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COMMENTARY

Choosing sides – asymmetric centriole and basal body assembly

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ABSTRACT

Centrioles and basal bodies (CBBs) are microtubule-rich cylindrical structures that nucleate and organize centrosomes and cilia, respectively. Despite their apparent ninefold rotational symmetry, the nine sets of triplet microtubules in CBBs possess asymmetries in their morphology and in the structures that associate with them. These asymmetries define the position of nascent CBB assembly, the orientation of ciliary beating, the orientation of spindle poles and the maintenance of cellular geometry. For some of these functions, the orientation of CBBs is first established during new CBB biogenesis when the daughter structure is positioned adjacent to the mother. The mother CBB organizes the surrounding environment that nascent CBBs are born into, thereby providing a nest for the new CBB to develop. Protists, including ciliates and algae, highlight the importance of this environment with the formation of asymmetrically placed scaffolds onto which new basal bodies assemble and are positioned. Recent studies illuminate the positioning of nascent centrioles relative to a modular pericentriolar material (PCM) environment and suggest that, like ciliates, centrosomes organize an immediate environment surrounding centrioles for their biogenesis and positioning. In this Commentary, I will explore the positioning of nascent CBB assembly as the first event in building cellular asymmetries and describe how the environment surrounding both basal bodies and centrioles may define asymmetric assembly.

KEY WORDS: Asymmetry, Basal body, Centriole, Pericentriolar material, Polarity

Introduction

Centrioles and basal bodies (CBBs) are characterized by their nine sets of triplet microtubule 'blades' that are arranged in a cylindrical structure (Fig. 1A). Although the two structures are analogous in their triplet microtubule organization, centrioles and basal bodies have unique functional roles (Fig. 1B,C). Centrioles function at the centrosomes to organize cellular microtubules, whereas basal bodies organize the microtubules of cilia. Centrosomes are comprised of a pair of centrioles surrounded by the pericentriolar material (PCM), which possesses microtubule-nucleating activity (Fig. 1B). Moreover, centrioles and basal bodies interchange their functions during the cell cycle when a centriole transitions to a basal body during G0/G1 to organize the primary cilium (Fig. 1C). Once cells enter the cell cycle, the cilium is resorbed and the basal body returns to its role as a centriole at the centrosome. The term centriole, basal body or CBB refers to when these structures are functioning at centrosomes, cilia or both, respectively. During the canonical cell cycle, duplication of new centrioles initiates at the G1/S-phase boundary of the cell cycle, producing a single centriole assembly event per centriole to ensure that future daughter cells are provided with two centrioles; one old mother from the previous cell cycle and one new daughter centriole from the current cell cycle.

Centrosomes temporally age such that, during cell division, each daughter cell inherits either an old or a new centrosome. This age is defined by the centrioles that reside within them; the mother centriole formed prior to the last cell cycle resides in the old centrosome, whereas the mother centriole from the last cell cycle is the daughter centrosome. Such asymmetries are important for both cell division and ciliogenesis. Asymmetries in centrosome age contribute to asymmetric cell division in stem cells, where the mother and daughter centrosome divide in a stereotypic fashion (Pelletier and Yamashita, 2012). In the case of ciliogenesis, CBB and centrosome age have differing effects on the timing of when the daughter cells form primary cilia. Older basal bodies (from the older centrosome) nucleate primary cilia more rapidly than cells that inherit the younger centrosome (Anderson and Stearns, 2009). This has important implications for the timing of when a cell becomes competent for cilia-dependent cell signaling (reviewed in Goetz and Anderson, 2010).

The positioning of the mother CBB establishes important polarity cues within cells. The mother centriole defines the site of nascent CBB assembly, the position and orientation of cilia formation and beating, the orientation of mitotic spindle poles and the maintenance of cellular geometry. During mitosis, the mother centriole is positioned within the centrosome perpendicular to the spindle axis, suggesting that the asymmetric orientation of centrioles is important for mitotic chromosome segregation (Vorobjev and Chentsov, 1980). Furthermore, proper positioning and orientation of motile cilia is required for effective ciliary beating to move fluid. Fluid motility is required for cellular motility and mucus clearance. Ultimately, asymmetric positioning and orientation are first established during centriole duplication.

During centriole duplication, assembly initiates at one location outside of the microtubule triplets at the base of and perpendicular to the mother centriole. Centrioles maintain this geometry and function as an orthogonally arranged pair (Fig. 1D). Viewed as a cylinder, the CBB has both a ninefold rotational symmetry and potential symmetry between the proximal (bottom) and distal (top) ends. Therefore, CBBs have a number of symmetries, all of which must be broken in order for these structures to function and duplicate themselves. First, as described above, the two members of a centriole pair are not equal; mother centrioles have structures and capabilities that their relatively immature daughters do not (Brito et al., 2012; Lange and Gull, 1995; Nigg and Stearns, 2011; Pelletier and Yamashita, 2012; Vorobjev and Chentsov, 1980). The mother centriole plays

