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Mechanisms of procentriole formation

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The centrosome comprises a pair of centrioles and associated pericentriolar material, and it is the principal microtubule-organizing centre of most animal cells. Like the genetic material, the centrosome is duplicated once and only once during the cell cycle. Despite the fact that both doubling events are crucial for genome integrity, the understanding of the mechanisms governing centrosome duplication has lagged behind the fuller knowledge of DNA replication. Here, we review recent findings that provide important mechanistic insights into how a single procentriole forms next to each centriole once per cell cycle, thus ensuring that one centrosome becomes two.

Centrosome structure and function

Being the principal microtubule-organizing centre (MTOC) of most animal cells, the centrosome directs several fundamental processes, including cell polarity, intracellular trafficking and cell division. The centrosome comprises two centrioles surrounded by pericentriolar material (PCM), which functions as the primary site of microtubule nucleation (Figure 1a). Centrioles are small cylindrical structures (~200 nm in diameter and ~400 nm long in human cells) characterized by nine sets of microtubules organized in a beautiful, radially symmetric manner (Figure 1b). In most species, microtubule triplets are present at the base or proximal end of centrioles, where the bulk of the PCM is also located (for discussion, see [1]), whereas microtubule doublets are present at their distal end. Although microtubule triplets are entirely replaced by singlets or doublets in some species, the nine-fold radial symmetry is a universal feature of centrioles (for review, see [2]).

Centrioles are fundamental for the structure, function and duplication of the centrosome. Thus, centrioles organize the PCM, as shown for instance by the fact that human cells or *Caenorhabditis elegans* embryos with compromised centrioles exhibit defective PCM recruitment [3,4]. Moreover, centrioles can serve as basal bodies for cilia, which sustain processes such as mechanosensation, chemosensation, and the generation of liquid flow (for review, see [5,6]). Of particular importance in the context of this review, the formation of new centrioles (hereafter referred to as 'procentrioles') is crucial for the duplication of the entire centrosome.

Procentriole formation: canonical and *de novo* pathways

In proliferating cells, procentrioles usually form through a canonical pathway in which their assembly occurs in association with existing centrioles (Figure 1c) [7–9]. The two centrioles present early in the cell cycle are loosely connected, and the vicinity of the base of each centriole provides an environment that promotes assembly of a procentriole, which is oriented approximately perpendicular to each centriole (for discussion, see [10,11]). In the green algae *Chlamydomonas reinhardtii*, in which basal-body formation occurs in a manner related to centriole formation, the 'cartwheel', a minute structure (~130 nm in diameter and ~70 nm long) with a nine-fold symmetry, is clearly apparent at the onset of basal-body assembly [12]. A cartwheel can also be observed at the onset of procentriole assembly in favourable preparations of human cells (for review, see [10]).

The cartwheel persists in the mature basal body in *C. reinhardtii* [12–14], whereas it disappears during centriole maturation in human cells (for review, see [10]). In *C. elegans*, a cylindrical structure dubbed the 'central tube' is similarly apparent at the onset of procentriole assembly [15]. Following assembly of the cartwheel or the central tube, procentrioles acquire microtubules and continue to elongate until they reach a size similar to that of their tightly associated centriole. Thereafter, it is thought that the loose connection between the two pairs of centriole–procentriole is relieved, enabling the two resulting centrosomes to separate and thus ensure bipolar spindle assembly. At the end of mitosis, the tight association between centriole and procentriole is severed in a process called disengagement or disorientation [7,8]. As a result, each daughter cell is endowed with two centriolar units akin to those present at the beginning of the cycle.

Besides assembling through the canonical pathway, procentrioles can also assemble through an alternative pathway in which they do not form in association with pre-existing centrioles. This *de novo* pathway is active during ciliogenesis, for instance in vertebrate epithelial cells of the oviduct or the trachea, where many procentrioles emerge in a single cell from electron-dense areas called fibrous granules and deuterosomes [16–18]. In addition, the *de novo* pathway becomes apparent in proliferating human cells after removal of pre-existing centrioles by a laser microbeam, after which procentrioles assemble in the cytoplasm (Figure 1d) [19,20]. An important distinction between the two pathways of procentriole formation is that the number of procentrioles assembled in somatic cells is not regulated with precision through the *de novo* pathway [16,19,20]. Moreover, some procentrioles assembled *de novo* after laser microbeam removal of pre-existing centrioles exhibit structural aberrations [19]. Therefore, although the *de novo* pathway is indispensable when many centrioles must be formed or when resident

