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Centriole structure

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Centrioles are among the largest protein-based structures found in most cell types, measuring approximately 250 nm in diameter and approximately 500 nm long in vertebrate cells. Here, we briefly review ultrastructural observations about centrioles and associated structures. At the core of most centrioles is a microtubule scaffold formed from a radial array of nine triplet microtubules. Beyond the microtubule triplets of the centriole, we discuss the critically important cartwheel structure and the more enigmatic luminal density, both found on the inside of the centriole. Finally, we discuss the connectors between centrioles, and the distal and subdistal appendages outside of the microtubule scaffold that reflect centriole age and impart special functions to the centriole. Most of the work we review has been done with electron microscopy or electron tomography of resin-embedded samples, but we also highlight recent work performed with cryoelectron microscopy, cryotomography and subvolume averaging. Significant opportunities remain in the description of centriolar structure, both in mapping of component proteins within the structure and in determining the effect of mutations on components that contribute to the structure and function of the centriole.

1. Introduction

The centriole is one of the most recognizable structures in all of biology. Nine triplet microtubules are elegantly arranged into a cylinder with a diameter of approximately 250 nm and a length ranging from 150 to 500 nm, depending on the cell type. This makes the centriole one of the largest protein-based structures in the cell. Two orthogonally arranged centrioles are found in centrosomes, the principal microtubule organizing centre in most animal cells. The basal body is closely related to the centriole, but serves the distinct cellular function of organizing a cilium or flagellum. Many cell types are able to convert a centriole in the centrosome to a basal body. Despite their critical functions, centrioles and basal bodies are not found throughout the entire eukaryotic world. The spindle poles in yeasts, higher plants, and many oocytes lack centrioles, and these cells use a variety of other structures to form and organize spindle microtubules. Basal bodies, however, are absolutely required for cilia assembly. There are many intriguing questions about centrioles and basal bodies, including what proteins are found in the structures, how such complex structures are built and how cells use centrioles and basal bodies to organize even larger structures, namely the centrosome and the cilium. Many of these questions arise from microscopic observations of centriole and basal body morphology—the topic of this paper.

Before describing centriole structure in detail, a quick tour of its basic parts is necessary to define terms. Most centrioles are made up of nine circularly arranged triplet microtubules. A triplet microtubule has a complete microtubule (the A-tubule) onto which two additional partial microtubules are assembled (the B- and the C-tubules, respectively). Being composed of microtubules, the centriole is an inherently polar structure with microtubule minus ends positioned at the proximal end of the centriole. The proximal end is also the site from which the new centriole is built, using the cartwheel, which consists of a hub at the centre and nine spokes, each contacting the A-tubule of a triplet microtubule to organize assembly. Within the centriole's centre and distal end is a luminal density. On the exterior of the microtubule scaffold are filaments that connect the two centrioles within a centrosome as well as distal and subdistal appendages found exclusively on the older of the two centrioles. We will explore these

structural features in greater detail by illustrating what is known about their ultrastructure as opposed to focusing on their molecular composition.

2. Historical perspective

Early cell biologists using light microscopes observed centrioles and centrosomes, as described elsewhere in this volume [1,2]. Early illustrations by van Beneden [3] show two dots within the centrosomes or spindle poles, which probably represent the centrioles. Boveri's contributions are discussed elsewhere in this volume [1]. However, the complexity of centriole structure was not appreciated until it was viewed by electron microscopy. The first report and published images of centrioles are attributed to Harven & Bernhard [4]. Prior to this work, electron micrographs of basal bodies in multi-ciliated cells had been published by Fawcett & Porter [5]. Ever since their detection using electron microscopy, there has been a rich history of structural work on centrioles and basal bodies. Because centrioles and basal bodies are closely related, being essentially the same entity in many cell types, we will draw morphological observations from both these structures.

