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The Rise of the Cartwheel: Seeding the Centriole Organelle

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The cartwheel is a striking structure critical for building the centriole, a microtubule-based organelle fundamental for organizing centrosomes, cilia, and flagella. Over the last 50 years, the cartwheel has been described in many systems using electron microscopy, but the molecular nature of its constituent building blocks and their assembly mechanisms have long remained mysterious. Here, we review discoveries that led to the current understanding of cartwheel structure, assembly, and function. We focus on the key role of SAS-6 protein self-organization, both for building the signature ring-like structure with hub and spokes, as well as for their vertical stacking. The resemblance of assembly intermediates in vitro and in vivo leads us to propose a novel registration step in cartwheel biogenesis, whereby stacked SAS-6-containing rings are put in register through interactions with peripheral elements anchored to microtubules. We conclude by evoking some avenues for further uncovering cartwheel and centriole assembly mechanisms.

1. Introduction

The centriole organelle is one of the largest protein assemblies of eukaryotic cells. Centrioles are evolutionarily conserved microtubule-based structures that recruit the pericentrolar matrix (PCM). In this manner, centrioles assemble the centrosome that constitutes the major microtubule organizing center (MTOC) of most animal cells, and is thus fundamental notably for cell polarity and division. In non-proliferating cells, one centriole, which is referred to as the basal body in this circumstance, docks below the plasma membrane, where its constituent microtubules elongate to form the axoneme of the primary cilium or the flagellum in some cells. Through this role, centrioles are critical for cell signaling and motility.

The centriole is a cylindrical structure typically ≈500 nm in height and ≈250 nm in outer diameter, which is characterized by a striking radial symmetry of nine sets of microtubule blades at the periphery. The centriole is polarized along its height, with a proximal side from where the organelle initiates assembly, and a distal part involved notably in docking below the plasma membrane prior to axoneme formation.

In this review, we focus on the cartwheel, a structure crucial for building the proximal part of the centriole at the onset of organelle assembly. We first mention how the cartwheel has been discovered and characterized at the ultrastructural level, starting with observations by electron microscopy (EM) of resin-embedded specimens, to the elucidation of its native 3D architecture using cryo-electron tomography (cryo-ET). Second, we discuss how self-organization of SAS-6 proteins leads to the formation of signature ring-containing structures bearing a hub and spokes. We also mention how cell-free reconstitution uncovered that SAS-6 proteins can stack vertically in an autonomous manner. Third, we introduce a novel registration step in centriole assembly, whereby a stack of several SAS-6 ring-containing structures that are twisted with respect to one another are put in register, possibly through the action of peripheral elements such as Bld10p/Cep135. We conclude the review by discussing some important open questions for the years ahead and possible avenues to pursue them.

2. The Birth of the Cartwheel

Theodor Boveri discovered the centrosome and the centriole more than 100 years ago.[1] Conducting optical microscopy of stained embryos in the sea urchin and in the parasitic roundworm *Parascaris equorum*, he spotted two structures that appeared to act as organizing centers of the mitotic spindle and named them "Centrosomen."[2] In addition, Boveri detected two dots within each centrosome that he termed "Centriol."[3]

The nature of the centriole remained mysterious for over 50 years after these remarkable early observations, until the advent of electron microscopy helped unveil what previously was an invisible world. The year 1954 saw the first EM observation of the basal body, which is now known to correspond to the

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DOI: 10.1002/bies.201700241

centriole. Imaging the base of the cilium, Fawcet and Porter observed that the "basal body does not appear as a solid ring but as a ring of nine small circles disposed around the cross section of the central cavity."[4] Two years later, Bernhard and De Harven observed that the centriole within the mammalian centrosome had a similar structure.[5] Rapidly, the community realized that the basal body and the centriole were the same organelle,[6] confirming a hypothesis formulated in 1898 already by Henneguy and Lenhossek.[7,8]

A few years after the first EM observations of centrioles, another structure internal to the organelle became apparent through the work of Gibbons and Grimstone, who studied the exceptionally long cartwheel and centriole (≈3600 nm and ≈4000 nm, respectively) of the unicellular termite symbiont *Trichonympha sp*.[9] These authors reported that "extending the whole length of the proximal region is a remarkable structure that, in transverse sections, resembles the hub and spokes of a cartwheel. The 'hub' is a small open circle about 250 Å in diameter, from which nine 'spokes' radiate out as rather delicate lines about 45 Å thick. Each of these connects with a small structure (a ridge, or perhaps a series of short fibrils) on one of the lateral surfaces of subfiber A." Remarkably, this initial description of the cartwheel is very similar to our current knowledge about its architecture (**Figure 1**).

In the following years, the presence of the cartwheel was reported in other systems with canonical centrioles, including mammalian cells.[10] However, the function of the cartwheel remained unclear. In 1968, working with *Paramecium aurelium*, Dippell[11] found that the cartwheel is apparent at the very onset of centriole assembly, before the addition of A-microtubules and the completion of microtubule triplets. This led her to suggest "an organizing role to the hub-and-spokes placing the cartwheel before the horse," a proposal that has been also made shortly thereafter for *Tetrahymena*.[12]

