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Current Opinion in Cell Biology

Deconstructing the centriole: structure and number control

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Centrioles are very small microtubule-based organelles essential for centrosome, cilia and flagella assembly, which are involved in a variety of cellular and developmental processes. Although the centriole was first described almost a century ago, the knowledge on its assembly mechanism remains poor. In the past decade, forefront functional studies have provided important data on the different players involved in centriole biogenesis. Centriole research has now started to profit from highly sensitive structural, imaging, and biochemical techniques that are unveiling how those players contribute to assemble such a small and complex structure. We will review those studies and discuss how this field will increasingly benefit from the newborn and exciting era of super resolution analyses.

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Current Opinion in Cell Biology 2012, **24**:4–13

This review comes from a themed issue on
Cell structure and dynamics
Edited by Jason Swedlow and Gaudenz Danuser

Available online 8th February 2012

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2012.01.003

Introduction

Centrioles are microtubule-based structures that are essential for the formation of centrosomes, cilia, and flagella. As centrosomes, centrioles regulate diverse processes, from polarity in interphase to spindle-pole organization in mitosis, being the major microtubule-organizing center of animal cells (Figure 1). As basal bodies, centrioles anchor at the cell membrane and are fundamental for cilia and flagella assembly, organelles with important functions in physiology and development across eukaryotes [1•,2,3]. It is thus paramount that centrioles are generated with high structural fidelity and stringent number control. Indeed, abnormalities in centriole/centrosome structure/number and function are implicated in different diseases including microcephaly and cancer [3]. Therefore, understanding centriole biology and characterizing the different players involved in regulating centriole biogenesis is of utmost importance.

As a core structure, the centriole exists in most branches of the eukaryotic tree-of-life [1•,4•]. There is a phylogenetic correlation between the presence of centriole/basal body and the proteins involved in its assembly (see below). Yet, other proteins involved in centriole function appeared in a taxon-specific fashion or diverged as species-specific adaptations (e.g. Plk4/Sak, Cep192/Spd2, and CP110). Together, these data suggest that the same protein module is at the basis of centriole architecture conservation, even if assembly regulation and function can be cell context dependent [1•,4•]. Here, we will review recent data on centriole biogenesis and number control.

Centriole biogenesis

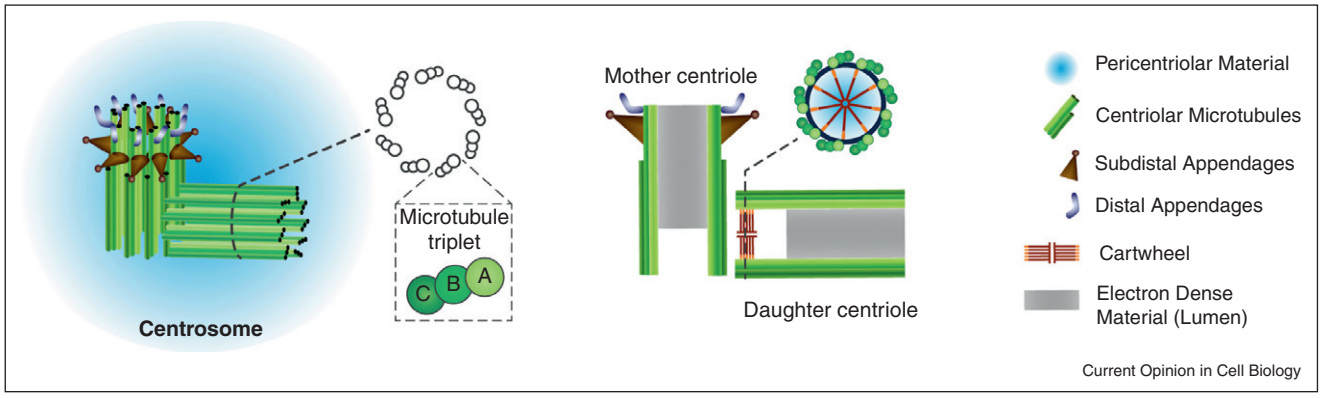
Centriole biogenesis is known to occur via two mechanisms. In the canonical pathway procentrioles form exclusively in association with a pre-existing centriole. Conversely, *de novo* centriole biogenesis occurs in the absence of pre-existing structures. While the canonical pathway is characteristic of most proliferating animal cells, the *de novo* pathway is activated in the absence of any centrioles in the cell or in cells that produce multiple cilia [2]. Although distinct, these mechanisms converge in using a common conserved protein set that triggers assembly and defines the centriolar structure ([1•,2,4•,5]; see below, and Figure 2).

The control of centriole assembly and number is tightly coordinated with the DNA replication cycle. At the end of mitosis/beginning of G1-phase, DNA strands are unwound for licensing factors to prime replication origins. Only after licensing is the replication machinery able to access and bind DNA and elicit replication during the following S-phase. Similarly, centriole disengagement in mitosis was suggested as the 'licensing' step for S-phase centriole biogenesis [6]. Both cycles rely on a temporal biphasic division where licensing and new structure synthesis are mutually exclusive. Additionally, several proteins (e.g. SCF complex, Separase and Plk1) have been proposed to have a role in both cycles ([7]; see below).