3. The microtubule scaffold

The most recognizable features of centrioles and basal bodies are the nine triplet microtubules that constitute its microtubule scaffold, although structural variations exist. For example, the small centrioles found in *Drosophila* and *Caenorhabditis elegans* embryos contain doublet and singlet microtubules, respectively (figure 1*a*). In humans and other organisms, the triplets are deployed in a circle with a diameter of approximately 250 nm and length of approximately 500 nm (figure 1*a*,*b*). At this size, the centriole is readily imaged in the electron microscope allowing for detailed morphological analysis of the structure. As an analogy, the ribosome—a complex and large cellular machine—occupies only a small volume compared with a centriole (at least 1000 ribosomes would fit in the volume occupied by a centriole). At a scale that is easier to recognize, if we set the size of ribosome to a tennis ball, then the centriole would be approximately the size of a 55 gallon drum, which has approximately the same ratio of height to diameter as a shorter (300 nm) centriole.

The triplet microtubules define the cylindrical barrel of centrioles and basal bodies. The triplets are at a small angle off the circumference giving the centrioles the look of a turbine. When viewed from the proximal end, the triplet microtubule blades have an anticlockwise twist (figure 1*b*). The triplets consist of a complete microtubule of 13 α- and β-tubulin-containing protofilaments, called the A-tubule, to which two successive 10 protofilament microtubules, called B- and C-tubules, are assembled (figure 1*b*; [10,11]). The B- and C-tubules share three protofilaments with the preceding tubule to make complete, 13 protofilament microtubules. However, these tubules are not like the elliptical A-tubule, or circular single microtubules. The B- and C-tubules are slightly concave where they are adjoined to the neighbouring tubule. This arrangement suggests that there is a special junction between the tubulin protofilaments at the interface between tubules. Discussed in more detail below, recent electron cryotomography on isolated basal bodies from *Chlamydomonas* and *Trichonympha* reveals specific non-microtubular connections between the A- and B-tubules, and the B- and C-tubules [10,11].

Like single microtubules, the triplet microtubules of centrioles and basal bodies require γ-tubulin for their formation [12]. Although triplet microtubules are absent in yeasts, both budding (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*) have contributed significantly to our understanding of centrosomes (figure 1*c*; see [13]), including our knowledge of γ-tubulin complex function in microtubule nucleation [14]. Cryoelectron microscope analysis of reconstituted γ-tubulin complexes using budding yeast components reveals a lock washer structure that forms a cap on the minus end of the microtubule (figure 1*c*, left). The structure consists of seven so-called tubulin small complexes that include two molecules of γ-tubulin, one molecule each of Spc97 (orthologue of GCP2) and of Spc98 (orthologue of GCP3), and the N-terminal domain of Spc110. Spc110 tethers the complex to the *S. cerevisiae* centrosome and is orthologous to kendrin/pericentrin, a component of the pericentriolar material (PCM) from which centrosomal microtubules are nucleated. The seven small complexes form a ring that exposes 13 of the 14 γ-tubulin molecules such that they can each template a protofilament of the microtubule [14]. The resulting cap in this *in vitro* reconstituted structure is very similar to the capped minus ends of microtubules that have been observed in yeast cells (figure 1*c*, right) [15], and at the A-tubule of the centriole [9]. Interestingly, elongation of centriolar microtubules has been observed when γ-tubulin is reduced in *Drosophila* and *C. elegans* [16,17].

The presence of triplet microtubules correlates with the organism's genome encoding the additional minor tubulin isoforms δ and ε [18], i.e. organisms lacking triplet microtubules in their centrioles, as discussed below, also lack the genes for δ- and ε-tubulins. These would include *C. elegans* and *Drosophila* with singlet and doublet microtubules, respectively, in their centrioles (figure 1*a*; a few other examples of centrioles lacking triplet microtubules exist, see [18]). Indeed, functional analyses of δ- and ε-tubulin encoding genes reveal their importance in the formation or maintenance of triplet microtubules. δ-tubulin was discovered first as the product of the *UNI3* gene of *Chlamydomonas* [19] that when mutated leads to loss of most of the C-tubules from the basal body [20]. In mammals, δ-tubulin is highly enriched in the testes, whereas in somatic cells, it displays both cytoplasmic and nuclear localization as well at the spindle poles during mitosis [21]. Gene silencing of δ-tubulin in *Paramecium* also resulted in loss of the C-tubule without affecting cilia or basal body formation [22]. This requirement for δ-tubulin in C-tubule formation and genetic interactions between δ-tubulin and α-tubulin [23], lead Li *et al*. [10] to speculate that δ-tubulin may be a component of a unique linker they observed between the B- and C-tubules.