The conserved major ultrastructural features of the cartwheel detectable by EM analysis of resin-embedded specimens are those described initially,[9] namely a central hub ≈20–25 nm in diameter and nine ≈40–45 nm-long spokes that emanate radially outwards from this hub (Figure 1). Furthermore, work in *Paramecium*[11] and *Chlamydomonas reinhardtii*[13] uncovered three additional ultrastructural features associated with the cartwheel (Figure 1). First, "amorphous disks similar in diameter to a basal body,"[13] visible below the cartwheel during early stages of procentriole assembly. Second, a density between the end of the spokes and the microtubule triplets, first mentioned as an "A-tubule attachment sheet"[13] and later renamed the pinhead.[6,14,15] A third ultrastructural feature entails cross-links between A-microtubules that are apparent strictly during the onset of procentriole assembly, perhaps reflecting a role during initial microtubule recruitment. These early EM analyses also established that the cartwheel is not a simple two dimensional (2D) structure, but instead a 3D assembly with an average height of ≈100 nm in most systems.[11–13,16] Furthermore, more recent work conducted with synchronized *Chlamydomonas* cells revealed variations in cartwheel height during the cell cycle, from ≈100 nm in procentrioles to more than ≈150 nm in more mature centrioles, before diminishing to ≈40 nm.[17]

3. The Native Cartwheel

As described above, conventional EM provided important information about cartwheel architecture, height, and conservation through evolution. However, the stepwise procedure to prepare samples, including dehydration, resin embedding, and staining with heavy metals, limited the final resolution that could be reached with this method. By contrast, cryo-EM is instrumental for preserving the native structure of samples and increasing the resolution.[18] Combining cryo-EM with tomography (cryo-ET), which enables one to reconstruct macromolecular structures in 3D from many micrographs taken at different angles,[19] revealed the native architecture of the cartwheel in the human centriole.[20] This provided the first evidence that the central hub is not a continuous tube, but rather a structure exhibiting periodicities along its ≈100 nm height. In addition, this work revealed the presence of an entity resembling the γ-TURC (γ-tubulin Ring Complex) at the minus end of the A-microtubule, and led to the suggestion that the B- and C-microtubules elongate bidirectionally from the wall of the A-microtubule (Figure 1). Moreover, a structure was found that was dubbed the stalk and which connects the outer wall of the parental centriole with the central hub of the cartwheel (Figure 1). The potential function and molecular composition of the stalk remains unclear to date. Interestingly, this analysis also confirmed previous observations that, in contrast to the situation in other systems, including *Chlamydomonas*[13] and *Tetrahymena*,[12] the cartwheel is absent in mature centrioles in human cells,[21] indicating that the cartwheel is dispensable for centriole maintenance in this case.

Although the above cryo-ET study of isolated human centrioles provided important knowledge about cartwheel ultrastructure, the resolution it provided remained limited. This issue was circumvented by analyzing the *Trichonympha sp*. centriole using cryo-ET coupled to sub-tomogram averaging, which led to a ≈38 Å resolution view of the proximal region of the organelle.[14,22] At this resolution, it became clear that the central hub of the cartwheel comprises a stack of rings exhibiting a vertical periodicity of ≈8.5 nm, from which emanate nine spokes. Moreover, this analysis uncovered that the spokes merge vertically two by two to form a ≈17 nm periodicity towards the periphery (Figure 1). Furthermore, a so-called Cartwheel Inner Density (CID), also exhibiting a radial nine fold symmetry, was found within the confines of the central hub. Careful examination of the electron density map also enabled subdivision of the pinhead into three sub-structures: a central-most pinbody and two more peripheral pinfeet-1 and -2, both contacting the A-microtubule (Figure 1).[14] Interestingly, this work also uncovered that the pinhead is a polarized structure along the proximal-distal centriole axis, with the two pinfeet being kinked towards the distal side (Figure 1, dark red and dark blue lines between the pinhead and the microtubule, respectively). Finally, this study revealed that the native structure of the A-C linker that connects adjacent microtubule triplets is also polarized along the proximal-distal centriole axis (Figure 1).

How distinct cartwheel elements and associated structures are organized during organelle assembly remains largely an open question. However, some clues came from a recent analysis using cryo-ET of isolated *Chlamydomonas* procentrioles,[23] which could