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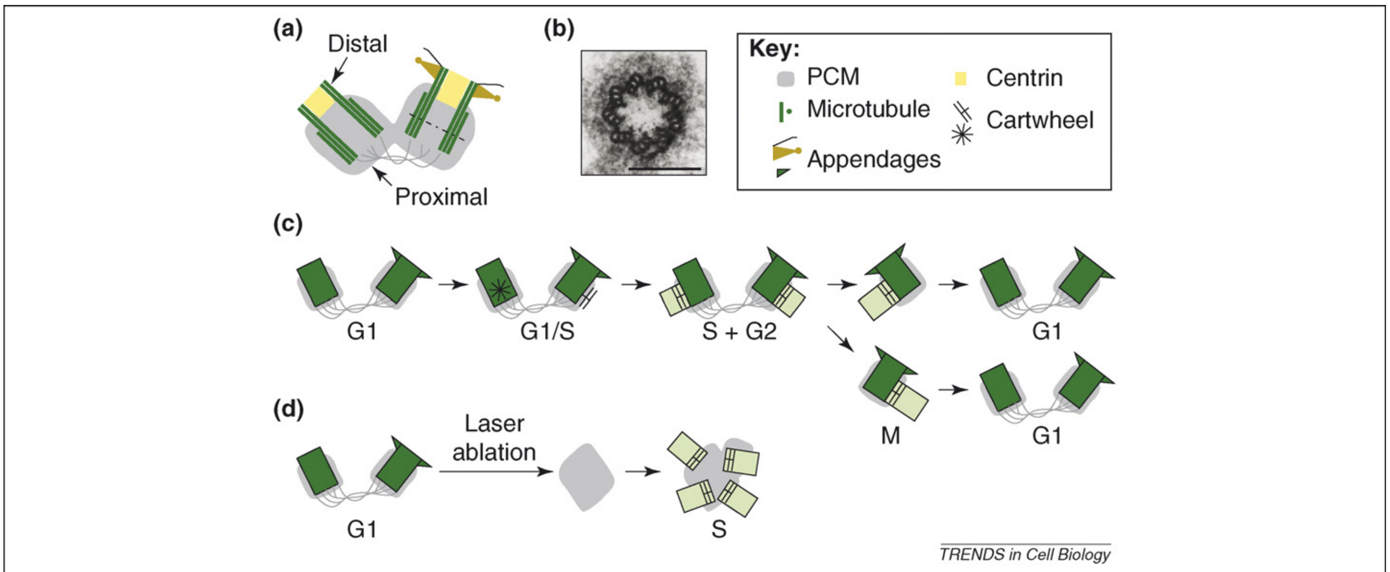

Figure 1. Centrosome structure and duplication. **(a)** Schematic representation of a centrosome in a human cell in the G_1 phase of the cell cycle, when the two centrioles are connected by a flexible linker. Note that the older centriole harbours distal and sub-distal appendages, which are important for anchoring microtubules, as well as for anchoring the centriole to the plasma membrane during primary cilium formation. Note also that the distance between the two centrioles in G_1 can vary between cell types. **(b)** Electron micrograph in a human cell, revealing a cross-section through the proximal part of a centriole [as schematized by the dashed line in panel (a)]. Scale bar: 200 nm. Image courtesy of Michel Bornens. **(c)** The canonical procentriole duplication cycle begins approximately at the G_1–S transition with the formation of a cartwheel at the proximal end of each centriole. This is followed by elongation of the procentrioles until, at the end of G_2, they reach almost the size of the mature centrioles. Procentrioles and centrioles then disengage from one another during mitosis, giving rise to two pairs of centrioles in the resulting G_1 cells. **(d)** *De novo* formation of procentrioles induced by ablation of the pre-existing centrosome begins by the aggregation of pericentriolar material (PCM). A variable number of procentrioles then assembles in this cloud of PCM. Note that the cartwheel has not been observed directly under these experimental conditions, but that its presence during *de novo* centriole formation is inferred from findings during ciliogenesis.

centrioles are lacking, procentriole assembly through the canonical pathway appears to be best suited for ensuring faithful centriole duplication in most proliferating cells.

In this review, we discuss recent findings that provide important insights into the mechanisms governing procentriole formation. We first discuss how components essential for this process have been identified in recent years in several experimental systems. Second, we review how the central tube and the cartwheel play a fundamental role in initiating procentriole assembly. Third, we discuss how procentriole assembly can be coupled to progression through the cell cycle. Fourth, we consider the mechanisms ensuring that in proliferating cells a single procentriole assembles next to each centriole once per cell cycle. Other important aspects of centriole formation, including post-translation modifications of centriolar microtubules, further maturation of centrioles or their role in directing cilium formation, are not covered here, owing to space constraints.

Searching for genes required for procentriole formation

The canonical pathway of procentriole formation has been described in exquisite detail at the ultrastructural level, starting several decades ago (for review, see [2]). By contrast, the identity of proteins required for this process has been discovered only recently. Several approaches have been successful in identifying components required for procentriole formation (Table 1). Forward genetic and RNA interference (RNAi)-based functional genomics screens uncovered five proteins essential for this process in *C. elegans*: the kinase ZYG-1 [21], as well as the coiled-coil proteins SPD-2 [22,23], SAS-4 [4,24], SAS-5 [25] and SAS-6 [26,27]. Apart from SAS-5, counterparts of these proteins have been identified in other species, raising the possibility that their function has been conserved through evolution. Accordingly, the ZYG-1 homologue PLK4 (also known as SAK) is required for procentriole formation in human cells [28] and in *Drosophila melanogaster* [29]. This is also the case for DSas-4, the *D. melanogaster* relative of SAS-4 [30], whereas the human SAS-4 relative CPAP (also known as CENPJ) is needed for PLK4-induced centriole over-duplication, a process in which several procentrioles form around a single centriole [31]. Conflicting results exist for SPD-2 homologues: one study reported that human Cep192 is required for procentriole formation [32], but three others concluded that Cep192 or *D. melanogaster* DSpd-2 are dispensable during this process [33–35]. By contrast, SAS-6 homologues seem to be invariably essential for procentriole formation, be it in *D. melanogaster* [36,37], *C. reinhardtii* [38], zebrafish (*Danio rerio*) [39] or human cells [40]. Overall, these findings support the view that the components initially identified in *C. elegans* are evolutionarily conserved players needed for procentriole formation.