The ε-tubulin gene was initially found in the human genome. The encoded protein was localized to centrosomes and shown to be essential for centriole duplication [24,25]. Analysis in *Chlamydomonas* [26], *Paramecium* [27] and *Tetrahymena* [28] reveal that ε-tubulin is required for basal body assembly and/or maintenance. The ultrastructural defects include the absence of the B- and C-tubules. A depletion time course in *Tetrahymena* shows progressive loss of microtubule triplets, leading to the loss of entire basal bodies [28]. These requirements for δ- and ε-tubulins in forming or maintaining triplet microtubules cannot be absolute, because *Drosophila* form triplet microtubules in the giant centrioles

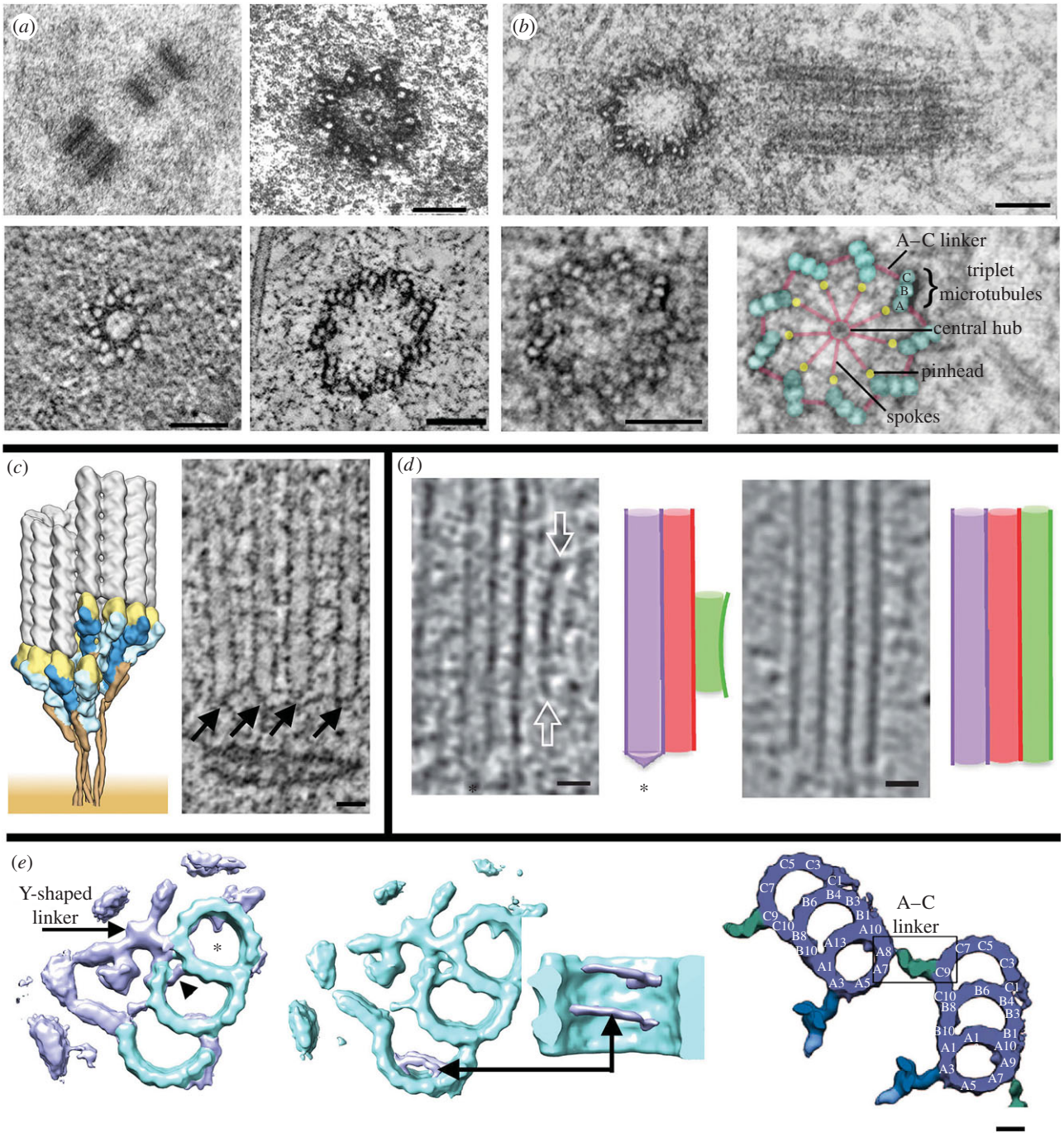

Figure 1. The microtubule scaffold and associated structures of the centriole. (*a*) Variations in centriole microtubule number. The top panels are *Drosophila* centrioles in longitudinal (left) and cross (right) section, showing doublet microtubules [6]. The lower panels are a cross section of a *C. elegans* centriole (left) showing singlet microtubules, and a human centriole (right) showing triplet microtubules. Bars, 100 nm. (*b*) Centrioles in mammalian cells. The top panel is a pair of orthogonally arranged centrioles in a centrosome. The bottom panels display cross sections of a centriole proximal region; the structural features are indicated on the right panel. Bars, 100 nm. (*c*) The left panel is a model of the yeast γ-tubulin complex (indicated in yellow and blues) anchoring a microtubule (grey) to a spindle pole body via a tether (brown, such as Spc110). See Kollman *et al.* [7] for details. The right panel is a tomographic slice of a budding yeast spindle pole body and attached spindle microtubules (as in O'Toole *et al.* [8]); arrows indicate the capped microtubule minus ends. Bar, 25 nm. (*d*). Capped minus ends are present on the A-tubule of assembling centrioles (left panel, asterisk; purple), B- and C-tubules have open ends (red, green). The A-tubule minus end cap is absent in mature centrioles (right panels). This figure is from Guichard *et al.* [9] with permission. Bars, 25 nm. (*e*). Models showing non-tubulin structures (purple) associated with the microtubule triplets (light blue) identified in cryoelectron tomograms of basal bodies in *Chlamydomonas* (as in Li *et al.