Centriole assembly onset

Centriole biogenesis obeys a hierarchical cascade of proteins and events, both poorly understood. Plk4 and Cep152 (Asl in *Drosophila*) are assembly onset triggers (Figure 2, Table 1). Both Plk4, a polo-like kinase family member, and Cep152/Asl are required for centriole biogenesis in *Drosophila* and human cells [2,8–11]. In

Figure 1



Structure of a vertebrate centrosome.
The centrosome is the major microtubule-organizing center of most animal cells. A centrosome (depicted on the left) is formed by two cylinder-shaped microtubule-based structures, the centrioles, which are surrounded by a protein matrix cloud, the pericentriolar material (PCM). Each centrosome is composed of a mature (mother) and an immature (daughter) centriole. While centrioles are initially built around a 9-fold symmetric scaffold, the cartwheel, only daughter centrioles maintain this internal feature at their proximal half in human cells (see longitudinal section of a centrosome, depicted on the right). The mother centriole has distal and subdistal appendages. Distal appendages are required for centrioles, as basal bodies, to anchor at the plasma membrane. Each human centriole is composed of microtubule blades in a triplet or doublet composition (see centriole cross-sections). Note that blades bind to the cartwheel through a single A-tubule, the only closed microtubule and the first to be assembled [20,21••,27]. Both B-tubules and C-tubules are incomplete microtubules, as they are fused to and polymerize along A-tubules and B-tubules, respectively (see main text).

Drosophila cells, Asl is required for Plk4 recruitment to the centriole and to bridge the interaction between Plk4 and CPAP/Sas4, another player in biogenesis, thus working as a scaffold for centriole assembly [9•]. Although these proteins are limiting factors for assembly onset, their time of action remains undetermined. It is feasible that the requirement for these triggers immediately precedes procentriole formation. Likewise, their activity can be essential during different cycle phases, for instance, to potentiate a favorable environment for accumulation of factors required for centriole assembly or as an activating priming mechanism for particular players.