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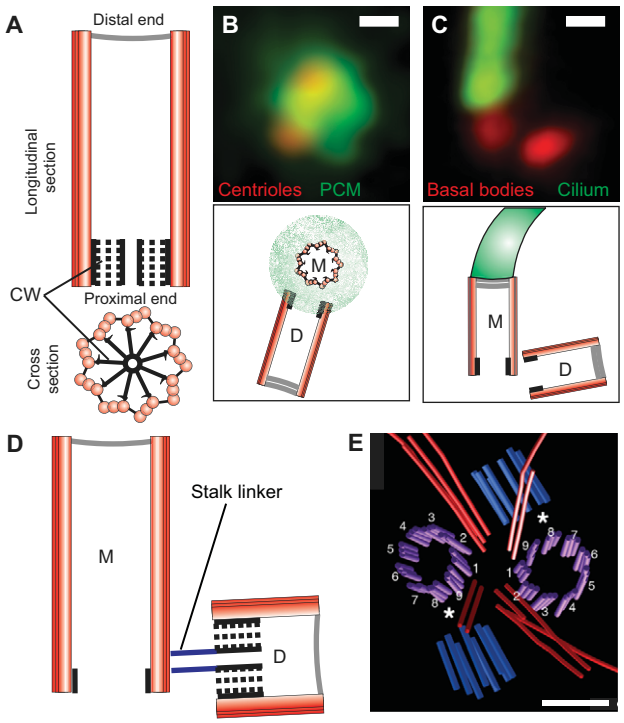


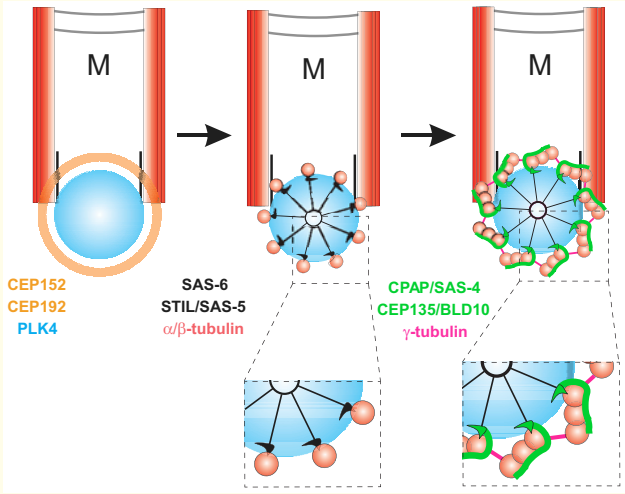
Fig. 1. Overview of CBB structure and function. (A) Structural organization of CBBs. The cartwheel (CW) resides at the proximal end with the minus ends of the polarized triplet microtubules (red). Microtubule plus ends orient towards the distal end. A diagram of a cross section through the cartwheel is shown underneath. (B) Image and schematic representation of centrioles (centrin in red) and PCM (γ-tubulin in green) in retinal pigmented epithelial cells (RPE1). M, mother centriole. D, daughter centriole. Scale bar: 0.5 μm. (C) In the image, the basal body is labeled in red using an antibody against centriole and spindle-associated protein (CSAP) as described previously (Backer et al., 2012) and the primary cilium is stained for Arl13B in green. M, mother basal body; D, daughter centriole. Scale bar: 0.5 μm. (D) General schematics of the orientation of new centriole assembly. M, mother centriole; D, daughter centriole. The diagram shown contains a stalk linker (purple), which attaches the wall of the mother centriole cylinder to the daughter pro-centriole through the cartwheel. (E) New basal bodies form at a defined triplet microtubule on the mother basal body in *Chlamydomonas*. Tomographic reconstruction of pro-basal bodies (blue) forming specifically at triplet microtubule (in purple) number 8 (asterisks) of the mother basal. The rootlet microtubules (shown in red) define the position of each triplet blade. Scale bar: 200 nm. Image taken from O'Toole and Dutcher (O'Toole and Dutcher, 2013) with permission.

a larger role in the centrosome in recruiting and organizing the proteinaceous matrix known as the pericentriolar material (PCM) (Fig. 1B) (Piel et al., 2000), and only the mother centriole becomes a basal body to nucleate the primary cilium (Fig. 1C) (Pazour and Witman, 2003). Second, assembly of a new CBB starts at the proximal end and builds towards the distal end by assembly of inherently polarized triplet microtubules thereby generating a polarized structure. As part of this process, the earliest structural event in CBB biogenesis, cartwheel formation, occurs at the proximal end (transiently in some cases), whereas the distal end has its own unique structure, the so-called CP110 cap in centrioles and the transition zone in basal bodies (Box 1). Sites of accessory structures specific to mature CBBs are found at distinct locations along the length of the CBB (reviewed in Azimzadeh and Marshall, 2010). Finally, new CBB assembly occurs at a defined position with regard to the ninefold rotational symmetry of the CBB. This last category of symmetry breakage – that of the ninefold symmetry of the microtubule triplets and the site of new CBB assembly – will be the focus here. New CBB assembly occurs in close proximity to an individual triplet microtubule of the mother CBB cylinder.

In this Commentary, I review historical and recent studies showing how the architecture of ciliate and algal basal bodies defines the location of asymmetric basal body assembly. Ciliate basal bodies have a well-established molecular and structural architecture surrounding the basal body; this architecture points to the site of new basal body assembly. I expand upon these ideas developed from studies in protists to incorporate recent data that suggests that the immediate centriole environment produced by the centrosome might be analogous to protist basal bodies.