* [10], left and centre panels). Left: the Y-shaped linker facing the basal body centre. Microtubule luminal structures such as the A- and B-tubule linker (arrowhead) and the A-tubule cone shaped structure (asterisk). The middle panels show microtubule luminal structures (arrows) present in the C-tubule in the distal region of the basal body. The right panel shows the location of the A-tubule to C-tubule linker (green) between microtubule triplets (purple) in *Trychonympha* ([11] with permission). Bar, 25 nm.

(approx. 2.5 μm long) found in their spermatids [29,30], despite an apparent absence of these minor tubulin isoforms in their genome. In addition, the small amount of these tubulin isoforms observed at centrioles and basal bodies is at odds with a structural or assembly cofactor role. Further, there is a lack of convincing immuno-electron microscopy data placing the proteins at the seams between the tubules of the triplet microtubules; in fact, the ε-tubulin in vertebrate cells is localized to the subdistal appendages (discussed below), and not found in the microtubule scaffold [24].

Microtubules typically have a hollow lumen that appears clear in electron micrographs. A newly identified feature of the triplet microtubules of basal bodies is the presence of densities within the lumen of the microtubules, referred to as microtubule inner proteins (MIPs) [10,11]. Cryoelectron tomographic studies of the doublet microtubules of the ciliary or flagellar axoneme also reveal MIPs [31,32]. Additionally, such densities have been reported in microtubules of the *Giardia* ventral disc [33]. Li *et al*. [10] used cryoelectron tomography and subvolume averaging to detect densities on the internal lumen of the A- and B-tubules in the mid-distal region of the *Chlamydomonas* basal body (figure 1*e*). Likewise, Guichard *et al*. [11] reported a density on the luminal wall of the A-tubule of the *Trichonympha* basal body. The densities are positioned at specific protofilaments within the triplet tubules of the basal body. In addition, Li *et al*. [10] identified a structure that bridges protofilaments of the C-tubule in the distal half of the basal body, which they propose acts as a brace (figure 1*d*, middle). Although this study was of the highly specialized *Chlamydomonas* basal body, it clearly raises the possibility that there may be microtubular luminal structures in all centrioles. This will probably be revealed in future studies using cryoelectron tomography and subvolume averaging of these structures.