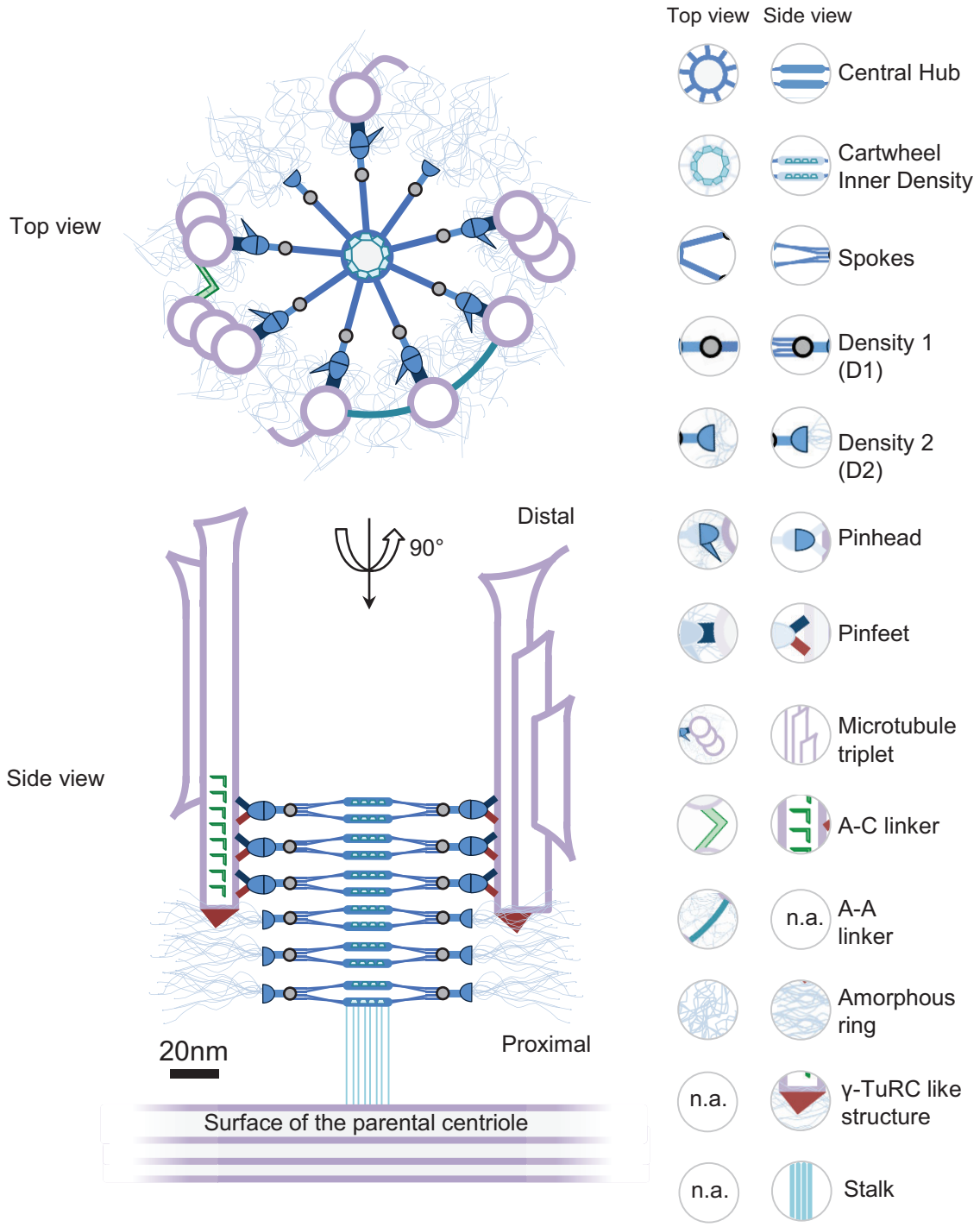

Figure 1. Schematic of the assembling cartwheel-containing procentriole. Top and side view, as indicated, of a procentriole assembling around a cartwheel emanating from the surface of the parental centriole. This schematic summarizes observations by EM in different species. See text for more information. n.a. indicates that the structure is not visible in this orientation.

be divided into three regions. First, a most proximal region that harbors only a cartwheel and no pinhead or microtubules; such a core cartwheel is characterized by two densities termed D1 and D2 (Figure 1). Second, a region in which the cartwheel is present, together with pinhead and microtubules and, third, a more distal region without cartwheel, but harboring pinhead and microtubules. Moreover, the previously described amorphous ring surrounding the core cartwheel was observed, as well as A-microtubules recruited onto the core cartwheel (Figure 1).[23] Interestingly, the presence of a core cartwheel without pinhead or microtubules suggests that such an entity may provide an initial scaffold onto which more peripheral elements then assemble.

4. Cartwheel Proteins

Despite the above discoveries made using EM starting in the late 1950s, the lack of knowledge about the nature of cartwheel proteins prevented understanding of the underlying assembly mechanisms and the potential function of the entire structure. The situation changed starting in the early 2000s, when forward genetic and RNA interference (RNAi)-based functional genomic screens in *C. elegans* led to the discovery of six proteins essential for centriole duplication: the kinase ZYG-1,[24] as well as the coiled-coil proteins SPD-2,[25,26] SAS-4,[27,28] SAS-5,[29,30] SAS-6,[29,31] and the more recently identified SAS-7.[32] Snapshots of centriole assembly captured with EM in one-cell *C. elegans* embryos revealed that the process begins with the formation and elongation of a central tube that is dependent on SAS-5 and SAS-6, and which might be the functional cartwheel equivalent in nematodes.[33] Subsequent studies demonstrated that SAS-6 homologues are essential for centriole duplication in many other systems, including *D. melanogaster*,[34,35] zebrafish (*Danio rerio*),[36] and human cells.[37]

Initial evidence that SAS-6 proteins are intricate to cartwheel structure came from work in *Chlamydomonas*, where mutant cells harboring a null allele in *bld12p*, the sole full-fledged SAS-6 homolog in this species, lack the cartwheel entirely.[38] Interestingly, whereas SAS-6 proteins appear essential for centriole formation in other systems, ≈15% of *Chlamydomonas bld12p* mutant cells nonetheless assemble a centriole. Therefore, while important, the cartwheel is not essential for centriole assembly in this system. However, such centrioles that are assembled without a cartwheel do not always exhibit a nine fold radial symmetry of microtubule triplets.[38] Overall, it can be concluded that the cartwheel is critical for centriole assembly and participates in setting the nine fold symmetry of the organelle, in line with the original suggestion regarding the role of this structure made in 1968.[11] Strikingly, in addition, immuno-EM analysis revealed that the Bld12p/CrSAS-6 protein localizes very close to the central hub,[38] which raised the possibility that SAS-6 proteins in general might be a key structural element of the cartwheel.[39]

SAS-6 proteins comprise an N-terminal globular domain containing the PISA motif (Present In SAS-6), which is its most evolutionarily conserved moiety, followed by a coiled-coil region predicted to be typically ≈45 nm long, and then a C-terminal part predicted to be unstructured.[40] Recombinant CrSAS-6 lacking the C-terminal part undergoes dimerization driven by the coiled-coil, as well as higher order oligomerization mediated by an interaction between N-terminal domains of adjacent dimers.[41,42] The resulting assembly can form a ring ≈22 nm in diameter, similar to that of the cartwheel hub in vivo, and corresponding to the predicted diameter of a ring containing 9 CrSAS-6 homodimers.[9] Remarkably, preventing higher order oligomerization of SAS-6 proteins by mutating a key interacting residue in the N-terminal domain abolishes centriole formation in human cells as well as in *C. elegans*,[41] and results in a lack of flagellum formation in *Chlamydomonas*.[42] Such experiments led to the proposal that SAS-6 ring oligomerization is at the root of cartwheel assembly.[41,42] Moreover, it has been reported that the C-terminal moiety of human HsSAS-6 promotes microtubule assembly in vitro,[43] raising the possibility of a direct interaction of SAS-6 proteins and the polymer.

