrioles and their low copy number per cell. Recently, proximity labeling has emerged as a proteomic approach that can overcome some of these obstacles. Proximity labeling takes advantage of enzymes that generate radical forms of biotin-containing compounds that, owing to their high reactivity and short half-lives, covalently react with and label nearby proteins (271, 272). Following in situ labeling, biotinylated proteins can be purified and analyzed by mass spectrometry. At present, the two enzymes most commonly used to generate biotinyl radicals are BirA-R118G (known as BioID) and variants of soybean ascorbate peroxidase named APEX or APEX2 (for further discussion, see References 271, 272).

One of the first applications of proximity labeling to cilia and centrioles was reported by Gupta et al. (273). In this study, BioID fusions were analyzed for a host of proteins that localize to the cilium–centriole interface, yielding an extensive proximity-based protein network containing known and novel components. In parallel, Mick et al. (239) and Kohli et al. (210) used APEX labeling to define a ciliary proteome. In both cases, the APEX enzyme was fused to cilia-targeted proteins that localize throughout the ciliary membrane. The labeling reactions therefore led to biotinylation of a range of known and novel ciliary proteins. These studies also investigated changes in ciliary proteome composition in mutant cells deficient in the IFT-B subunit IFT27 (239) and

in cells stimulated to disassemble their cilia (210). These examples illustrate how ciliary APEX labeling may be used to investigate how the ciliary proteome changes during dynamic cellular processes or in specific disease states. With ongoing improvements to proximity labeling methodology, ciliary and centriolar proteomics is likely to be a powerful complement to the functional screening approaches described above.

Finally, we note that advances in light and electron microscopy are also providing important new insights into the biology of cilia and flagella. Although a full discussion of such approaches is beyond the scope of this review, we note that super-resolution fluorescence microcopy methods are providing increasingly detailed molecular maps of ciliary and centriolar structures (118, 169, 170, 274–276). For example, 3D-STORM imaging has been used to generate a map of proteins that form the distal appendages and transition zone, identifying for the first time distinct functions and localizations for distal appendage blade versus distal appendage matrix proteins (274, 275). Additionally, EM approaches are revealing the in situ organization of cilia (277–281) and the elaborate structure of the centriole (4, 282). Given the nanometer scale of many key ciliary and centriolar structures, the continued application of these technologies is likely to be an important complement to genomic and proteomic approaches.

The past several years have seen tremendous advances in our understanding of cilium and centriole biology. The key steps in the biogenesis of cilia and centrioles have been defined, and many of the important proteins have been identified. As the molecular players are now largely known, a key challenge for the future is to define molecular mechanisms and to better understand the roles of cilia and centrioles in normal physiology and disease. Some critical questions that remain to be addressed are listed below. With the advent of new technologies and a growing interest in the biology of cilia and centrioles, we anticipate exciting new findings as answers to these questions emerge.

FUTURE ISSUES

1. Can cells sense the presence of cilia or centrioles, and if so, what are the underlying mechanisms?
2. How is centriole biogenesis restricted to a single new procentriole per parent centriole in each cell cycle?
3. Given differences in how cilia are assembled and disassembled in different cell types or organisms, which aspects of these processes are invariant and which exhibit plasticity?
4. How are centriole and cilium function regulated through transcriptional, translational, and posttranslational means and in different tissues and cell types?
5. What are the physiological consequences of dysregulated cilium disassembly?
6. How do centriolar and ciliary defects lead to the phenotypes observed in microcephaly and ciliopathies?
7. Can insights into centriole and cilium biogenesis be leveraged for therapeutic benefit?

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Errata

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