In addition to these components, a genome-wide RNAi-based screen in *D. melanogaster* cells identified three other genes essential for procentriole formation, *Ana-1, Ana-2 and Ana-3* [41], but the mechanism of action of these proteins awaits further analysis. Moreover, screening of *C. reinhardtii* mutants led notably to the additional identification of the protein Bld10p, which might be related to human Cep135 [42,43], and of the tubulin isoform δ-tubulin, which ensures assembly of triplet centriolar microtubules (for review, see [44]). The role of yet other players in

Table 1. Principal proteins implicated in procentriole or basal-body formation in *C. elegans, D. melanogaster, C. reinhardtii* and *H. sapiens*[a]

Protein[b]	Species	Inactivation	Bases for inferred centriole formation defect	Refs
ZYG-1	*Ce*	RNAi; mutation	EM; no procentriole in mitosis; no central tube	[15,21,49]
Sak	*Dm*	RNAi; mutation	EM+IF; no centrioles in mitosis	[29]
PLK4	*Hs*	RNAi	IF; reduced number of centrioles in mitosis	[28]
SPD-2	*Ce*	RNAi; mutation	EM+IF; no procentriole in mitosis	[22,23]
DSpd-2	*Dm*	Mutation	*IF; no defect; normal number of centrioles*	[34,35]
Cep192	*Hs*	RNAi	IF+EM; no procentriole in mitosis [32]; other report differs [33]	[32,33]
SAS-6	*Ce*	RNAi	IF+EM; no procentriole in mitosis; no central tube	[15,26,27]
DSas-6	*Dm*	Mutation	IF+EM; reduced number of centrioles	[36,37]
Bld12p	*Cr*	Mutation	EM; no flagella in most cells, structurally defective basal bodies	[38]
HsSAS-6	*Hs*	RNAi	IF+EM; no procentriole in mitosis	[26,40]
SAS-5	*Ce*	RNAi; mutation	IF; monopolar spindle with single centriole	[15,25]
SAS-4	*Ce*	RNAi	IF+EM; no procentriole in mitosis; central tube still forms	[4,15,24]
DSas-4	*Dm*	Mutation	EM; no centrioles in mitosis	[30]
CPAP	*Hs*	RNAi	IF; no centriole over-duplication upon Plk4 overexpression	[31]
TBG-1	*Ce*	RNAi	IF; delayed procentriole formation	[27]
γ-Tubulin	*Hs*	RNAi	IF; no procentriole in mitosis	[52]
Ana-1	*Dm*	RNAi	IF; reduced number of centrosomes in mitosis	[41]
Ana-2	*Dm*	RNAi	IF; reduced number of centrosomes in mitosis	[41]
Ana-3	*Dm*	RNAi	IF; reduced number of centrosomes in mitosis	[41]
Bld10p	*Cr*	Mutation	EM; absence of basal bodies	[42]
Cep135	*Hs*	RNAi	IF; no centriole over-duplication upon Plk4 overexpression	[31]
Mps1p	*Sc*	Mutation	EM; no spindle-pole body duplication	[48]
MPS1	*Hs*	RNAi	IF; no procentrioles in S-phase cells [65]; other report differs [64]	[64,65]
Sfi1p	*Sc*	Mutation	EM; no spindle-pole body duplication	[46]
hSFI1	*Hs*	N/A	GFP fusion localizes to centrosome, function not yet reported	[46]
Cdc31p	*Sc*	Mutation	EM; no spindle-pole body duplication	[45,48]
Centrin	*Cr*	RNAi; mutation	DIC+EM; variable flagellar number	[76–78]
Centrin-2	*Hs*	RNAi	IF+EM; single centriole in each spindle pole	[79]
PLK2	*Hs*	RNAi	IF; no centriole over-duplication in S-phase-arrested cells	[80]
Centrobin	*Hs*	RNAi	IF+EM; no procentriole in mitosis	[81]
CP110	*Hs*	RNAi	IF; no centriole over-duplication in S-phase-arrested cells	[28,62]
NPM/B23	*Hs*	Antibody	IF; antibody injection inhibits centrosome duplication	[61]
PLK1	*Hs*	RNAi	IF; no centriole over-duplication in S-phase-arrested cells	[82]