Box 1. Centriole assembly: structure and molecules

The convergence of genetic and proteomic analyses with structural imaging of mutants has enabled the dissection of many of the stages of centriole biogenesis (reviewed in Brito et al., 2012; Gönczy, 2012; Pearson and Winey, 2009; Jana et al., 2014; Dutcher, 2007). The site of new centriole formation is defined by the Polo-like kinase, PLK4, and its recruitment factors, CEP152 and CEP192, near the wall of the mother centriole (M), which precedes cartwheel formation (see figure). Cartwheels comprise an inner structure formed from Sas-6 and STIL (known as Sas-5 in *C. elegans* and Ana2 in *Drosophila*). The Sas-6 protein forms the central hub and spokes that extend towards the triplet microtubule blades (see figure). The outer cartwheel bridges the inner cartwheel and the microtubule blades; here, CEP135 (also known as Bld10) link Sas-6 to the triplet microtubules and CPAP (also known as CENPJ and, in *C. elegans*, Sas-4). CEP120 and SPICE1 proteins localize CEP135/Bld10 to the centriole to form the linkage between the inner cartwheel and the microtubule cylinder structure (Lin et al., 2013). Microtubules are then assembled and stabilized in a manner that is dependent upon CEP135/Bld10, CPAP/Sas-4, CEP120, centrobin, SPICE1, Poc1, Poc5 and γ-tubulin. The proximal ends of CBBs are capped with the cartwheel, and the distal ends are capped with centrin and the CP110 protein, which limits centriole length.



Nascent CBBs form near the mother CBB

Historical electron microscopy studies identified important structural events that are associated with daughter CBB formation adjacent to the mother CBB (reviewed in Dutcher,

2007; Gönczy, 2012; Pearson and Winey, 2009; Jana et al., 2014). The close proximity of the assembly of the daughter CBB to the mother structure led to the term 'templated' duplication, suggesting that the mother CBB somehow specifies the organization of the daughter. However, several lines of evidence suggest that the mother CBB does not template the assembly of the daughter CBB. First, the two CBB microtubule structures are separated by a spatial gap of ~150 nm. In addition, tubulin does not exchange between the new and old structures suggesting that a template tubulin structure is not provided to the daughter (Kochanski and Borisy, 1990; Pearson et al., 2009). Finally, centrioles can also form at locations other than next to the mother (Klos Dehring et al., 2013; Sandoz and Biosvieux-Ulrich, 1976; Zhao et al., 2013). Thus, although they are closely juxtaposed, the formation of new CBBs does not require a structural template to be provided by the mother CBB, and is therefore unlike DNA replication. However, the close association might reveal a mother-CBB-associated platform on which the self-assembly of CBBs occurs.

Despite the clear separation of CBB triplet microtubules, recent cryo-tomography studies have captured an intriguing structure that directly connects mother and daughter centrioles. A stalk of ~110 nm links the microtubule wall of the mother centriole to the daughter cartwheel (Fig. 1D) (Guichard et al., 2010). Similarly, a proximally localized structure that has been identified in protist (ciliate and algal) basal bodies, called the generative or amorphous disk, might also play an analogous role in linking the mother and daughter basal bodies during the early stages of assembly (Allen, 1969; Dippell, 1968; Cavalier-Smith, 1974). The mother CBB functions to provide a platform onto which new CBBs self-assemble. This site might be required to recruit assembly components to increase the efficiency of assembly. Further understanding of these early structures that form at the proximal end of CBBs during assembly might answer whether and how mother CBBs contribute to the recruitment of assembly factors for new assembly (Box 1).

Despite the finding of structural features that appear to couple mother and daughter CBBs early in CBB assembly, the fundamental role of this association remains poorly resolved. Most likely the spatial gap between mother and daughter CBBs contains components that constitute the stalk linker and additional components that have been described (Fig. 1D) (Guichard et al., 2010; Mardin and Schiebel, 2012). As imaging tools become more advanced, the molecular basis of the links between nascent CBBs, their mothers and their surrounding environment might be resolved. Despite the lack of detailed understanding, it is apparent that the immediate environment surrounding existing CBBs contributes to the formation and position of the new CBBs. Moreover, the localized site of assembly that is defined by the mother CBB establishes the position and orientation of CBB biogenesis within cells.

Asymmetric basal body biogenesis

A defined site of new basal body formation

New CBBs form at approximately right angles at the base of mother CBBs. Whether the position of new CBB assembly occurs at a defined site relative to the nine symmetrically placed triplet microtubule blades remains an important question in centriolar systems. However, because each triplet microtubule blade of algal and ciliate basal bodies are easily distinguished by the accessory structures that protrude asymmetrically from them, the defined site of new basal body assembly in these systems is well established. In *Chlamydomonas*, basal bodies are important for cellular asymmetries and flagellar function (Bahe et al., 2005; Dutcher, 2007; Feldman et al., 2007; Holmes and Dutcher, 1989; Mardin et al., 2010; Marshall, 2012; Geimer and Melkonian, 2004; O'Toole and Dutcher, 2013; Dutcher, 2013). New basal body assembly occurs near a defined triplet microtubule (triplet microtubule number 8) relative to the accessory structures of the mother and this ensures transmission of intracellular patterning to the next generation of cells (Fig. 1E) (O'Toole and Dutcher, 2013). Mother centrioles instruct the positioning of new basal bodies, the nucleus, and polarized structures within *Chlamydomonas* cells (Holmes and Dutcher, 1989; Feldman et al., 2007). Similar to algal systems, new basal body assembly in ciliates follows a fixed profile for positioning new basal bodies (Beisson, 2008; Dippell, 1968; Iftode and Fleury-Aubusson, 2003; Wloga and Frankel, 2012).