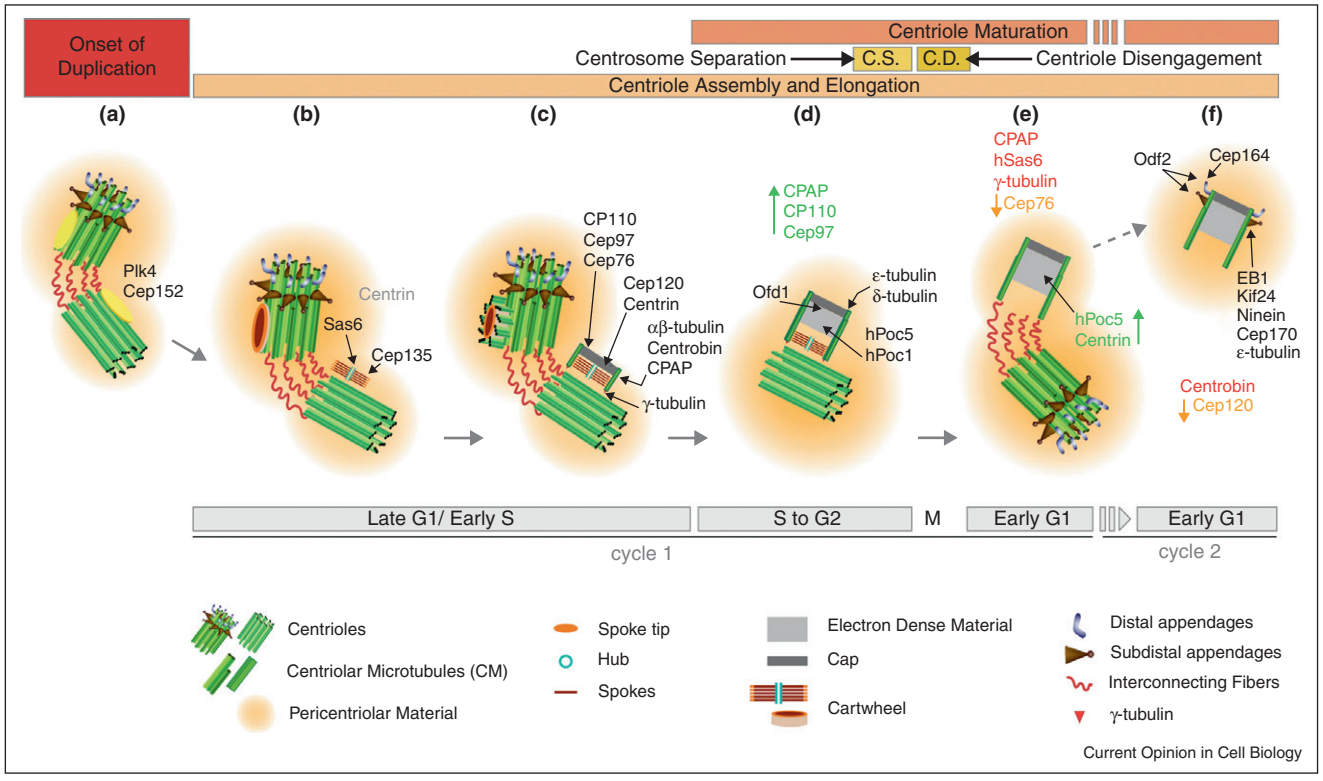
Building the cartwheel

The first visible sign of procentriole formation is the cartwheel (Figures 1 and 2). Originally described in *Paramecium* and *Chlamydomonas*, this structure can self-organize *in vitro* in the presence of basal body components [12], suggesting its building blocks have ninefold symmetry intrinsic properties. The cartwheel component Sas6 localizes to the cartwheel center, and in different organisms, Sas6 mutants either have defective centrioles (with abnormal/inexistent cartwheel) or lack them altogether [2]. Combined, these data revealed Sas6 is a key cartwheel assembly molecule. Recently, two cutting edge studies showed Sas6 oligomers are at the basis of the cartwheel conserved ninefold symmetry [13••,14••]. Using X-ray crystallography, analytical gel-filtrations, and *in silico* structural modeling, these studies demonstrated that Sas6 forms rod-shaped homodimers that oligomerize via the N-terminal domain. Sas6 homodimers come together at a defined angle to form a ring-like assembly from which nine spokes radiate, a composition that recapitulates cartwheel organization [13••,14••]. Sas5 in *C. elegans*, and its very divergent ortholog STIL1/Ana2 in *Drosophila*, are binding partners of Sas6, being required for both centriole biogenesis and for the ability of overexpressed Sas6 to generate hub-like structures [2,15,16•]. Along with Sas6, Bld10/Cep135 is another cartwheel component (Figures 2 and 3). While *Chlamydomonas* Bld10 mutants have no centrioles [17], *Drosophila* Bld10 seems dispensable in the early steps of assembly as mutants still assemble procentrioles, albeit shorter [1•,18]. Recently, studies in *Paramecium* showed Bld10 is required for Sas6 maintenance at mature basal bodies [19]. Together, Sas6 and Bld10 cooperate to establish and stabilize the cartwheel ninefold symmetry, respectively. The presence of some close-to-normal cartwheel-free centrioles in different mutant organisms suggests additional structures may also play a role in centriole architecture definition. It is possible that the PCM, microtubule blades, or even their connections, have self-assembly properties or create structural constraints that act in concert with cartwheel assembly to aid ninefold symmetry enforcement (Figures 1 and 2). Indeed, precisely configured microtubule singlets appear before the cartwheel in *Paramecium* [20].

Centriolar microtubule recruitment

Centriolar microtubules are extremely stable structures usually composed of microtubule triplet blades. Tomography studies have been particularly informative in understanding centriolar microtubule assembly. Blades (singlets, doublets or triplets) connect to the cartwheel

Figure 2



Building a human centriole: model of the centriole duplication cycle.
The centriole duplication cycle is a tightly ordered multistep event. Despite the existence of a myriad of centrosome-associated molecules [29], a core of only a few proteins was found to be conserved and indispensable for centriole biogenesis. Initially identified in *Caenorhabditis elegans*, Spd2, Sas6, Sas4, and Sas5 have orthologs in humans and *Drosophila melanogaster* (Cep192/Spd2, hSas6/dSas6, CPAP/dSas4, and Stil/Ana2, respectively). Although triggers of centriole biogenesis, Zyg1 (*C. elegans*) and Plk4/Sak (humans and *Drosophila*) kinases are functionally equivalent but not orthologs [1•,4•]. **(a)** While still temporarily undefined in the cell cycle, the onset of centriole assembly is triggered by Plk4 and Cep152/Asl. Spd2 is an additional duplication trigger in *C. elegans*, as it is required for the recruitment of Zyg1. The main function of the human and Drosophila orthologs (see above) is PCM recruitment [2]. **(b)** hSas6, Cep135 and centrin are among the first molecules to accumulate at the site of procentriole formation. SAS6 is involved in the assembly of the cartwheel, the first visible structure in the centriole cycle. **(c)** Centriole elongation phase starts almost simultaneously with, or right after, cartwheel assembly. γ-tubulin localizes to the procentriole proximal region to nucleate centriolar microtubules around the prebuilt cartwheel scaffold (see text and Figure 1 for more details). **(d)** Between S and G2 phases, daughter centriole elongation is fully active (see text for more details). While microtubule stabilization is further guaranteed by both ε-tubulin and γ-tubulin, control of centriole length relies on the counteracting activities of CPAP and CP110, as well as on Ofd1 and hPoc5. Likewise, Poc1 was described has having a role in centriole length control in human cells (see main text for details). Centrosome separation, a prerequisite for assembly of a bipolar spindle in mitosis, takes place in late G2-phase. During mitosis, centrioles disengage (loss of orthogonal configuration and separation of the two centrioles of a centrosome), an event dependent on Plk1 and separase [6,42•]. Centriole disengagement is required for the onset of duplication (see main text for details). **(e)** After mitosis, a fully mature centrosome is formed and an organizational shift of several proteins takes place as the cell cycle progresses. Particularly, CPAP, hSas6 and γ-tubulin delocalize/are degraded from the daughter centriole, while hPoc5 and centrin levels increase at its distal lumen. **(f)** A daughter centriole differentiates into a mother upon full maturation. Centriole maturation involves acquisition of PCM proteins, as well as development of distal and subdistal appendages. Centriole duplication and cell cycle stages are indicated at the top and bottom of the image, respectively. Key molecules and structural units are represented. Proteins represented in black indicate temporal and spatial localization during centriole assembly; proteins represented in red indicate moment of their displacement from the daughter centriole; proteins represented in green or orange indicate increasing or decreasing levels at the daughter centriole, respectively.