In addition to CrSAS-6, the protein Bld10p is essential for cartwheel assembly in *Chlamydomonas*.[44,45] In contrast to *bld12p* mutants, *bld10p* mutants do not assemble centrioles at all. Moreover, immuno-EM analysis revealed that the Bld10p protein localizes to the cartwheel, both along and at the periphery of the spokes, where the pinhead lies, leading to the suggestion that Bld10p may serve to bridge the cartwheel proper with peripheral elements.[6,44] Compatible with this view, a C-terminal deletion of *bld10p* (ΔC2) leads to the assembly of cartwheels with shorter radial spokes and centrioles with eight or nine fold symmetry.[44]

Bld10p is an evolutionary conserved protein whose human relative is Cep135, and which is part of a set of fourteen centriole proteins that was present in the last common ancestor of all extant eukaryotes, but that was lost in some lineages thereafter.[46,47] Based on its predicted coiled-coil nature and a length of 1000–1600 amino acids, depending on the species, the predicted length of Bld10p/Cep135 proteins is of ≈150–240 nm (number of residues × 0.1485 nm axial rise[48]). How such a presumably long protein can be placed in the emerging procentriole remains to be clarified. An N-terminal fragment of Cep135 binds to microtubules in vitro and in vivo,[47] whereas a C-terminal fragment interacts with the C-terminal part of HsSAS-6,[48] further indicating that Bld10p/Cep135 proteins may bridge the central cartwheel with peripheral microtubule elements.

How do CrSAS-6 and Bld10p cooperate to ensure cartwheel assembly? Some clues emerged from engineering CrSAS-6 mutants with altered oligomerization and symmetry properties, which form 5- to 10-fold radially symmetrical rings in vitro, depending on the specific mutant.[51] Interestingly, CrSAS-6 mutants that lead primarily to six fold symmetries in vitro led to a majority of eight fold symmetric structures when transferred to *Chlamydomonas* cells. Remarkably, however, when combined with the *bld10p* mutant (ΔC2) that weakens interactions between cartwheel and microtubules, the same CrSAS-6 mutant could now generate six fold symmetric structures on occasion in vivo.[51]

Taken together, these observations indicate that CrSAS-6 is the major component of the cartwheel hub, forming a vertical stack of nine fold symmetrical rings with a ≈8.5 nm periodicity. CrSAS-6 coiled-coils emerge radially from this ring and merge every ≈17 nm towards the periphery, forming part of the spokes that feed into the pinhead structure, with Bld10p perhaps mediating the interaction between CrSAS-6 and microtubule triplets.

5. Cartwheel Registration

Although the analysis of SAS-6 proteins and associated components such as Bld10p has uncovered some of the mechanisms of ring assembly, the cartwheel is not a simple ring: SAS-6-containing rings must somewhat stack to achieve the 3D structure observed in vivo.[22]

Using cryo-ET on a cell-free assembly system, we reported last year that full length CrSAS-6 (CrSAS-6_FL) forms large condensates containing ring assemblies.[23] However, radial spokes were seemingly not organized in a proper manner, since