[a]Related proteins are grouped. Note that proteins required for spindle-pole body duplication in *S. cerevisiae* are mentioned in addition when human homologues were identified. Note also that tubulin isoforms are not mentioned, except for γ-tubulin, and that SPD-5 (*Ce*), NEDD1 (*Hs*) and CDC25B (*Hs*) are not mentioned, because their requirement for procentriole formation probably reflects their function in ensuring centrosomal recruitment of γ-tubulin [27,52,83]. The DSpd-2 row is indicated in italics to reflect the fact that this protein does not appear to be required for procentriole formation. Abbreviations: *Ce*, *C. elegans*; *Cr*, *C. reinhardtii*; *Dm*, *D. melanogaster*; EM, electron microscopy; *Hs*, *H. sapiens*; IF, immunofluorescence; *Sc*, *S. cerevisiae*.

[b]ZYG-1: zygote defective 1, Sak: Snk/Plk-akin kinase, PLK4: polo-like kinase 4 (kinases); SPD-2: spindle defective 2, DSpd-2, Cep192: centrosomal protein 192 (coiled-coil proteins); SAS-6: spindle assembly 6; DSas-6, Bld12p: bold 12, HsSAS-6 (coiled-coil proteins); SAS-5: spindle assembly 5 (coiled-coil protein); SAS-4: spindle assembly 4, DSas-4, CPAP: centrosomal P4.1-associated protein (coiled-coil proteins);TBG-1: γ-tubulin 1, γ-tubulin (PCM component); Ana-1: anastral spindle phenotype 1 (coiled-coil protein); Ana-2, Ana-3: anastral spindle phenotype 2, 3 (no domain found); Bld10p: bold 10, Cep135: centrosomal protein 135 (coiled-coil proteins); Mps1p: monopolar spindle 1, MPS1 (kinases); Sfi1p: Suppressor of fermentation induced loss of stress resistance 1, hSFI1 (scaffold proteins); Cdc31p: cell division cycle 31, Centrin, Centrin-2 (EF-hand proteins); PLK2: polo-like kinase 2 (kinase); Centrobin (coiled-coil protein); CP110: centrosomal protein 110kDa (coiled-coil protein); NPM/B23: Nucleophosmin (nucleoplasmin domain); PLK1: polo-like kinase 1 (kinase).

procentriole formation in metazoan organisms was uncovered because they are homologous to components essential for duplication of the centrosome-related spindle-pole body in the budding yeast *Saccharomyces cerevisiae*. These proteins include the Centrin-related EF-hand protein Cdc31p [45] and its interacting partner Sfi1p [46], which is thought to form filaments crucial for initiating the duplication process [47], in addition to the Ser–Thr kinase Mps1p [48]. Furthermore, analysis of individual RNAi-mediated inactivation phenotypes in human cells extended the list of proteins required for procentriole formation (Table 1). Although these approaches resulted in the identification of a significant number of proteins participating in procentriole formation, our understanding of how exactly most of them contribute to the assembly of this unique structure is still incomplete. Nevertheless, analysis of some of these proteins has begun to shed light on the structural determinants underlying the earliest stage of procentriole formation, as discussed in the following section.

Central tube and cartwheel: initiating procentriole formation

Significant novel insight into the initial stages of procentriole assembly has come from electron tomographic reconstruction of early *C. elegans* embryos, which revealed the existence of two distinct steps (Figure 2a) [15]. First, a central tube forms next to the base of each centriole. Second, microtubules assemble around the circumference of the central tube. The first step requires the function of SPD-2, ZYG-1, SAS-5 and SAS-6, whereas the second step is dependent upon SAS-4 [15]. Systematic kinetic analyses and molecular epistasis experiments indicate that SPD-2 is required for the centriolar localisation of ZYG-1, which, in turn, is needed to enable SAS-5 and SAS-6 to localize to centrioles [15,49]. SPD-2 and ZYG-1 are recruited to the centriole before central tube formation becomes apparent [15], suggesting that they have a regulatory rather than a structural role. By contrast, SAS-5 and SAS-6 are recruited slightly later [15,49], and the amount of SAS-6 on the