through the A-tubule (Figure 1). Purified human centrioles were used to show that A-tubules nucleate from a gamma-tubulin ring complex (γ-TuRC)-like structure [21••]. The authors suggested that the proximal γ-TuRC-like structure works as a cap, enforcing unidirectional growth of A-tubules. Accordingly, uncapped B-tubules and C-tubules assemble bidirectionally, using the A-tubules and B-tubules as elongation templates, respectively [21••]. A very important question is how the open B-tubules and C-tubules are assembled. While δ-tubulin and ε-tubulin are required for proper doublet and triplet formation in *Paramecium* and *Chlamydomonas*, these proteins are absent from *Drosophila*, suggesting other factors play an important role in defining those microtubules [2]. Recently, cryo-EM tomographic analysis of the *Chlamydomonas* basal body revealed longitudinal structural

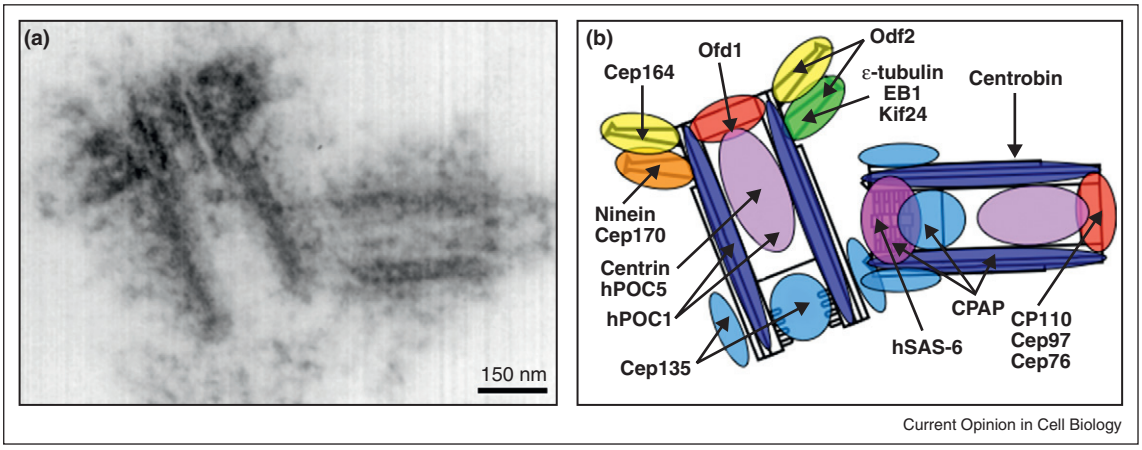
Table 1

Centrosomal proteins, localization and interaction partners

Centrosomal proteins	Homologs	Interaction partners	Centrosomal localization		
			PCM, mother, daughter procentriole	Proximal distal along	Region
Plk4/Sak	Plk4 (Dm), Zyg1 (Ce)	Cep152, CPAP, Sas6, FBXW5, β-TrCP/Slimb	M, D, Pr	P, A	Outer walls, lumen
Cep152	Asl (Dm), Cep152 (Dr)	CPAP, Plk4	M, D, PCM	P	Outer walls
Cep192	Spd2 (Ce, Dm)		M, D, PCM	A	Outer walls
hSas6	Sas6 (Ce, Dm, Dr, Tt, Pm), CrSas6/Bld12p (Cr)	Sas5, Ana2, Zyg1	Pr	P	Cartwheel (spokes, hub)
Cep135	Bld10 (Dm, Pm), Bld10p (Cr)	C-Nap1	M, D, Pr	P	Cartwheel, lumen, outer walls
Centrin	Cdc31 (Sc,Cp), CEN2/3 (Pt), CEN1 (Tt) VFL2 (Cr)	hPoc5, CP110	M, D, Pr	Di	Lumen
Cep120	Cep120 (Mm), Uni2 (Cr)	Ninein, Cep164, Cep290	M, D, Pr	A	Outer walls
CPAP	Sas4 (Ce, Dm)	γ-tubulin, αβ-tubulin, Cep152, Plk2, Plk4	M, D, Pr	P, A, Di	Cartwheel, lumen, walls
γ-tubulin	γ-tubulin (Dm, Pt), Gtu1 (Tt), Tbg1 (Ce), Tubg (Mm), Tug (Cr)	CDK5RAP2, CPAP, Cep170	M, D, Pr, PCM	P	Lumen
Centrobin	CG5690 (Dm), Cntrob (Mm), Nud1p (Sc), Cdc11p (Sp)	αβ-tubulin, Plk1, NEK2	D, Pr	P	Lumen, outer walls
CP110	CP110 (Dm)	Centrin, Cep97, Kif24 Cep76, Cep290,	M, D, Pr	Di	Cap
Cep97	Cep97 (Dm)	CP110, Cep76, Kif24	M, D, Pr	Di	Cap
Cep76	Cep76 (Xl, Dr)	CP110, Cep97, Kif24	M, D, Pr	Di	
ε-tubulin	Bld2 (Cr), Tube1 (Dr, Mm, Xl)	EB1	M, D, Pr	Di, A	Subdistal appendages, outer walls
δ-tubulin	Tubd (Dr, Mm, Xl), Uni3 (Cr), δPT1 (Pt)		M, D, Pr	P	Outer walls
hPoc5	Poc5 (Cr, Pm)	Centrin	M, D	Di, A	Lumen
hPoc1	Poc1 (Dm, Cr, Tt, Pm)	αβ-tubulin	M, D, Pr	Di, A	Lumen, walls
Ofd1	Ofd1 (Mm), BUG11 (Cr)	γ-tubulin	M, D, Pr	Di, A	Lumen
Odf2	Odf2 (Mm)	Ninein, Trichoplein	M	Di	All appendages
Cep164	XP_929307 (Mm), NP_611787 (Dm), XP_697015 (Dr)		M	Di	Distal appendages
Ninein	Nin (Dr, Mm)	γ-tubulin, Odf2, Trichoplein, EB1	M	Di	Subdistal appendages
EB1	Mal3 (Sp), Bim1 (Sc)	CDK5RAP2, FOP, Cep290, Cep170	M	Di	Subdistal appendages
Cep170	Cep170 (Mm)	Plk1, EB1	M	Di	Subdistal appendages
CAP350	Cep350 (Mm)	FOP	M, D	A	