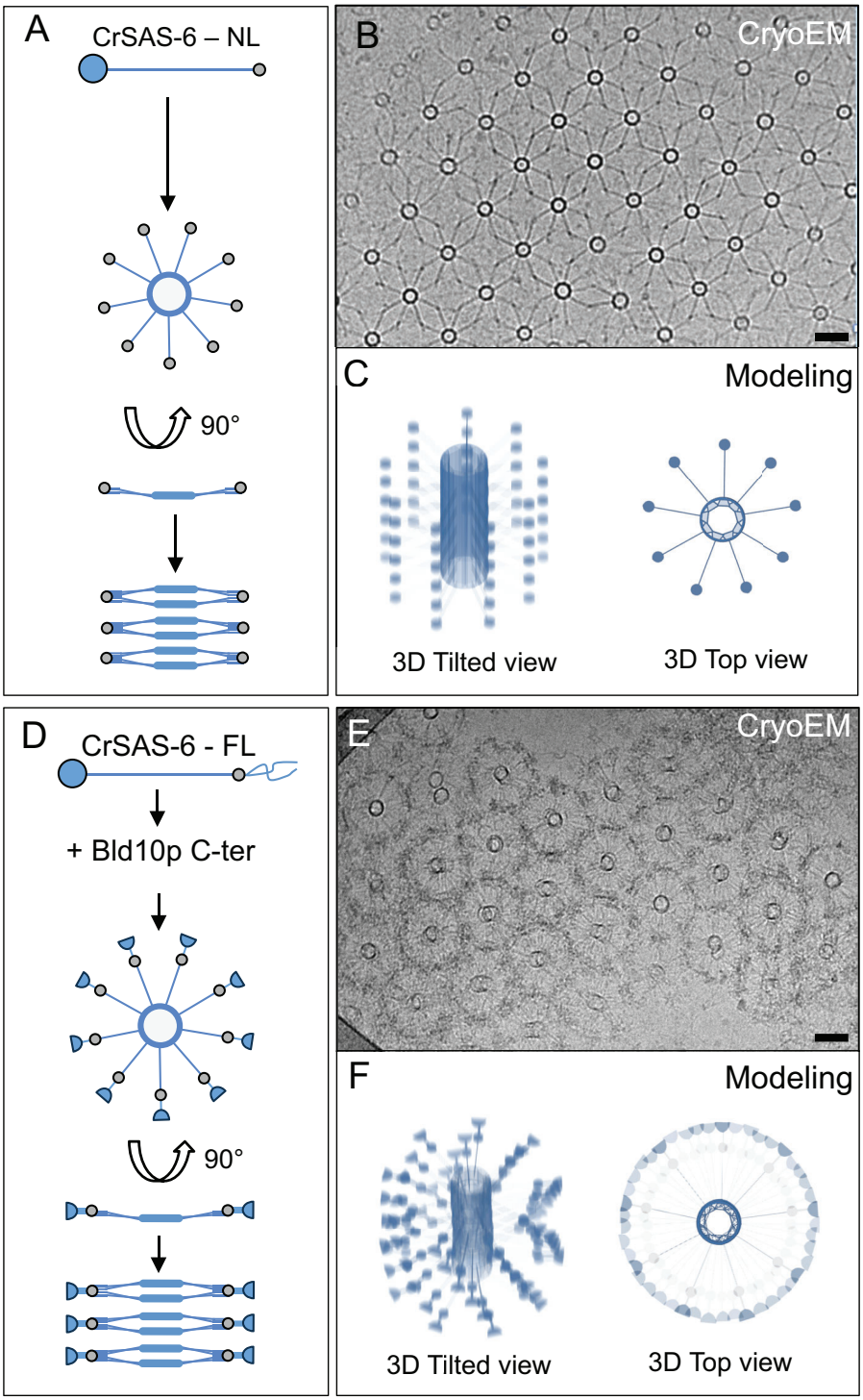

Figure 2. CrSAS-6 ring stacking in vitro. A-C) In vitro cartwheel-like assembly with CrSAS-6_NL. A) Model of cartwheel stacking with initial ring formation followed by stacking. B) Cryo-EM image of lattice organization of CrSAS-6_NL stacked cartwheel. C) Model representing tilted and top 2D views, as indicated, of a ≈100 nm high cartwheel-like assembly with spokes in register. Note similarity with experimental result in (B). D–F) In vitro core cartwheel-like assembly using CrSAS-6_FL+Bld10p_Cter. D) Model of cartwheel stacking triggered by the interaction of the C-terminus of CrSAS-6 with Bld10p. Note that the spokes of superimposed pairs of SAS-6 rings are not in register (not visible in this side view, but see panel (F). E) Cryo-EM image of honeycomb organization of in vitro reconstructed cartwheels. Note electron densities encircling the extremities of radial spokes, which tend to be incomplete on the lattice periphery, perhaps because interactions between CrSAS-6_FL and Bld10p are less favorable in that location. F) Model representing tilted and top 2D views of a ≈100 nm high reconstituted cartwheel with spokes not in register. B and E) reprinted with permission from.[23] Scale bars: 50 nm.

they could not be visualized clearly. Importantly, by contrast, CrSAS-6 proteins lacking the C-terminal part (CrSAS-6_NL) formed a 2D lattice of stacked rings in vitro. In addition, these cartwheel-like entities displayed clear D1 densities, as well as spokes superimposed in register and exhibiting the same vertical periodicities as observed in the cartwheel in vivo (**Figure 2**A-C). These results suggest that the C-terminal part of SAS-6 proteins negatively regulates ring stacking in vitro, and further raise the possibility that such negative regulation is alleviated by an interacting protein during organelle assembly. An obvious candidate for such a putative role was Bld10p. Accordingly, CrSAS-6-FL no longer formed condensates in the presence of a Bld10p fragment, but instead large honeycomb arrays of cartwheel-like structures (Figure 2D–F). Cryo-ET analysis revealed that these structures contained stacks of CrSAS-6 with radial spokes, as well as two densities located at the same distances from the hub margin as D1 and D2 in the cartwheel in vivo.[23]

Despite the remarkable similarity with the in vivo situation, the organization of spokes was barely visible in assemblies of CrSAS-6_FL/Bld10p proteins generated in vitro, although some stacking took place.[23] Interestingly, in addition, a large electron dense circle ≈100 nm in diameter was clearly detected (Figure 2D). To explain why spokes might be barely visible in such assemblies, it is important to remember how data is collected in transmission EM. Indeed, the image on the camera corresponds to a 2D average of all densities in the 3D layer of resin or ice, in essence analogous to what is obtained through a Z-projection in confocal microscopy. In the case of cartwheel assemblies, this implies that the spokes will appear as clear radial densities only if they are in register (Figure 2C). The same might apply to densities D1 and D2 if their visualization likewise depended on their stacking. By contrast, if spokes are not in register and instead exhibit a twist between each layer, then the resulting image will bear a clear central hub, whereas it will be difficult to distinguish the spokes, and perhaps also the D1 and D2 densities (Figure 2F). Therefore, one interpretation of the honeycomb assemblies obtained with CrSAS-6_FL/Bld10p is that while ring stacking does occur, the layers are not in register (Figure 2E–F), such that D1 and D2, whilst sometimes detectable as distinct entities, often appear as a large electron dense circle instead. That this is a plausible explanation can be appreciated by comparing the models of a cartwheel in register to one with twisted layers as illustrated in Figure 2C and F.