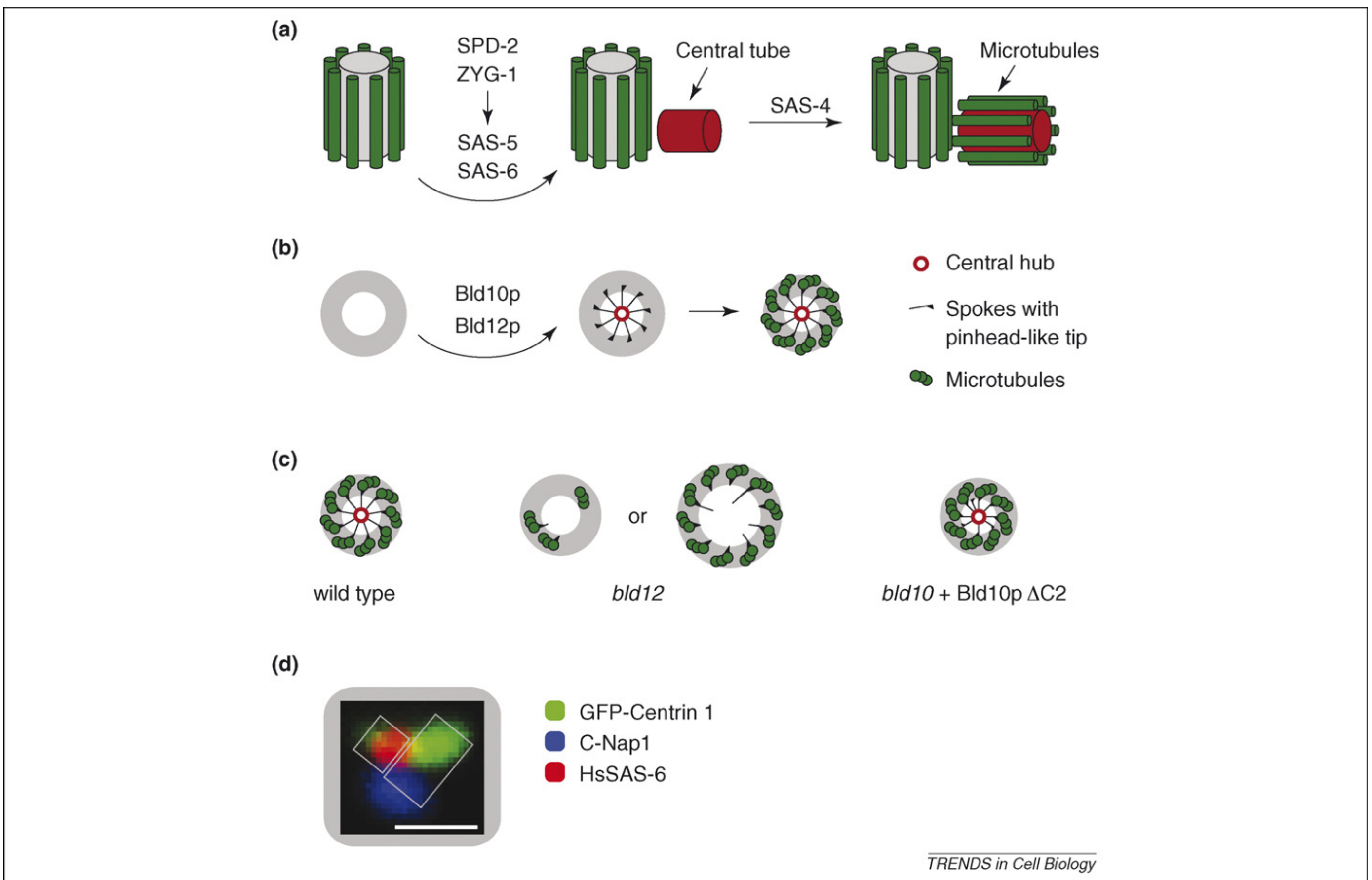

Figure 2. Initial steps in procentriole formation in *C. elegans* and *C. reinhardtii*. **(a)** Procentriole assembly in *C. elegans* embryos starts with the formation of a central tube and is followed by the addition of microtubules. Proteins essential for each step are indicated above the arrows. Note that SPD-2 is needed for the presence of centriolar ZYG-1, which, in turn, is needed for that of SAS-5 and SAS-6 (adapted from [15]). **(b)** Schematic cross-section of basal-body formation in *C. reinhardtii.* The onset of this process is characterized by the presence of an amorphous ring, which is followed by the assembly of a cartwheel composed of a central hub and nine spokes, and thereafter by the addition of microtubules (adapted from [38]). **(c)** Schematic cross-section through the proximal part of the basal body in *C. reinhardtii* cells of the indicated genotypes. In *bld12* mutants, the central part of the cartwheel is absent and the majority of the resulting structures are fragmented, with two or three microtubule triplets (left, three triplets shown). Closed basal bodies with variable numbers of centriole triplets (right, 10 triplets shown) are found more rarely. In a *bld10* mutant expressing a C-terminally truncated form of Bld10p, shorter cartwheel spokes are present, in addition to a basal body with a smaller diameter and only eight microtubule triplets. Note that although there are only eight microtubules, nine spokes are nevertheless present. **(d)** Centriole–procentriole pair in a human HeLa cell in the G_2 phase of the cell cycle stained with antibodies against GFP. GFP–Centrin 1, green; C-Nap1, blue; HsSAS-6, red. GFP–Centrin 1 marks the distal ends of the centriole and, to a lesser extent, of the procentriole, whereas C-Nap 1 marks the base of the centriole. Only one centriole–procentriole pair is shown, and its estimated orientation is highlighted in grey. Note the localisation of HsSAS-6 at the proximal part of the procentriole. Scale bar: 500 nm.

procentriole increases in parallel with central tube growth [50], compatible with the idea that SAS-6 is one of its structural components. Further support for this view comes from the observation that HsSAS-6 localizes to the proximal part of the procentriole in human cells, where the cartwheel is located (Figure 2d) [31,40]. Suggestively, in addition, HsSAS-6 is not present during the G_1 phase of the cell cycle, when the cartwheel is also absent [10,40]. Moreover, overexpression of *D. melanogaster* DSas-6 in syncytial embryos results in the formation of tube-like structures that might be somehow related to the central tube and the cartwheel [36]. Overall, these findings indicate that SAS-6 related proteins are part of the central tube and the cartwheel, and that they are thus in a prime location to play a crucial part at the onset of procentriole assembly.