FOP	Fgfr1op (Mm)	CAP350, EB1	M, D	A	Outer walls
Kif24	Kif24 (Mm)	CP110, Cep97	M	Di	Subdistal appendages
CDK5RAP2	Cnn (Dm), CDK5RAP2 (Mm)	Cdc20, PCNT, γ-tubulin, EB1	M, PCM	Di	Outer walls, appendages
C-Nap1	Cep250 (Mm)	Cep135	M, D	P	Linker
Plk1	Cdc5 (Sc), Plk1 (Dr, Mm), Plk1/2 (Ce), Plo1 (Sp), Polo (Dm)	Cep170	PCM		
Plk2	Plk2 (Dm, Mm), Plk2b (Dr)	CPAP	M, D		
β-TrCP	Slimb (Dm), β-TrCP (Mm)	SKP1, Plk4	Centrosome		
Cul1	Cul1 (Ce, Mm, Sp), Cul1a (Dr)	SKP1, SKP2, PPP1CA	M	Di	
Stil	Ana2 (Dm), Sas5 (Ce)	Sas6 (Ce, Dm)	M, D, BB	P, Di	

A list of human proteins involved in centriole duplication along with known homologs, interaction partners, and centriolar localization details. The criterion for homolog listing was to only include those for which functional data are available. Centrosomal interaction partners of the human proteins are shown, unless stated otherwise. Centriolar localization is categorized into three classes: (1) mother centriole (M), daughter centriole (D) or procentriole (Pr); (2) proximal region (P), distal region (Di) or along the centriole (A); and (3) detailed ultrastructural description. List of species abbreviations: Ce, *Caenorhabditis elegans*; Cr, *Chlamydomonas reinhardtii*; Dm, *Drosophila melanogaster*; Dr, *Danio rerio*; Mm, *Mus musculus*; Pt, *Paramecium tetraurelia*; Sc, *Schizosaccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Tt, *Tetrahymena thermophila*; Xl, *Xenopus laevis*. Empty spaces correspond to unknown information. Owing to space limitations, it is not possible to include the references of all the original papers used to obtain the data presented in this table. However, this information has been compiled in a number of publications [1•,3,59].

Figure 3



Ultrastructural localization of human centriolar markers in a fully mature centrosome.
(a) Electron micrograph of a longitudinal section of a centrosome isolated from human lymphoblastoma cells (KE37 cell line). Picture adapted from [58].
(b) Schematic representation of the picture shown in **(a)**. The localization of several centriolar markers, as determined through ultrastructural and immunofluorescence studies, is indicated (the information was gathered from the various reports referenced throughout this review). Note that owing to the differential localization of appendages, there are several proteins that are mother centriole specific (Cep164, Cep170, ε-tubulin, EB1, Kif24, Ninein and Odf2). Conversely, Sas6 only localizes to the daughter centriole of a fully mature centrosome, as it delocalizes (by displacement or degradation) from mother centrioles as they reach full maturation.

variations along its length, as well as uncharacterized non-tubulin structures and binding sites ([22••], Figure 4a). These data constitute a road map for elucidating the basal body/centriole assembly mechanism, in particular how its many components might fit into the structure.