How centriolar length is controlled remains an open question. Centrioles in animal cells are on the order of approximately 500 nm in length. However, centrioles are sometimes shorter, such as 150 nm in the worm *C. elegans* [34] or very long such as the *Drosophila* 2.5 µm sperm basal body. Cells must also have a mechanism to control the distal elongation of procentrioles prior to mitosis and then limit elongation once the appropriate length is reached. While molecules that are required for assembly of a full-length centriole or that function in length control have been identified, none has been associated with any particular recognized structure that may set or control centriole length. Poc5 has been implicated in centriole growth by an unknown mechanism [35]. Poc5 is found in the distal lumen of the centriole where its binding partner centrin (a small calcium binding protein) has also been localized [35,36]. While the majority of studies in vertebrate cells have not revealed a role for centrin in centriole duplication or structure (see Dantas *et al*. [37]), the protein is important in basal body assembly or stability in *Tetrahymena* [38]. Elongation also requires a complex of CPAP (SAS4)/SPICE/CEP120 [39,40]. The centriolar protein, CP110, has a capping function limiting the length of centrioles in cooperation with CEP97 and KIF24 along with other proteins [41]. Part of this function involves the conversion of one of the centrioles to a basal body to organize a cilium. Finally, centrobin has been implicated in centriolar elongation [42]. The complexity of the triplet microtubule scaffold as shown by Li *et al.* [10] and by Guichard *et al*. [11], taken together with the number of structural and regulatory proteins involved suggests that revealing the molecular mechanism of centriole elongation, termination and stabilization will be a daunting task.

Once assembled, the triplet microtubules of the centriole scaffold are very stable. This feature allowed Kochanski & Borisy [43] to differentially label centrioles and track their behaviour during the cell cycle. It is also well known that cold treatment of vertebrate cells leads to the loss of microtubules, but that the centrioles persist. Finally, Pearson *et al.* [44] used fluorescence recovery after photobleaching to show very little turnover of green fluorescent protein-tagged α-tubulin in *Tetrahymena* basal bodies. The triplet microtubules themselves, as discussed with respect to δ- and ε-tubulin function, contribute to the stability of the centriole. Further stability arises from chemical modifications of α- and β-tubulin within the microtubule triplets as well as by additional associated components. Microtubules in the centrioles are highly modified; they are detyrosinated, acetylated, polyglutamylated and contain Δ2-tubulin. It is possible that centrioles contain the most highly modified tubulin of any microtubule structure within the cell (nicely reviewed in Janke & Bulinski [45]). Tubulin glutamylation in centrioles is known to be important for their stability [46]. The precise function of other tubulin modifications at centrioles is probably important as well but has yet to be elucidated.

Beyond tubulin modification, the nine microtubule triplets of the centriole must be held together by structures that contribute to their stability. Although the cartwheel at the proximal end of the centriole imparts ninefold symmetry, it is not thought to stabilize the centriole once assembly is complete, as the cartwheel is disassembled in mature centrioles. Cross-sectional images of centrioles and basal bodies show a linker between the A- and C-tubules in many different organisms (figure 1). Recent cryoelectron tomography and subvolume averaging studies have revealed high-resolution detail of this structure [10,11]. In *Chlamydomonas* [10], the linker is approximately 130 Å in length, anchored at a specific A-tubule protofilament (A6), and linked to various sites on the neighbouring C-tubule depending on the position along the long axis of the basal body. This variable attachment is thought to contribute to the slight left-hand twist of the triplet microtubules of the basal body arising from changes in triplet angle. The A–C linker in the proximal end of the *Trichonympha* basal body connects the A8 and C9 protofilaments and is composed of two clear densities [11]. Surprisingly, candidate molecules serving as constituents of these linkers have not yet been identified. Presumably connectors would be important not only during assembly, but also for centriole stability. Compromised basal body structure leads to cytoplasmic infiltration (ribosomes in the lumen of the basal body) and triplet loss [47]. These phenotypes reveal that the microtubule scaffold separates different cellular compartments—inside and outside the centriole—and that the intertriplet linkers may contribute to this partitioning.

Finally, cryoelectron tomography of *Chlamydomonas* basal bodies has revealed additional features of the centriole interior [10]. These authors describe a Y-shaped linker between the A- and B-tubules of the triplet microtubules (figure 1*e*). This linker has a central stem (bottom of the Y) that contacts both the A- and B-tubules at the protofilaments where the tubules contact each other facing the basal body lumen. The structure then has two long arms (top of the Y) that point into the lumen of the basal body. The Y-shaped linker is found at each triplet around the entire circumference of the basal body lumen and along 250 nm of the basal body length in the region distal to the cartwheel [10]. Likewise, similar structures were observed in the basal bodies of *Trichonympha* [11]. It remains to be seen whether there are similarly elaborate structures, or any such structures, on the luminal face of the triplet microtubules in centrioles.