How could the central tube or the cartwheel initiate procentriole assembly? Analysis of *C. reinhardtii* mutants sheds light on this important question. In wild type cells, the cartwheel consists of a central hub from which emanate nine symmetrically distributed spokes capped by a pin-head-like structure (Figure 2b). The cartwheel is the first nine-fold symmetrical structure apparent at the onset of procentriole assembly, before the addition of microtubules at the tip of the spokes [12]. Although this observation led to the long-held belief that the cartwheel is a structural scaffold onto which centriolar microtubules assemble [16,51], compelling evidence for this hypothesis has come only recently from the analysis of *bld12* mutants [38].

The majority of basal bodies in a null mutant allele of the SAS-6 homologue *bld12* are fragmented into pieces comprising two or three microtubule triplets (Figure 2c) [38]. The few basal bodies that retain a closed circular arrangement lack the central hub of the cartwheel, where Bld12p normally localizes [38]. Remarkably, in addition, these rare basal bodies are defective, with the number of microtubule triplets varying from seven to eleven, and with some triplets being shortened at the proximal end (Figure 2c). Similarly, open centrioles with disorganized and missing microtubule triplets are observed in *D. melanogaster DSas-6* mutants [36]. Collectively, these findings indicate that the cartwheel indeed acts as a scaffold for

Box 1. Hypothesis: structural basis for the nine-fold symmetry of procentrioles

The universal nine-fold symmetry of procentrioles, centrioles and basal bodies is apparent already in the cartwheel, which has been well described in *C. reinhardtii* [12–14] and in *Tetrahymena thermophila* [84]. The cartwheel comprises a central hub from which emanate nine spokes. These spokes are capped by a pinhead-like structure onto which the triplet microtubule is assembled (Box 1, Figure I, left). The central hub is crucial for the nine-fold symmetry of the resulting basal body, as illustrated by the defects apparent in *C. reinhardtii bld12* mutants, which lack the central hub (Figure 2c) [38]. Interestingly, in addition, SAS-6 proteins localize to the central hub, both in *C. reinhardtii* and in *T. thermophila* [38,85]. Given that the central hub is ~15 nm in diameter in *C. reinhardtii* (inferred from [14,38]) and that each CrSAS-6 molecule is estimated to be ~6 nm in diameter (based on the known dimensions of proteins of similar size, see http://www.rcsb.org/pdb/), we propose that the central hub could contain a nonameric ring of CrSAS-6 (Box 1, Figure I). Such a nine-fold structural imprint might then be translated from the hub to the spokes and further on to the triplet microtubules. Alternatively, other structures with a nine-fold symmetry might be assembled by proteins of the SAS-6 family and potentially interacting partners. Structural characterization of complexes containing SAS-6 proteins will be instrumental in addressing these possibilities. Scale bar in inset: 10 nm.

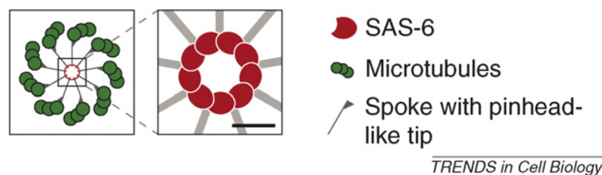

Figure I.

procentriole assembly. Importantly, they also suggest that the nine-fold symmetry of the cartwheel is necessary for imparting the related nine-fold radial organization of the resulting centriole. A speculation that stems from these observations taken together is that SAS-6 proteins assemble a structure that dictates the nine-fold symmetry of the procentriole (Box 1).

The analysis of *C. reinhardtii bld10* mutants offers further mechanistic insights. Bld10p is a large, coiled-coil protein that is essential for basal-body formation and which localizes in the vicinity of the terminal, pinhead-like structure of the cartwheel spokes [42,43]. Cells with a null mutant allele of *bld10* do not have basal bodies, but expression of truncated Bld10p lacking the C terminus partially rescues this defect, resulting in basal bodies with suggestive structural defects. Indeed, ~25% of them have shorter cartwheel spokes and thus a smaller lumenal diameter, and, possibly as a result, only eight microtubule triplets. This observation suggests that an appropriate cartwheel diameter is necessary to accommodate all nine microtubule triplets. Furthermore, although these smaller basal bodies harbour only eight triplet microtubules, their cartwheels nevertheless have nine spokes, with some being disconnected from microtubules (Figure 2c). Taken together, these findings indicate that, in addition to the nine-fold symmetry imparted by the cartwheel, an appropriate centriolar diameter is a prerequisite for centrioles to bear nine microtubule triplets. Importantly, the cartwheel is also observed during *de novo* procentriole formation in ciliogenesis in mammalian cells [16], which depends upon HsSAS-6 function [18]. Therefore, the cartwheel appears to be generally crucial for proper procentriole assembly.