The rapid cellular motility of ciliates depends on the exquisite organization of hundreds of basal bodies and their associated undulating cilia that line the cell cortex (Fig. 2A), and their organization has been a focus of a number of studies (Beisson and Sonneborn, 1965; Frankel, 1989; Kirschner et al., 2000; Ng and Frankel, 1977). To achieve this organization, new basal bodies must form at an established site within the local environment of the mother basal body. In their studies of cortical inheritance in *Paramecium*, Beisson and Sonneborn stated, "Newly formed molecules do not enter a vacuum, but a structured cell, the molecules of which are an essential part of the determinism for locating, orienting, and patterning new molecular formations" (Beisson and Sonneborn, 1965). The perpetuation of basal body and cilia organization along the length of the ciliary rows or kinety of a cell is termed 'cytotaxis' (Sonneborn, 1964) or 'structural inheritance' (Beisson, 2008). In *Paramecium* and other ciliates, nascent basal bodies form anteriorly and adjacent to pre-existing mother basal bodies whose polarized appendage structures define the basal body assembly site (Fig. 2) (Beisson and Jerka-Dziadosz, 1999; Iftode and Fleury-Aubusson, 2003; Kirschner et al., 2000; Pearson and Winey, 2009). The position of new ciliary units is dictated by the local architecture of the parental ciliary unit from which each new cilium develops. Moreover, individual units organize within the global cortical polarity; basal bodies are positioned and oriented along the anterior–posterior axes of the cell (Fig. 2). Thus, the propagating event in cytotaxis is the asymmetric positioning of new basal bodies.

The molecular and structural environment surrounding basal bodies

New basal bodies assemble at defined positions relative to the geometry of the existing mother basal bodies and their associated appendages. Three major appendages protrude asymmetrically from *Tetrahymena* and *Paramecium* basal bodies, and make asymmetries in their basal body architecture easy to identify. There are two microtubule-based structures: the post-ciliary microtubules and the transverse microtubules (Fig. 2B,C). The post-ciliary microtubules are nucleated from the posterior base of basal bodies and extend radially upward towards the plasma membrane and cell posterior. Transverse microtubules form anteriorly to basal bodies and also extend tangentially towards the plasma membrane, but are oriented such that they extend leftwards (from the point of view of the cell) towards an adjacent cortical row. These basal body appendages are generally thought to act as scaffolds that position basal bodies by defining where they assemble and/or restricting their movement following

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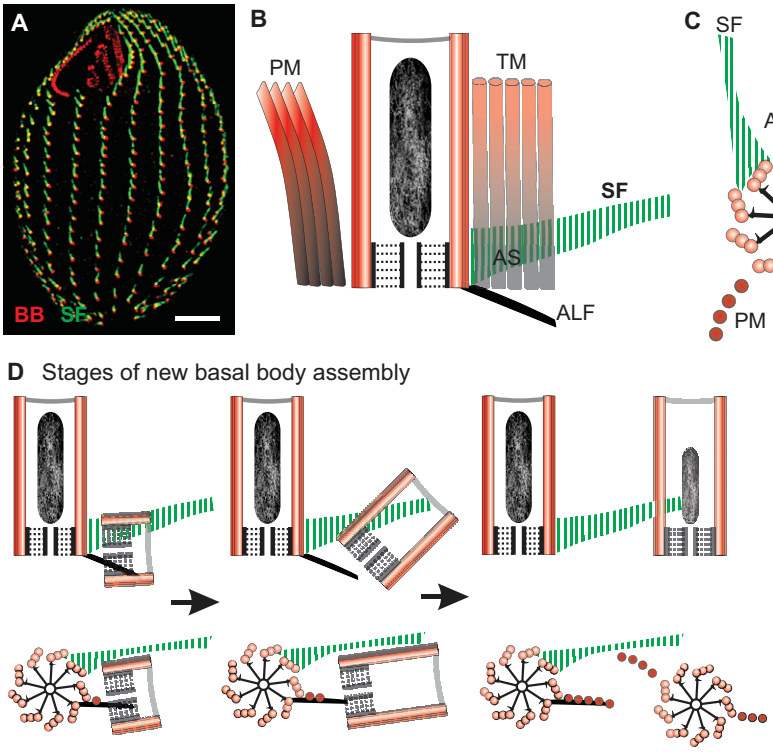

Fig. 2. *Tetrahymena* basal body organization and assembly. (A) Basal body organization in *Tetrahymena* cells with basal bodies (centrin in red) and striated fibers (striated fiber in green) shown. Scale bar: 5 µm. (B,C) Longitudinal (B) and cross section (C) schematic diagrams of the *Tetrahymena* basal body and associated structures. PM, post-ciliary microtubules; TM, transverse microtubules; SF, striated fiber (i.e. *Tetrahymena* kinetodesmal fiber); AS, new basal body assembly site; ALF, anterior left filament. Schematics adapted from Pearson and Winey (Pearson and Winey, 2009) with permission. (D) Structural stages of new basal body assembly in *Tetrahymena*. Nascent basal bodies form at the base of the mother basal body and move along the striated fiber. The presence of ALF occurs coincidently with new assembly and positioning. The ALF is shown to position basal bodies at the cell cortex as suggested previously (Jerka-Dziadosz et al., 2013). Illustrations adapted from Allen (Allen, 1969) with permission.

assembly. However, the mechanism for how these microtubule structures position basal bodies is not understood.