Both CPAP/Sas4 and centrobin interact with tubulin and have been proposed to sequester tubulin dimers to promote their incorporation at centriolar microtubule plus ends [23–26]. CPAP/Sas4 is a conserved protein required for centriole assembly, which stably accumulates at pro-centrioles upon interaction with γ-tubulin, and that is required for microtubule recruitment [1•,2,27]. Centrobin is also required for centriole biogenesis, and disruption of centrobin–tubulin interaction destabilizes pre-existing centrioles, suggesting this interaction is necessary for centriole stability [26]. It remains unknown how these different proteins work together to enhance centriolar microtubule nucleation and growth.

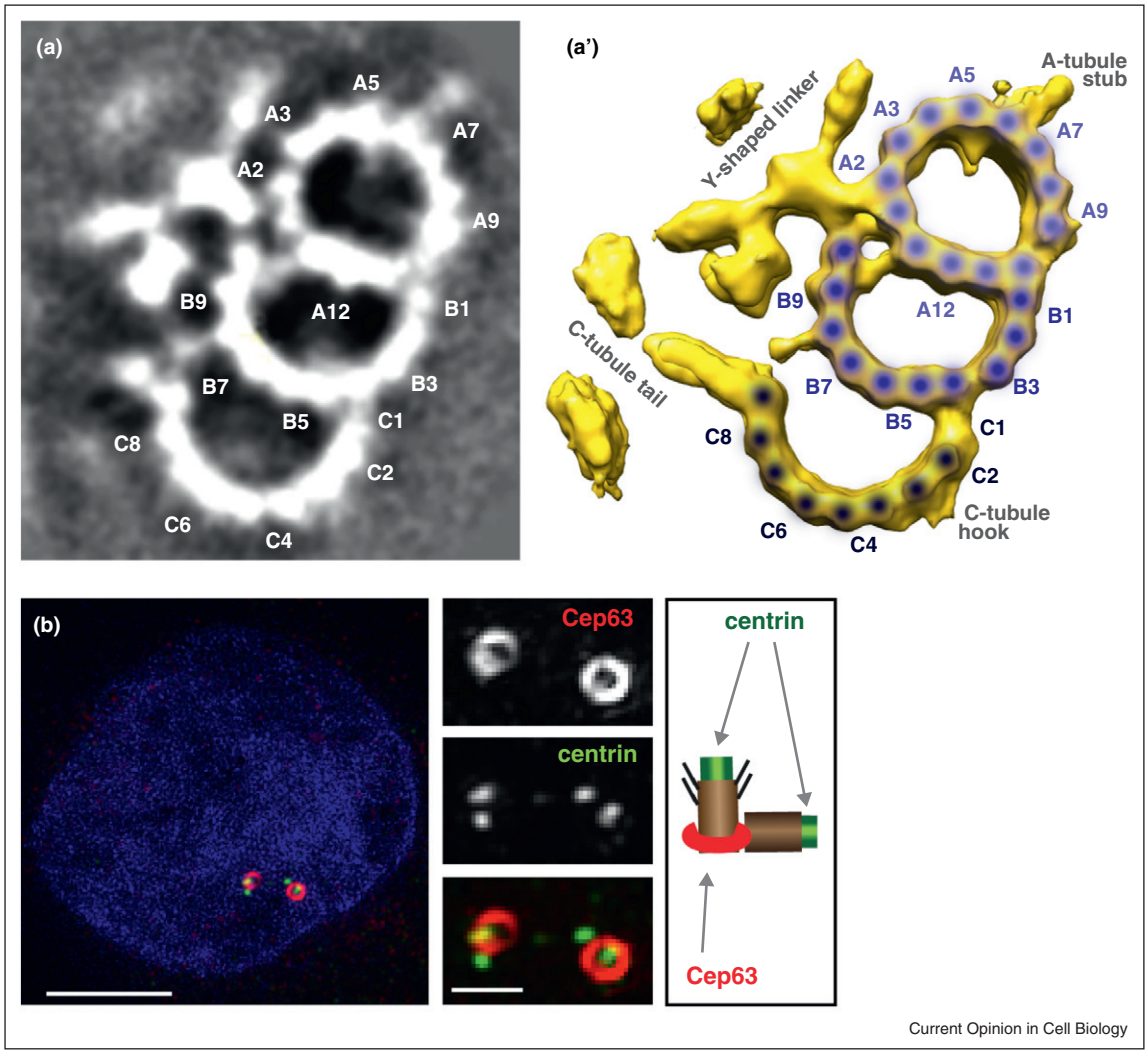
Centriole elongation regulation

Although an organism can have centrioles with different sizes, the mechanisms that determine their length and maturation status are still being discovered. Particularly intriguing is the observation that only procentrioles can elongate (which links centriole maturity and elongation), suggesting the existence of a centriole-intrinsic length control mechanism. In that respect it is very interesting that there are molecules that localize differently to pro-centrioles, daughter, or mother centrioles, such as Cep120 and centrobin that localize preferentially to procentrioles [2,26,28]. Several new differentially localized markers have recently been identified through isolation and mass spectrometry analysis of centrosomes [29]. It will be important to identify the role of these new components in the future.