Could it be that such a "twisted stack" reflects a bona fide intermediate in cartwheel assembly? We reasoned that one way to address this possibility was to examine published EM images of physiological cartwheel assembly intermediates and compare them to the assemblies of CrSAS-6_FL/Bld10p obtained in vitro (**Figure 3**). For instance, a procentriole lacking microtubules

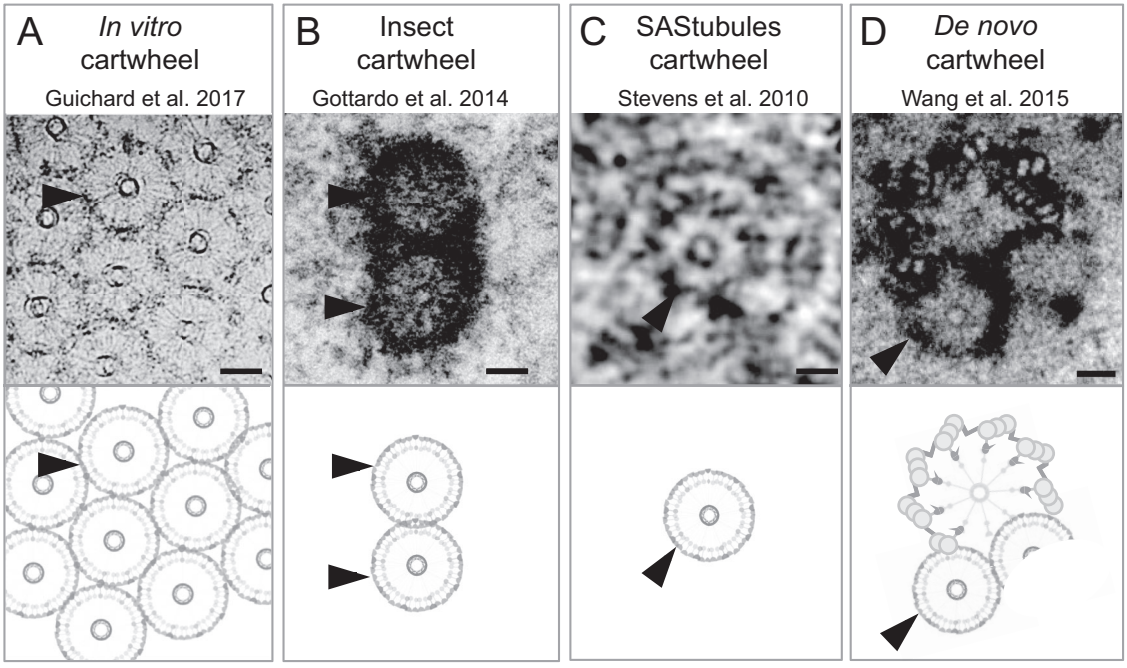

Figure 3. Registration of cartwheel spokes during procentriole assembly. A) Cryo-EM image (top) and corresponding schematic representation (bottom) of CrSAS-6_FL/Bld10p_Cter cartwheel reconstituted in vitro. Note the honeycomb organization and the electron dense circle ≈100 nm in diameter encircling the extremities of radial spokes (arrowheads). B) EM image (top) and corresponding schematic representation (bottom) of cartwheel-like entities lacking microtubules during meiotic prophase in males of the butterfly *Pieris brassicae*.[52] Note presence of electron dense circle ≈100 nm in diameter in this case also (arrowheads). Reprinted with permission from.[52] C) EM image (top) and corresponding schematic representation (bottom) of DmSAS-6/Ana2 tubule formation in *Drosophila* spermatocytes. Again, an electron dense circle is apparent here (arrowheads). Reprinted with permission from.[52] D) EM image (top) and corresponding schematic representation (bottom) of de novo cartwheel and centriole assembly in human cells. Note that the cartwheel onto which microtubule triplets are assembling (top) no longer exhibits the circular electron dense material present in what appears to be an earlier assembly stage (bottom left).

forms at the base of the parental centriole during meiotic prophase in males of the butterfly *Pieris brassicae*.[52] (Figure 3B). Suggestively, the cartwheel that forms in this instance bears a central hub and an electron dense circle ~100 nm in diameter, yet spokes are barely visible (Figure 3B). Likewise, overexpression of DmSAS-6 and Ana2 in *Drosophila melanogaster* spermatocytes leads to the assembly of so-called SAS tubules,[53] which are also surrounded by an electron dense circle (Figure 3C). Moreover, analogous intermediates have been observed during de novo centriole formation in human cells, with adjoined cartwheels sharing part of the same electron dense circle, clear central hubs and again less visible spokes (Figure 3D).[54]

Based on these suggestive observations, we hypothesize that a twisted cartwheel state might exist in vivo prior to registration and then microtubule addition.

6. Open Questions and Speculations

Important advances have significantly increased knowledge of cartwheel structure and function in recent years. At the same time, many new questions have arisen, which will be interesting grounds for further investigations.

6.1. Where to Start from

One important question is how cartwheel number is regulated, and from where exactly it arises. Part of the answer may come from the observation that the procentriole assembles in the vicinity of a particular microtubule triplet of the parental centriole in several unicellular organisms. Thus, the *Chlamydomonas, Paramecium,* and *Tetrahymena* centrioles contain asymmetric structural elements that enable specific triplet microtubules to be identified.[17,55,56] Interestingly, the cartwheel assembles next to a given microtubule triplet of the parental centriole in these species. This raises the possibility that the parental centriole is not a perfect radially symmetric structure, but instead a cylinder with distinct molecular ensembles next to given triplet microtubules, as is the case in flagella.[57] What may be the root of such putative asymmetries around the parental centriolar cylinder? In human cells, a complex containing the kinase Plk4 and the SAS-5/Ana2-related protein STIL provides a scaffold for recruiting HsSAS-6 to a single focus around the parental centriole.[58] By analogy with the situation in *Chlamydomonas, Paramecium,* and *Tetrahymena,* it is tempting to speculate that the single focus marking cartwheel assembly in human cells may also form next to a given microtubule triplet of the parental centriole, which is not recognizable by ultrastructural analysis.