The third type of basal body appendage is the striated fiber. In *Tetrahymena* this is the kinetodesmal fiber, in *Paramecium* the striated rootlet, and in *Chlamydomonas* the striated microtubule-associated fiber (Fig. 2) (Allen, 1969; Holmes and Dutcher, 1989; Iftode and Fleury-Aubusson, 2003; Jerka-Dziadosz et al., 1995; Lechtreck and Melkonian, 1991; Munn, 1970; Sperling et al., 1991). Striated fibers extend from a defined triplet microtubule site on the basal body at the base of mature basal bodies and, as their name suggests, are composed of fibers with striations of ~30-nm periodicity. Striated fibers project anteriorly from basal bodies during their maturation. New basal bodies assemble and follow the path of the striated fibers emanating from the mother basal body (Fig. 2D). Even after separation from the mother, the mature daughter basal body remains associated with the striated fiber of the mother basal body through attachment using its post-ciliary microtubules (Fig. 2D) (Allen, 1969; Iftode and Fleury-Aubusson, 2003). This association might facilitate the structural inheritance of basal body cortical organization, which maintains the normal order of cilia. A protein known to comprise the striated fibers is the microtubule-associated striated fiber assembling protein (SF-assemblin), which was originally discovered in the green algae *Spermatozopsis similis* (Lechtreck and Melkonian, 1991). However, the molecular organization and function of striated fibers that allows them to direct new basal body assembly and positioning remains unexplored.

Although the appendages described above are generally permanent residents of the basal body, recent studies have identified transient basal body appendages that provide insights into the initiation and propagation of new basal body assembly, and their positioning at the cell cortex once they are assembled. For instance, the anterior left filament (ALF) (Fig. 2B–D) is a transient appendage that appears coincident with new basal body assembly and then disappears once the basal body docks at the cell cortex (Jerka-Dziadosz et al., 2013). ALF formation in *Paramecium* requires centrin-3, which is necessary for basal body positioning at the cortex. A similar centrin protein (centrin-2) marks the site of new basal body assembly (Ruiz et al., 2005; Stemm-Wolf et al., 2005) and is required for CBB biogenesis, stabilization, and/or its positioning in most organisms where its function has been tested (reviewed in Dantas et al., 2012; Pearson and Winey, 2009).

The asymmetric localization and importance of centrins in basal body formation and positioning in ciliates and algae argues that they play a role in determining the site of nascent basal body biogenesis in these organisms. Moreover, studies in yeast suggest that the centrin and its binding partner Sfi1 are also important for the asymmetric position of the yeast centrosome (spindle pole body) during its duplication (Kilmartin, 2003). The conserved Sfi1 family of proteins comprises at least 13 members in *Tetrahymena*. They localize asymmetrically relative to the basal body and this localization identifies previously unrecognized molecular domains surrounding the basal body (Stemm-Wolf et al., 2013). Each Sfi family member localizes to distinct regions that radially surround the basal body, and several (Sfr8, Sfr10 and Sfr11) localize to the site of new basal body assembly, as does centrin. This suggests that transient protein and structural asymmetries define the unique basal body architecture that is important for new basal body assembly. These domains, combined with structures such as the striated fiber, are likely to be responsible for organizing the formation of new basal bodies along ciliary rows at predictable positions. Proteomic work also identified basal body components (BBCs) (Kilburn et al., 2007) that localize to the future site of basal body formation, among these are Bbc14, Bbc20, Bbc31 and Bbc71 (also known as Poc1), although it is not known whether and how these proteins function in the biogenesis and positioning of basal bodies. Thus, molecular asymmetries surrounding basal bodies suggests that, like the structural environment surrounding basal bodies, there is a local

molecular environment that dictates the position of new basal bodies.

In summary, mother basal bodies in ciliates organize a site sandwiched between the striated fiber, the transverse microtubules and the ALF structures for new basal body biogenesis (Fig. 2B,C). This narrow region harbors the structural environment for assembly; however, its detailed structure and the functional components therein are not well established. Nevertheless, they point to a crucial role of the local environment during basal body biogenesis.

Basal body biogenesis within the local environment

The impact of the above-described molecular and structural environment surrounding mother basal bodies on the assembly of triplet microtubules of the new basal body is less clear. It has been suggested that, in *Paramecium*, the striated fibers are important for basal body triplet microtubule assembly mainly based on three observations (Iftode and Fleury-Aubusson, 2003). First, new basal body assembly occurs in the immediate proximity of striated fibers (Allen, 1969; Iftode and Fleury-Aubusson, 2003; Lechtreck and Grunow, 1999). Second, γ-tubulin is required for basal body assembly and associates with both basal bodies and striated fibers (Ruiz et al., 1999; Shang et al., 2002). Third, triplet microtubules adjacent to the striated fiber are longer and more mature compared with those distal to the striated fiber (Iftode and Fleury-Aubusson, 2003). However, it remains to be determined how exactly the striated fiber promotes the assembly of basal body microtubules. The identification of additional components that comprise striated fibers is expected to facilitate future functional studies.