Different studies showed that length control is the result of molecules with antagonistic functions. Depletion of CP110 in human cells impairs length control resulting in overly long centrioles [23,30]. Likewise, CPAP overexpression originates longer centrioles [23,24,30], a phenotype that depends on centrobin [26]. Additionally, hPoc1 levels also correlate with centriolar length [31]. Conversely, overexpression of Kif24, a microtubule depolymerizer, rescues centriole elongation induced by CP110 and Cep97 depletion [32•]. The fact that CP110 localizes at the distal end of the centriole suggests it may act as a cap-like structure that regulates centriole elongation [2]. It will be important to understand how these different players influence centriolar microtubule polymerization, in particular how the balance between polymerization and depolymerization results in homogeneous centriole size.

Centriole elongation is tightly regulated by the cell cycle, with completion of centriole elongation taking place during G2 and mitosis phases [2]. Several players might have a role at the later stages (Figure 2; [24,33]), particularly, (1) CPAP overexpression-dependent centriole over-elongation occurs during G2-phase [30]; (2) hPoc5 is required for elongation of centrioles distal half and is recruited during G2-M transition [34]; and (3) depletion

Figure 4



Imaging centrioles and basal bodies at higher resolution.
(b) 3D-SIM image of a DT40 cell stably expressing protein G-tagged Cep63, co-stained with antibodies against protein G (represented in red) and centrin-3 (represented in green). Centrosomes are shown at high magnification. Schematic shows relative positions of centrin and Cep63 staining. Cep63 forms a ring around the proximal part of the mature centriole. Scale bar = 3 μm; inset scale bar = 0.5 μm. Images courtesy of Joo-Hee Sir and Fanni Gergely. For more info see Sir *et al.* [43]. **(a-a′)** Cryo-EM reconstruction of the basal body triplet of *Chlamydomonas*. **(a)** Longitudinal section of a basal body triplet (selected triplet protofilaments are labeled). **(a′)** Docking of the crystal structure of tubulin into the corresponding density map. Tubulin molecules of each microtubule are represented as dots in different shades of blue. Non-tubulin densities associated with the triplet are exclusively represented in yellow. Several non-tubulin densities are observed.
Images courtesy of Sam Li and David A. Agard. For more info see Li *et al.* [22••].

of Ofd1 (another distal centriole elongation regulator) increases centriole length mainly during G2-phase [35]. Some of these regulators might be subject to proteolysis as proteosome inhibitors induce abnormal daughter centriole elongation [36••]. Finally, as CPAP phosphorylation and tubulin glutamylation were shown critical for centriolar microtubule stabilization and elongation in human cells [2,37], one can speculate post-translational modifications to be an equally important regulating mechanism of centriole elongation.

Centriole maturation

Procentrioles become daughter centrioles after going through one mitotic phase. In turn, daughters differentiate into mother centrioles upon acquiring distal and subdistal appendages and reaching full maturation during the following cell cycle (Figure 2). While distal appendages are involved in the recruitment of basal bodies to the membrane during ciliogenesis, subdistal appendages are implicated in microtubule anchoring (reviewed in [38]). EB1, FOP and CAP350 form a complex at subdistal

appendages, which together with ninein, plays a role in centrosomal microtubule anchoring and nucleation [2,38,39]. Likewise, ε-tubulin and Cep170 exclusively associate with the subdistal appendages of mature centrioles, while Cep164 localizes to distal appendages and is involved in primary cilia formation, and Odf2 is crucial for the assembly of both appendage types (reviewed in [2,38]). Although different players are already known to be involved in appendage assembly, the precise timing of assembly and function of distal and subdistal variants still remain uncharacterized.

Controlling centriole numbers

Centrosome amplification is a common cancer hallmark. Although multiple centrioles can cluster to nucleate bipolar spindles, the increased incidence of unbalanced merotelic attachments can compromise chromosome segregation fidelity and generate genomic instability (reviewed in [3]). Centriole number defects can arise from cytokinesis failure, simultaneous assembly of many procentrioles around a mother centriole, or multiple duplication rounds during one cell cycle. The number of centrioles in a cell depends on a tightly controlled centriole cycle: (1) it occurs once per cell cycle during S-phase (temporal control) and (2) only one centriole (litter control) forms per and near each existing centriole (spatial control).

A series of elegant experiments showed that upon daughter centriole ablation in S-phase-arrested HeLa cells, the mother regains the ability to form another single daughter [40]. These data suggest mother-daughter engagement limits duplication to a single round per cycle. The spatial control limits arbitrary procentriole assembly while inhibiting *de novo*. Indeed, the mother centriole organizes and maintains a compact PCM region, the only area in the cell where new centrioles form [40]. Additionally, the PCM cloud size correlates with the number of daughter centrioles [40], consolidating the PCM as an optimized and limiting area for centriole biogenesis onset. On the contrary, S-phase centriole duplication is contingent on the Plk1-dependent and separase-dependent disengagement during the preceding mitosis, suggesting further coordination between the centriole and cell cycles [6,41••,42•]. Cohesin was identified as a centriole engagement factor [42•], and disengagement was recapitulated when wild-type (but not protease-dead) human separase was added to human centrioles in *Xenopus* extracts [42•]. The role of Plk1 in centriole disengagement and duplication is still unclear. Plk1 was recently proposed essential for an uncharacterized early-mitotic centriole modification that dictates the centriole ability to duplicate during the following S-phase [42•]. Indeed, Plk1 is required for centriole reduplication in S-phase-arrested mammalian cells [40]. Additionally, Plk1 is involved in PCM recruitment to mitotic centrosomes [43]. Plk1 may thus have several functions in centriole transformation, including involvement in disengagement (which might be required for relieving the spatial proximity between a centriole pair), accumulation of PCM, maturation, and duplication of daughter centrioles. Cep63 was recently identified as a centrosome component that recruits Cep152/Asl to the mitotic centrosome [43]. Using super-resolution microscopy, this work showed that together with Cep152/Asl, Cep63 forms a ring between the wall of the mother centriole and the PCM, thus revealing substructures within the previously thought amorphous PCM cloud (Figure 4b; [43]).