4. The centriole lumen

In the centriole lumen within the microtubule scaffold, one finds the very well-defined cartwheel structure, the poorly

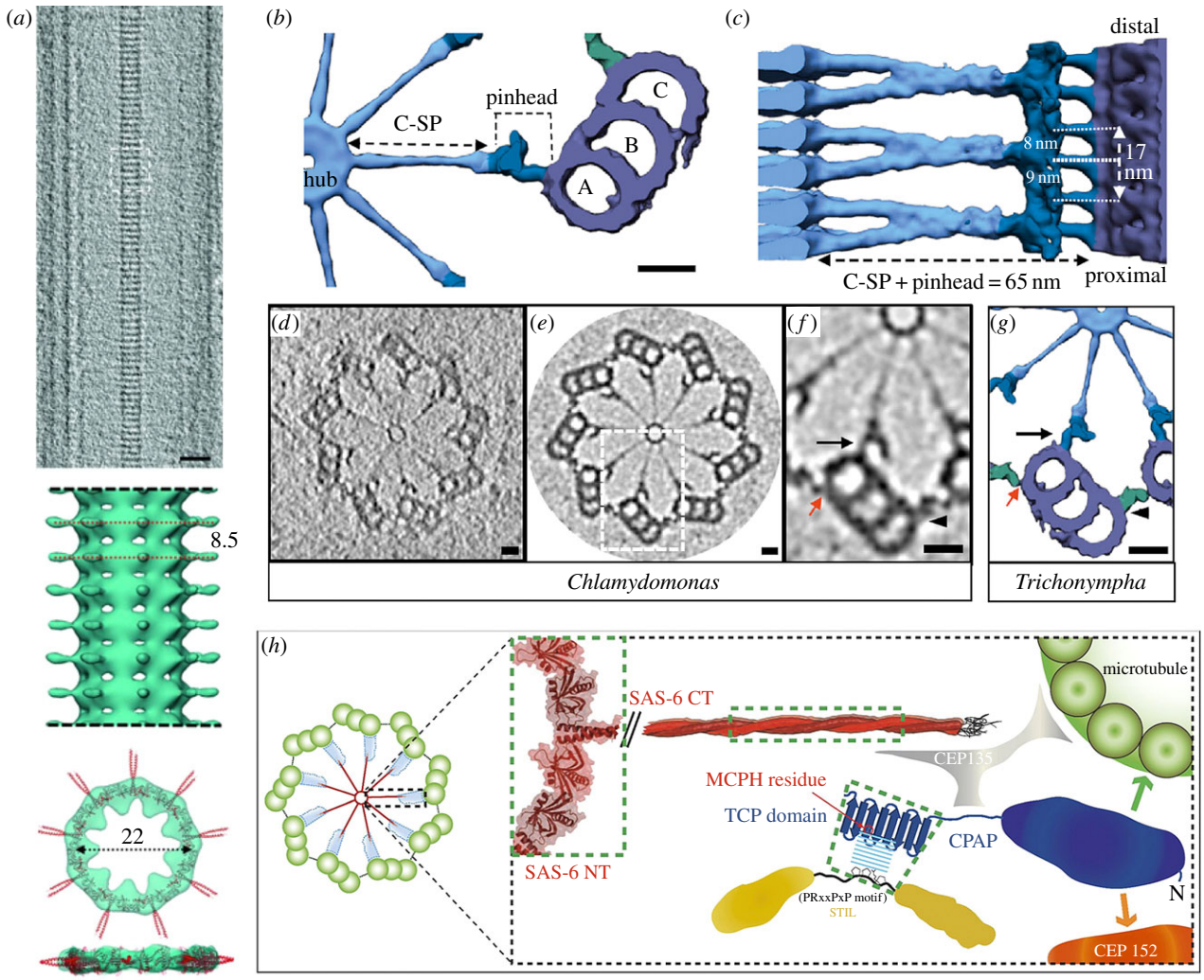

Figure 2. Detail of the cartwheel structure. (*a*) Cryoelectron tomographic slice (top) and models (lower) of the hub in *Trichonympha* reveal the stacking and dimensions of the Sas6 ring at the hub from [48] with permission. (*b–g*) Selected slices from cryoelectron tomograms and models comparing the spoke and pinhead structures in *Chlamydomonas* and *Trichonympha* from [11] with permission. Cross-sectional (*b*) and longitudinal (*c*) views of the cartwheel (light blue), pinhead (medium blue), microtubule triplet (purple) and the A–C linker (green). Dimensions are shown, and C–SP is the 'cartwheel spoke.' Tomographic sections of the proximal region of *Chlamydomonas* basal bodies (*d*), were used for three-dimensional volume averaging (*e*), and higher magnification image (*f*) shows the structural similarity to the *Trichonympha* basal body (*g*). The black arrow indicates the pinhead–A-tubule connection. The red arrows and black arrowhead indicate the A-C linker connection to the A-tubule and the C-tubule, respectively. Bars, 25 nm. (*h*) A molecular model for the cartwheel hub, spoke and pinhead based on crystallographic data and known protein–protein interactions from [49] with permission.

defined luminal density and other features. While the cartwheel is clearly implicated in the assembly of new centrioles and basal bodies, the function—if any—of the luminal density is not known. We describe both these structural categories in detail below.

The cartwheel, as the name suggests, is a structure resembling the hub and spokes of a wheel (the wheel being the microtubule scaffold when viewed end-on (cross section of the centriole; figure 2)). The cartwheel defines the proximal end of the centriole, being close to the other centriole in the centriole pair, and is near the site of new centriole assembly. The basal body cartwheel is found at the base of the structure distal from the axoneme of cilia or flagella. The hub is an approximately 22 nm diameter tube that can be tens of nanometres long in a centriole. Spokes are found along the entire length of the hub. In ciliated cells, the hubs and associated spokes in basal bodies can be quite long, for instance the nearly 4 um long cartwheel in *Trichonympha* [48]. A density, referred to as the cartwheel inner density, has been observed