The formation of the ALF structure coincides with new basal body assembly and had thus been suggested to promote the formation of new basal bodies (Jerka-Dziadosz et al., 2013). However, it appears that the primary function of ALF is to position basal bodies near the cell cortex (Jerka-Dziadosz et al., 2013) after their assembly. Consistent with this model, mutations that impair ALF formation (e.g. in centrin-3) do not affect the formation of new basal bodies but instead prevent their appropriate migration and positioning to the cell cortex (Jerka-Dziadosz et al., 2013).

The above studies in ciliates, such as *Paramecium* and *Tetrahymena*, describe a structural environment in which new basal bodies are assembled. Daughter basal bodies form at the base of their mother and are assembled in tight association with striated fiber structures that appear to support basal body microtubule assembly and, generally, basal body positioning at the cell cortex. Despite newly identified molecular components that localize to the region of assembly, the events and components that initiate basal body assembly and positioning in ciliates remain unclear. A key component for the initiation of centriole duplication in metazoans is Polo-like kinase 4 (PLK4) (Box 1). However, the regulators that perform the role(s) of PLK4 in ciliate basal body assembly remain to be discovered, as PLK4 is not conserved in ciliates (Carvalho-Santos et al., 2010; Hodges et al., 2010). Regardless, combined studies from divergent basal body (ciliates) and centriolar systems might inform both the environment of assembly as defined in ciliates and the regulators of assembly that have been discovered in centriole-containing systems.

Asymmetric centriole biogenesis

In contrast to the architecture surrounding ciliate basal bodies, centrioles are embedded in a matrix of electron-dense material known as the pericentriolar material (PCM), which appears amorphous in thin-section electron microscopy micrographs (Bornens, 2002), but was recently found, by using super-resolution fluorescence imaging, to be organized in a modular architecture (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012; Sonnen et al., 2012; reviewed in Lüders, 2012; Mennella et al., 2013). This modular architecture is defined by unique protein domains that form concentric rings encircling the mother centriole (Fig. 3A). The details of this modular architecture provide insights into the asymmetric positioning of centriole assembly and reveal the functional importance of the PCM in new centriole biogenesis.

Morphology of the pericentriolar material

The PCM at the proximal end of the centriole adapts a radial pattern of different protein domains, a feature that was shown for human pericentrin (PCNT) and its homolog in *Drosophila*, pericentrin-like protein (PLP, also known as Cp309) (Fig. 3A) (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012; Sonnen et al., 2012). Here, the C-termini of PCNT and PLP are positioned at the mother centriole, whereas the N-termini project outwards from the centriole where they organize a filamentous scaffold in the PCM. Moreover, the organization of PCNT/PLP displays a ninefold symmetry, suggesting the pericentrin scaffold is initiated from the centriole triplet microtubule blades and projects this symmetry outward in a radial and toroidal architecture (Fig. 3A,C). Additionally, the components of the PCM are structured as a series of concentric rings that extend along the length of PCNT, from its C- to the N-terminus. In human cells, this organization spatially moves outwards from the centriole walls in the following order: CEP120, CEP192, CEP152, CDK5RAP2, NEDD1 to TUBG1 (Fig. 3A; Lawo et al., 2012), whereas the *Drosophila* PCM organization is similar, but somewhat less complex (Mennella et al., 2012). Many of the above components are important for centriole assembly and their organized architecture promotes centriole biogenesis (Box 1). For example, CEP192 and CEP152 recruit PLK4 to the site of new centriole biogenesis, and perhaps the organization within the PCM initiates these first events. Moreover, the mother centriole and the PCNT/PLP scaffold are required to maintain the discrete PCM domains that are described above (Lawo et al., 2012; Mennella et al., 2012; Sir et al., 2013). The architecture of the PCM suggests that there are unique functional domains in what was previously thought to be a merely ‘amorphous structure’.

Centrosomes are dynamic during the cell cycle. PCM proteins increase in amount and their distribution expands to cover a greater area surrounding the centrioles during mitosis (reviewed in Palazzo et al., 1999). Similarly, the above-described radial, ninefold rotationally symmetric architecture of the PCM is also dynamic such that the PCM, which is closely associated with the centriole during interphase, expands during mitosis and becomes less toroidal (Fig. 3C) (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012; Sonnen et al., 2012). Both PCNT and CDK5RAP2 (Cnn in *Drosophila*) modulate the PCM expansion; when the amount of either protein is increased, the size of the PCM increases (Loncarek et al., 2008; Lawo et al., 2012; Conduit et al., 2014; Megraw et al., 1999). These studies suggest that these components modulate the dynamics of the PCM. Following expansion that reaches its maximum during metaphase of mitosis, the centrosome reduces in its size during anaphase and is reorganized for the start of the cell cycle and perhaps centriole biogenesis.