Depletion of Plk4 in human cells reduces centriole number, while overexpression induces multiple daughter formation around a mother. Moreover, Plk4 can trigger *de novo* assembly both in centriole-depleted tissue culture cells and in unfertilized *Drosophila* eggs, a naturally centriole-free system (reviewed in [3]). Clearly, litter control depends on the tight regulation of Plk4 levels and activity. Indeed, Plk4 levels are regulated by degradation via the $SCF^{Slimb/\beta TrCP}$ ubiquitin ligase complex [36••,44–47,48•]. Additionally, PP2A phosphatase is involved in regulating Plk4 activity and stability by counteracting Plk4 autophosphorylation activity [49]. As a kinase, it is reasonable that Plk4 primes other proteins involved in centriole duplication and is thus required at a different temporal setting. Indeed, centrosomal Plk4 levels and activity have been shown to peak during mitosis [45,47], while paradoxically, centriole duplication ensues in the following S-phase. Moreover, an autoregulatory feedback loop limits Plk4 stability, as trans auto-phosphorylation of multiple sites of a phosphodegron is crucial to promote optimal Plk4 degradation [46,48•].

The data discussed above show the importance of post-translational modifications in centriole number control, such as phosphorylation and ubiquitination, Recent studies suggest that an intricate balance between kinase and phosphatase activity regulates those processes: (1) Plk2-dependent CPAP phosphorylation favors centriole duplication in HeLa cells [37]; (2) Zyg1-dependent Sas6 phosphorylation is determinant for new centriole formation [50]; (3) PP2A is required for centriole formation in human culture cells, fly, and worms [51–53], promoting Zyg1 and Sas5-dependent Sas6 recruitment to the nascent centriole [52,53]; (4) in human cells the SCF component Fbxw5 targets Sas6 for degradation, while being itself targeted upon Plk4-dependent phosphorylation [54].

Future perspectives

The centriole is an undeniably important organelle. The difficulty in isolating this very small organelle has made it difficult to study using conventional techniques. For example, where much advancement has been made on control mechanisms of other microtubule-based

structures, such as the spindle, the centriole small scale has precluded further studies on its size control. In recent years, the combination of sensitive high-resolution structural analysis with biochemical, comparative genomics, and cell biological approaches, has both led to the identification of several players and suggested mechanisms defining centriole structure and number. We are yet far from understanding how those molecules work together, both in time and space, to orchestrate such an elaborate process.

Recently, the structures of human, worm, and green algae centrioles and basal bodies were described using tomography [21••,27,55,22••], revealing new details on the composition, stability and assembly mechanisms of those structures. Additionally, new data on the establishment of the conserved centriole ninefold symmetry were revealed by X-ray crystallography, cryo-EM, and rotary metal-shadowing EM [13••,14••], while novel components were identified using highly sensitive mass spectrometry approaches [29]. Moreover, different studies have used super resolution microscopy to map molecules to the centriole structure at nanoscale resolution, unravelling the presence of novel molecular complexes and structures [43,56,57] (Figure 4a). PALM (Photoactivated Localization Microscopy), STORM (Stochastic Optical Reconstruction Microscopy), and SIM (Structural Illumination Microscopy) have proven to be powerful microscopy techniques in analyzing the centriole with nanoscale detail [43,57]. Unquestionably, we are witnessing the advent of an era of highly sensitive and quantitative ultrastructural approaches that are paramount to elucidate the centriole structural and molecular details [22••,43,57]. The combination of super-resolution methods with comprehensive *in vitro* and *in vivo* assays will provide new insights into the molecular mechanisms regulating centriole biogenesis, stability, and function.

Acknowledgements

We apologize to colleagues whose work was not discussed or cited owing to space constraints. We would like to thank Zita Santos for help with scientific illustrations and discussions. We would also like to acknowledge Sam Li and David Agard, and Joo-Hee Sir and Fanni Gergely for kindly providing panels a and b, respectively, of Figure 4. We also thank Jadranka Lončarek, Meng-Fu Bryan Tsou, Inês Bento, Gaëlle Marteil, and Filipe Leal for critically reading the manuscript. DAB and SMG are supported by EMBO long-term fellowships. The laboratory and MBD are supported by an EMBO installation grant, an ERC starting grant, and grants from the Portuguese Foundation for Science and Technology (FCT).

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