If the cartwheel is crucial for initiating procentriole assembly, why does this process always begin at the base, rather than the more distal part, of the centriole in the canonical pathway? One possibility derives from the combined observation that the bulk of the PCM is found around the base of the centriole (for discussion, see [1]) and that the PCM is needed for efficient centriole formation. Indeed, centriole formation is markedly impaired in *C. elegans* embryos or human cells depleted of γ-tubulin [27,52], a major PCM constituent essential for efficient microtubule nucleation, thus mirroring results obtained originally in *Paramecium tetraurelia* [53]. Conversely, expansion of the PCM in S-phase-arrested Chinese hamster ovary (CHO) cells through overexpression of the PCM protein pericentrin results in the formation of a larger number of centrioles [11]. Therefore, it is plausible that the base of the centriole dictates the site of procentriole assembly by organizing a focus of PCM to which factors necessary for procentriole assembly are recruited [11]. An extension of this view is that the cartwheel might form preferentially in such a PCM-rich environment. Could the PCM also play a role during *de novo* procentriole formation? Compatible with this view, procentrioles formed after ablation of resident centrioles emanate from regions that bear resemblance with the PCM and which contain γ-tubulin [19]. Taken together, these observations indicate that, in both the canonical and the *de novo* pathways, a region enriched in PCM recruits the cartwheel and thus dictates the site of procentriole assembly.

Cell cycle control of procentriole formation

How is procentriole assembly regulated temporally? Its first manifestation apparent by electron microscopy occurs some time between late G_1 and early S phase in most proliferating cells (for review, see [54]). However, the prior presence of the cartwheel might have gone unnoticed, given the probable absence of microtubules at the earliest stages of assembly. Careful temporal analysis of CHO cells expressing green fluorescent protein (GFP)-labelled Centrin 1, which marks the distal end of centrioles and procentrioles, led to the conclusion that procentriole assembly begins around the time of the G_1–S transition [55]. An alternative strategy that has been used to investigate when procentriole assembly initiates is to arrest cells at given stages of the cell cycle and then assay the ability of centrioles to duplicate (for review, see [54]). This approach established that the G_2 and M phases of the cell cycle are not competent for procentriole assembly. By contrast, procentriole assembly can occur in cells arrested in S phase. In some cell types, such as CHO cells or human U2OS cells, centrioles can even undergo repeated rounds of duplication during S-phase arrest. The situation is less clear for the G_1 phase; some studies argue that this phase of the cell cycle cannot sustain procentriole assembly [56] (for review, see [54]), whereas recent findings indicate that this process can in fact occur during mimosine-induced G_1 arrest, although at a slower pace than during S phase [55]. Compatible with the idea that procentriole assembly initiates in G_1, the first steps of spindle-pole body duplication in *S. cerevisiae* can

occur even upon inactivation of the Ser–Thr CDK2 homologue Cdc28p, which is essential for the G_1–S transition [57].

Although CDK2 activity might not be essential, procentriole assembly normally initiates around the time of the transition between the G_1 and S phases of the cell cycle in vertebrate cells. This period is characterized not only by a rise in CDK2 activity but also by the concomitant downregulation in the activity of the ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C) in a complex with Cdh1 (for review, see [58]). What is the nature of the centriolar components that are regulated at this time and which trigger onset of procentriole assembly? First, it is possible that such components are regulated by the rise in CDK2 activity. For instance, this has been proposed for nucleolar phosphoprotein nucleophosmin (NPM, also known as B23), which is present in the loose connection between the two centrioles in cells in the G_1 phase of the cell cycle [59]. Phosphorylation of NPM–B23 by CDK2 displaces NPM–B23 from the centrosome, and this event appears to be required for procentriole assembly [60,61]. Another CDK2 substrate of potential importance in this context is the coiled-coil protein CP110, the depletion of which prevents PLK4-induced centriole formation [31,62]. Yet another CDK2 substrate that has been implicated in procentriole formation in mammalian cells is the kinase MPS1 [63], the homologue of which is needed for duplication of the spindle-pole body in *S. cerevisiae* [48]. Although the role of MPS1 in centrosome duplication in mammalian cells is controversial [63–65], recent findings indicate that phosphorylation of MPS1 by CDK2 is required to prevent degradation of MPS1 at centrosomes [66]. Second, an alternative and potentially non-mutually exclusive possibility to a rise in CDK2 activity being important is that centriolar components are controlled through downregulation of APC/C^{Cdh1}. For instance, the available evidence indicates that APC/C^{Cdh1} targets HsSAS-6 for degradation [40]. Therefore, it is tempting to speculate that the diminution of APC/C^{Cdh1} activity towards the end of the G_1 phase promotes procentriole assembly by enabling the accumulation of HsSAS-6. This possibility appears especially plausible because SAS-6 proteins are components of the central tube and the cartwheel and are thus ideally positioned for linking cell cycle progression with the onset of procentriole assembly.