6.2. Amorphous Ring and Pinhead, Two Faces of the Same Coin?

Forty years ago, an amorphous ring was described at the base of the procentriole in *Paramecium*.[11] Last year, we reported the presence of a similar amorphous ring in *Chlamydomonas* below the proximal part of the procentriole, surrounding the core cartwheel sticking out from the microtubule barrel.[23] Moreover, we showed that, with the addition of an interacting Bld10p fragment, CrSAS-6_FL forms a core cartwheel in vitro, which exhibits a large electron dense circle and sometimes contains D1 and D2 densities.[23] Intriguingly, Bld10p has been proposed to be a major pinhead component and its homologue Cep135 can interact with microtubules.[6,15,45,49,50,59] Taken together, these considerations lead us to speculate that peripherally located disorganized regions of Bld10p may constitute the amorphous ring; perhaps a conformational change in Bld10p then leads to it forming the pinhead and thus connecting the core cartwheel with microtubule triplets (**Figure 4**).

Given that the in vitro evidence suggests that the association of CrSAS-6_FL and a Bld10p fragment might first lead to the assembly of a twisted core cartwheel, with the spokes not in register, one can further propose that registration is attained after linkage with the microtubules locks the core cartwheel in place, perhaps with contributions from flexible linkers connecting adjacent microtubule blades.[21] Moreover, elements such as Bld10p that play a role in the connection between cartwheel and microtubules are expected to be important for establishing the chirality with which microtubule triplets assemble around the cartwheel.[15] This is in line with the finding that >50% of centrioles harbor misoriented microtubule triplets in *Tetrahymena bld10p* mutants.[60]

6.3. From a Ring to a Stack

Atomic structural information of SAS-6 proteins from a number of species revealed a ring ≈22 nm in diameter and ≈4.5 nm in height.[41,42,61] Intriguingly, however, fitting of the predicted SAS-6-based ring structures into the *Trichonympha sp.* cryo-ET map of the cartwheel shows that such rings are separated by a gap of ≈4 nm, without apparent molecular density, as if rings were "floating."[22] By contrast, the structure of the N-terminal domain plus the first seven heptad repeats of the coiled-coil domain of *Leishmania major* SAS-6 revealed the presence of rings piled onto one another without intervening gaps.[61] How can one explain such a lack of spacing compared to the situation in vivo or to the in vitro cartwheel reconstitution experiments with CrSAS-6_NL? One possible answer lies in the coiled-coil region, which is absent for the most part from the *Leishmania* construct utilized for crystallization. Based on these and other considerations, we propose that the coiled-coil of SAS-6 proteins somehow leads to structural tension that separates the SAS-6 rings by a few nanometers. The exact underpinning of such a postulated mechanism remains to be determined.

6.4. Cartwheel Inner Density: The Last Russian Doll?

The discovery of ever smaller components within the centrosome over the years is reminiscent of uncovering Russian dolls: the centriole within the centrosome, the cartwheel within the centriole and now the cartwheel inner density (CID) within the cartwheel.[14] Interestingly, cryo-ET and sub-tomogram averaging revealed that the CID exhibits contacts with the interface mediating higher order oligomerization of SAS-6 proteins.[14] Therefore, it is tempting to speculate that the CID serves a