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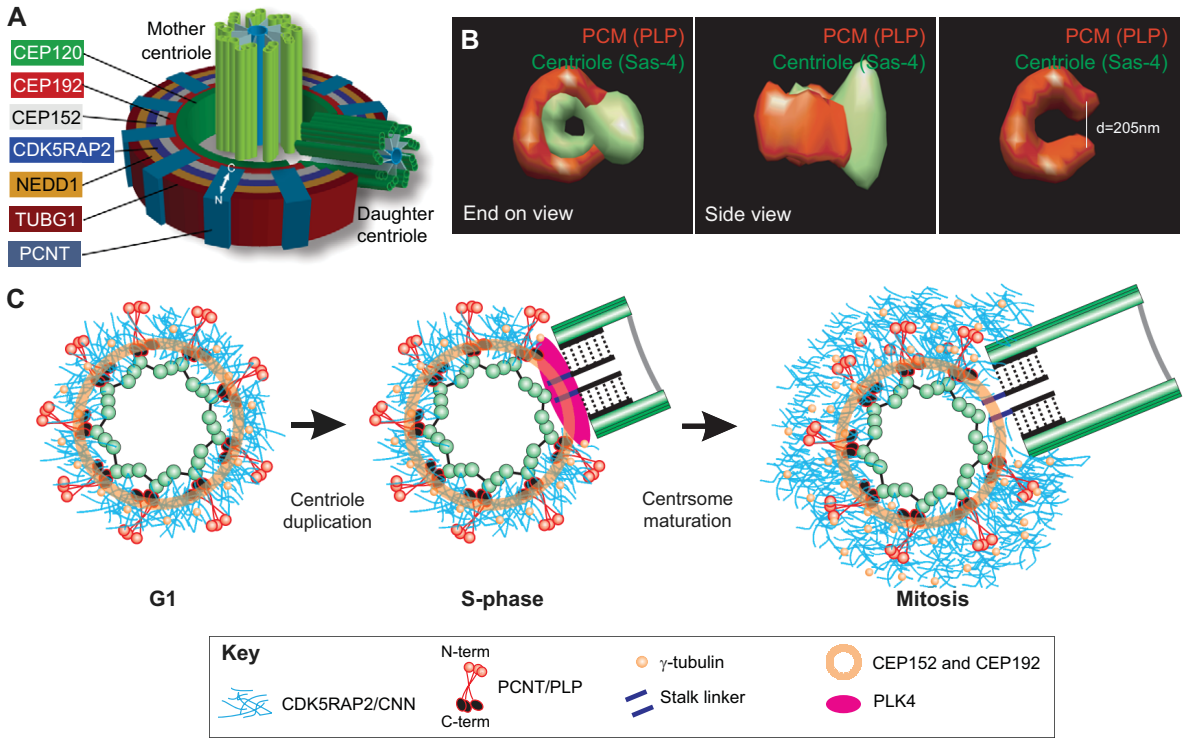

Fig. 3. Vertebrate centriole and PCM organization and assembly. (A) Modular organization of the PCM into toroidal domains. The C-termini of pericentrin (PCNT; in blue) are positioned near the centriole triplet microtubule blades, whereas the N-termini project away from the centriole. Concentric rings of protein domains localize to the surrounding architecture with CEP120 (green) closest and γ-tubulin (dark red) furthest from the centriole wall. Image taken from Lawo et al. (Lawo et al., 2012) with permission. (B) The PCM 'molecular gate'. The gap (shown in the image on the right) is thought to accommodate the newly forming centriole (marked with Sas-4, green). Shown here are different views of the volume rendering of *Drosophila* PCM organization in G2 cells with PLP in red and the centriole marker Sas-4 in green. Image adapted from Mennella et al. (Mennella et al., 2012) with permission. (C) Model of PCM organization around centrioles during the cell cycle highlighting centriole and PCM dynamics. In G1, the PCM retains a toroidal organization and, during S-phase, the nascent centriole is accommodated by loss of one PCNT/PLP cluster. Centriole duplication occurs through CEP152 and CEP192-dependent loading of PLK4 at a single focus, where new centriole biogenesis will initiate and begin with cartwheel formation. Upon mitotic entry, the PCM expands its general organization and γ-tubulin-dependent microtubule nucleation capacity. The illustration is based on work described in Fu and Glover, 2012, Lawo et al., 2012, Mennella et al., 2012 and Sonnen et al., 2012.

Building new centrioles in their pericentriolar environment

The PCM is important for nascent centriole biogenesis. Laser ablation of centrosomes and centrioles is followed by *de novo* centriole formation, with the new PCM forming first, before centriole biogenesis proceeds (Khodjakov et al., 2002). Disruption of PCM components in *C. elegans* abrogates centriole formation (Dammermann et al., 2004), and overexpression of PCM components, such as pericentrin, in S-phase-arrested U2OS cells, a human osteosarcoma cell line, increases the number of centrioles, arguing that the size of the PCM affects centriole duplication (Loncarek et al., 2008). Thus, it is clear the PCM promotes the formation of new centrioles and is negatively regulated in order to limit centriole replication.