Regulating procentriole number

Although the above findings could aid our understanding of the temporal regulation of procentriole assembly, they do not necessarily explain why just one procentriole assembles at the base of each centriole in proliferating cells. Important insight into this question comes from cell fusion experiments [67]. When a cell in the G_1 phase is fused with a cell in the G_2 phase, the G_1 cell cycle status is dominant and drives the common cytoplasm into S phase without an intervening mitosis. During this S phase, a new procentriole assembles next to each centriole coming from the G_1 cell, but not next to those originating from the G_2 cell. Therefore, there is a centrosome-intrinsic block that prevents repeated duplication events [67]. Recent findings indicate that this block is due to the presence of the procentriole [11]. When a laser microbeam is used to ablate specifically one procentriole in S-phase-arrested HeLa cells, a new procentriole invariably assembles to replace the ablated one. This occurs despite the fact that another procentriole is still associated with the second centriole present in the cell, indicating that the duplication block is strictly limited in space [11].

It has been suggested that this duplication block is maintained by the tight association between each centriole–procentriole pair, which initiates at the onset of procentriole assembly and is severed during mitosis through the disengagement step [68]. Consistent with this, *Xenopus laevis* egg extracts can promote duplication of centrioles isolated from human cells only when these centrioles are disengaged [69]. Intriguingly, depletion of separase, a protease best known for its requirement in promoting sister chromatid separation in anaphase, prevents centriole–procentriole disengagement in this system [69]. Conversely, when a splice variant of Shugoshin1 (sSgo1), a protein that protects sister chromatids from separase activity during prophase, is depleted in human cells, centriole–procentriole pairs disengage prematurely [70]. Taken together, these findings suggest that, during mitosis, separase cleaves a substrate, which might be sSgo1, and that this cleavage results in disengagement of the centriole–procentriole, thus licensing each of them for another round of procentriole formation during the subsequent cell cycle.

Although it is attractive, this model alone does not readily explain why several procentrioles can assemble from the base of one centriole in some cases, as in CHO or U2OS cells blocked in S phase [71,72]. Similarly, overexpression of PLK4, of HsSAS-6, or of the human papillomavirus type 16 E7 oncoprotein each induce assembly of multiple procentrioles from a single centriole [28,31,40,73], further indicating that the duplication block is not based solely on structural constrains, at the least in these cases. One plausible alternative is that a given component needed for procentriole assembly is limiting around the G_1–S transition and is recruited in a cooperative manner. This could favour procentriole assembly at just one site, and thus conceivably prevent concurrent growth at other sites. Upon laser microbeam-mediated ablation of this sole procentriole [11], the process would be reiterated, leading to the assembly of another procentriole. A distinct possibility is that the duplication block imparted by the presence of a procentriole is regulatory in nature. Studies in *C. elegans* indicate that the ZYG-1 kinase might play such a role. ZYG-1 localizes to centrioles primarily at the onset of procentriole assembly in wild type embryos, but it remains at that location if procentriole assembly is prevented following SAS-6 depletion [21,49]. This observation raises the possibility that the loss of centriolar ZYG-1 upon procentriole assembly might normally serve as a mechanism to prevent further assembly events [50]. Could PLK4 play a similar role in human cells? Although PLK4 localises to centrioles throughout the cell cycle [28], PLK4 kinase activity might potentially be modulated in time and diminish upon procentriole assembly. Interestingly, overexpression of PLK4 induces excess procentriole assembly in cells arrested in S phase, wherein one procentriole

has already assembled [31]. This suggests that an increase in PLK4 overrides the regulation normally imposed by the existing procentriole. The finding that depletion of the phosphatase CDC14B results in supernumerary centrioles in human cells reinforces the notion that the duplication block imparted by the procentriole is regulatory in nature. However, the relevant substrate of CDC14B in this context remains to be identified [74].

Hence, in summary, although the exact mechanisms restricting the occurrence of procentriole assembly to one per centriole remain to be clarified, it appears reasonable to suggest that it will be proved that they impinge on the same components that are crucial for initiating procentriole assembly.

Concluding remarks

Although our understanding of the mechanisms governing centrosome duplication is still less complete than that regarding DNA replication, considerable advances have been made in recent years. Components that are essential for procentriole formation have been identified in several model systems, and initial insight has been gained into how they contribute to building this beautiful structure. Moreover, the mechanisms restricting the occurrence of procentriole assembly to one per centriole per cell cycle are being clarified. Exciting challenges lie ahead of the field. These include elucidating how proteins of the SAS-6 family can assemble a structure amenable to initiating procentriole assembly, and clarifying the molecular tenets by which this event occurs just once per cell cycle next to each centriole. Moreover, it will be interesting to investigate to what extent the components that are essential for regulated procentriole assembly contribute to proliferation control, given that cancer cells often exhibit aberrations in centrosome number, the severity of which correlates with tumour progression [75]. With many of the tools in hand to address these and related questions, the path is wide open for elucidating one of the fundamental outstanding mysteries in cell biology.

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