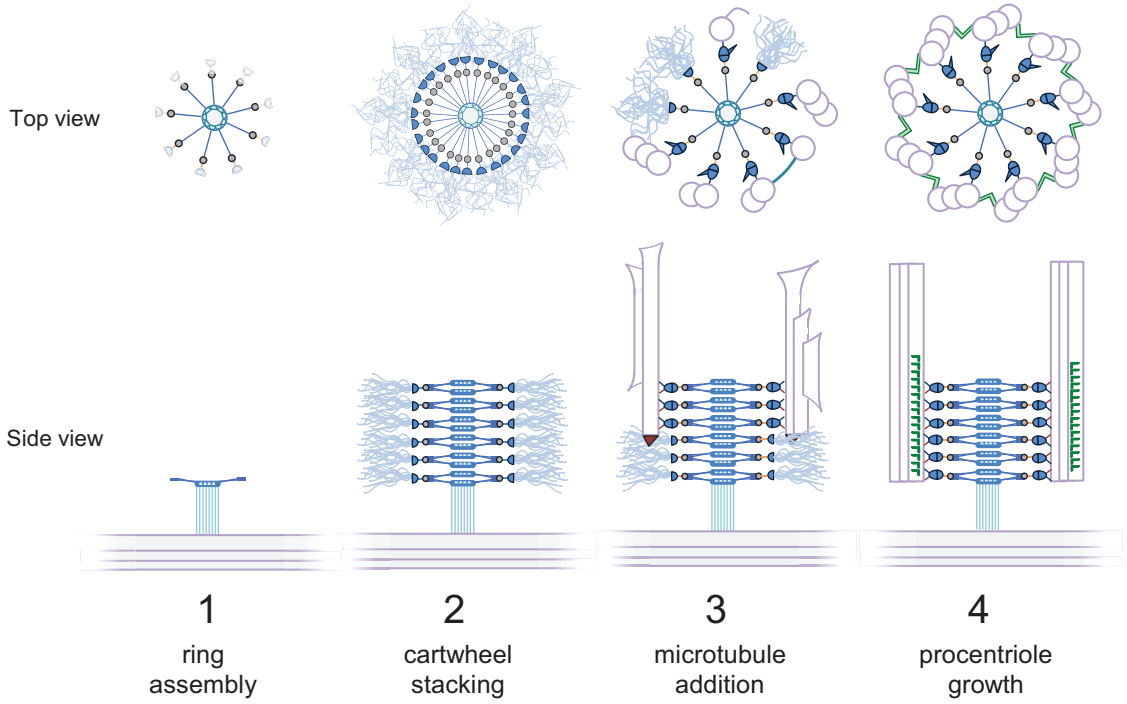

Figure 4. Working model of cartwheel assembly and registration. Four speculative steps characterizing cartwheel and procentriole assembly; top and side views, as indicated. See text for more information; structures represented like in Figure 1. 1: Ring of nine SAS-6 homodimers self-assemble through interactions mediated by adjacent N-terminal domains. 2: Interaction of CrSAS-6 with Bld10p promotes stacking. Note that pairs of SAS-6 rings are not in register at this stage, as can be seen in the top view. Note also that whereas the C-terminal domain of Bld10p interacts with CrSAS-6, its N-terminal domain is not organized in the absence of microtubules, thus potentially generating the amorphous ring. 3: Assembly of the A-microtubule interacting with the N-terminal domain of Bld10p triggers a conformational change that results in its incorporation into the pinhead. Furthermore, the B- and C- microtubules form at the surface of the A- and B- microtubules, respectively, not necessarily in synchrony, as illustrated. 4: Complete assembly of the procentriole around the cartwheel. The amorphous ring is no longer visible once all pinhead elements are formed. Note A-C linkers connecting microtubule triplets.

scaffolding function necessary to enable formation and maintenance of the exceptionally high cartwheel present in *Trichonympha sp.*[14] and thus be restricted to parabasalid organisms with such unusual centrioles. Such an exceptionally high cartwheel may be important to confer supplementary mechanical stability to centrioles subjected to strong flows in the intestine of the host termite. Alternatively, the CID may be present and important in other species as well, but escaped detection to date owing to the lack of detailed tomographic analysis.

6.5. *C. elegans* as an Outlier?

Differences from the canonical picture in cartwheel and centriole structure occur in numerous other species and are bound to be fertile grounds for unraveling potential variations in the mechanisms governing organelle assembly and function. One interesting case in point is that of *C. elegans*. Indeed, crystallographic and biophysical analysis of CeSAS-6 revealed that whereas the worm proteins can form higher oligomers *in vitro*, they adopt a helical structural rather than forming a stack of rings.[62] This may explain why a central tube instead of a canonical cartwheel has been reported in this organism in vivo.[33] This provocative finding raises the possibility that the same molecular interface leading to the higher oligomerization of SAS-6 proteins may have evolved in two distinct ways to impart a nine fold symmetrical arrangement of microtubules in the centriole organelle.

6.6. Role and Molecular Composition of the A-C Linker

Apart from the cartwheel, the procentriole harbors other conserved ultrastructural elements, including the A-C linker, which bridges the A-microtubule of a given triplet to the C-microtubule of an adjacent one (Figure 1). Interestingly, microtubule triplets that form in cartwheel-less centrioles of *bld12* null mutants can be connected by the A-C linker, and adopt a relatively normal position with respect to one another.[38] This led to the hypothesis that the A-C linker may dictate the angle between microtubule triplets and, thereby, participate in ensuring the nine fold radial symmetry of centrioles.[6,63] However, centriole-like entities can form in only ≈15% of *bld12* mutants, and sometimes harbor an incorrect number of microtubule triplets, together indicating that the A-C linker is not sufficient to efficiently impart the signature nine fold symmetry of the organelle. What is the molecular nature of components contributing to the A-C linker? Although a definitive answer to this question awaits further experiments, a promising candidate is the evolutionary conserved protein Poc1.[64] Interestingly, *poc1* null mutants in *Tetrahymena*[65] exhibit subtle defects in the A-C

linker, as well as loss of entire microtubule triplets in the proximal region and a change in the microtubule triplet twist along the centriolar length.[66] In addition, in human cells, POC1 localizes early during the assembly process to the proximal part of the procentriole, whereas its depletion reduces centriole numbers.[64] Together, these observations raise the possibility that Poc1 is important for A-C linker function and is perhaps a component of this structure. However, because the A-C linker is present, albeit in an altered form, in *Tetrahymena poc1* null mutants,[66] other proteins must at the least play a partially redundant role.

Another circumstance during which peripheral elements such as the A-C linker might play an important role is de novo centriole formation, for instance during development of early rodent embryos or some parthenogenetic species, as well as following experimental ablation of resident centrioles in proliferating human cells. Surprisingly, whereas HsSAS-6 is required for de novo centriole formation, the interaction between its N-terminal head domains that normally drives higher order oligomerization is not needed in this case.[54] One possible explanation is that peripheral components such as the A-C linker or pinhead components play an instructive role during de novo centriole assembly. Alternatively, centriole assembly might be promoted by an internal structure such as the CID in this case. Regardless of the actual mechanism, de novo centriole assembly lacks precision, since an abnormal number of centrioles can be generated, including ones exhibiting structural abnormalities.[54]

7. Conclusions

In conclusion of this review, much progress has been made to date in discovering and understanding the cartwheel. In recent years, the key role of SAS-6 proteins in forming and stacking the characteristic ring-containing elements has been elucidated, and we proposed here a novel registration step that may follow during the assembly process. We expect future investigations of cartwheel assembly architecture to yield further fundamental insights into the mechanisms governing formation of the centriole organelle. Once this is achieved, it should be possible to go from the smallest Russian doll to the largest one, in other words to engineer the entire organelle from the orderly assembly of its component parts.

Abbreviations

CID, cartwheel inner density; cryo-ET, cryo-electron tomography; EM, electron microscopy.

Acknowledgements

We thank Anne-Marie Tassin, Michel Bornens, George Hatzopoulos, and Maeva Le Guennec for critical reading of the manuscript. PaG and VH are supported by the Swiss National science Foundation (SNSF) PP00P3_157517. Work on centriole assembly in the lab of PG is supported by the European Research Council (AdG 340227).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

basal bodies, cartwheel, centrioles, cilia, SAS-6

Received: December 13, 2017
Revised: January 21, 2018
Published online: March 6, 2018

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