This raises the question of how the PCM is organized to promote the delicate balance that is required for the assembly of only one daughter centriole and to perhaps also position the nascent centriole at a defined site within its architecture. Prior to the expansion of the PCM that occurs during mitosis, the PCM architecture is finely altered during new centriole biogenesis (Fig. 3C). The scaffold of toroidal PCNT/PLP is disassembled next to a specific triplet microtubule to create a gap and break in the circumferential symmetry of the PCNT/PLP organization (Fig. 3B) (Lawo et al., 2012; Mennella et al., 2012). This asymmetry has been coined the 'molecular gate' and appears to be formed by the loss of one of the nine postulated PCNT/PLP clusters that form around the centriole (Mennella et al., 2012). The molecular gate is ~200 nm wide, consistent with the diameter of pro-centrioles (150–200 nm). Thus, a compelling model is that the PCM, represented by PCNT/PLP, forms a spatial gap to promote the asymmetric assembly of the daughter centriole at a single triplet microtubule. An important test of this model is to understand when the molecular gate is formed relative to centriole formation. Perhaps the gate is formed prior to centriole assembly during G1 in order to make way for daughter centriole self-assembly. Alternatively, new assembly could initiate gate formation. Whether the molecular gate is formed simply by the removal of PCNT/PLP clusters or by insertion of other factors that displace PCNT/PLP is not known. In addition, it is important to determine what happens to the PCM architecture when centrioles over-duplicate in multi-ciliated cells, when multiple centrioles form from the same mother centriole. This concept might also be applicable in cancer cells and experimental conditions where multiple centrioles coincidentally assemble from the same mother centriole. It will be interesting to determine whether the plasticity of the PCM is consistent with the number of new centrioles formed and whether centrosome over-duplication in cancer represents a state where the molecular gate is unregulated, thereby leading to promiscuous centrosome amplification.

The formation of the molecular gate reveals that the centrosome architecture is modulated to accommodate new

centriole assembly but it remains to be determined when and why this gate forms. Perhaps gate formation is initiated coincident with the docking of early centriole assembly factors, such as CEP152, CEP192 and PLK4 (Box 1). For example, PLK4 initially organizes as a toroid at the mother centriole during G1 but then forms a focus at the site of new centriole biogenesis prior to the loading of the cartwheel proteins Sas-6 (also known as SASS6) and STIL (Kim et al., 2013; Sonnen et al., 2012). PLK4 and its loading factors, CEP152 and CEP192, could trigger the reorganization of the PCM to accommodate the centriole. Alternatively, the molecular gate might form during the separation of centrioles at the metaphase-to-anaphase transition, an event that licenses centrioles for replication in the next cell cycle (Tsou and Stearns, 2006). The separation of centrioles is likely to be initiated by separase, a cysteine protease (Tsou et al., 2009), and surprisingly also requires cleavage of cohesin by separase (Nakamura et al., 2009; Schöckel et al., 2011). An additional substrate of separase is the pericentrin B (kendrin) isoform that regulates centriole separation (Lee and Rhee, 2012; Matsuo et al., 2012) and is organized in a toroid manner similar to PCNT (Mennella et al., 2013). Moreover, when cleaved, the N-terminus of pericentrin B is removed from centrosomes (Lee and Rhee, 2012; Matsuo et al., 2012). This invokes a model where localized cleavage of pericentrin B clears the path for new centriole formation in the subsequent cell cycle by allowing the attached sister centrioles to separate and remove the PCM scaffold from a single site on mother centrioles, thereby producing the molecular gate. It seems plausible that this molecular gate serves two purposes: first, to establish a platform to concentrate the factors that are required for efficient centriole biogenesis and, second, to limit the number of nascent centrioles to only one.

After centriole biogenesis is initiated (Box 1), the association between the PCM and newly forming centrioles might play an important role in promoting the formation of centriole microtubules as proposed for the *Paramecium* striated fiber (Iftode and Fleury-Aubusson, 2003). Indeed, a number of microtubule assembly factors (including γ-tubulin) associate with the PCM. Perhaps the pericentrin-dependent organization of the PCM that surrounds the daughter centriole also prevents its movement, particularly at the distal end, whereas anchorage of the cartwheel to the mother centriole wall limits its proximal end movements. This is also consistent with the tight association of centrioles when pericentrin B cannot be cleaved by separase (Lee and Rhee, 2012; Matsuo et al., 2012). I predict that the organized PCM is functionally analogous to the highly structured environment that surrounds ciliate and algal basal bodies. This structural environment might serve to both position centrioles and to positively and negatively modulate their biogenesis.

Conclusions and perspectives

CBB assembly occurs in an environment that influences its position, polarity and regulation, ultimately dictating the orientation of ciliary beating and flow, the orientation of spindle poles and the maintenance of cellular geometry. The orientation of new CBB formation is the first step in establishing the relative positions of the two CBBs. This orientation is obvious in ciliate and algal model systems where asymmetries in basal body organization dictate a point of assembly and a path for positioning. A potentially analogous environment exists in centriolar systems where the modular organization of the PCM might accomplish similar objectives. It remains to be determined whether a predetermined triplet microtubule functions as a platform for centriole biogenesis, as is found for basal body biogenesis in unicellular organisms. Markers for centriole asymmetries, such as those found in ciliates, are required to draw this conclusion. To reach this goal, future studies will need to define the site of new assembly on a molecular level. Understanding the landscape of this unique site, and the asymmetries surrounding centrioles, will help to elucidate how the environment surrounding centrioles influences the initiating events in centriole biogenesis and positions and stabilizes the daughter.

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Competing interests

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