in the lumen of the hub. Importantly, the major component of the hub and spokes is the Sas6 protein. The X-ray crystal structure of Sas6 shows that dimers of this protein have a globular head and an extended coiled-coil tail. The globular heads are capable of forming a ring with ninefold symmetry (reviewed in [50]). Furthermore, the hub structure has been revealed in exquisite detail in a cryoelectron tomography study of the gigantic cartwheel found in *Trichonympha*, which reveals an apparent stack of Sas6 rings [11,48]. This bit of evolutionary protein engineering elegantly organizes the nine triplet microtubules of the scaffold. The assembly of the cartwheel is the focus of another section in this text [51].

The coiled-coil domain of Sas6 points away from the hub to begin the spoke, but is not long enough to reach the microtubule scaffold implicating other proteins in the structure. Bld10/Cep135 appears to be a component of the spokes. Importantly, spokes connect the cartwheel's hub to the A-tubule of the triplet microtubules. The connection occurs at a density (called the pinhead, or sometimes the 'foot') that appears to be very important in centriole formation and stability. Structural work on a complex of the centriolar components CPAP (Sas4) and STIL

(Sas5) found in this region of the centriole has led to an initial model for this domain [49] (figure 2). Furthermore, the cryo-electron tomographic analysis of both *Trichonympha* and *Chlamydomonas* pinheads reveals conserved structural features in this interesting complex [11].

The cartwheel has attracted significant attention, because it is among the earliest recognizable features of assembling centrioles and basal bodies. Based on Dippell's classic model of basal body assembly [52], hub and spokes come together prior to triplet microtubule formation. The assembly of the triplets starts with the A-tubule, followed by the B- and C-tubules, respectively. The sequential addition of tubules is also seen in human cells [9]. A different mechanism is seen in *Chlamydomonas*, as microtubules are formed more distally [53]. Of course, there may be variation between organisms in the order of assembly events. However, it is clear from numerous genetic studies that cartwheel formation is a critical early event in centriole and basal body assembly.

Despite its critical role in establishing centriole structure during assembly, the cartwheel may be neither universal nor permanent in these structures. Sas6 was discovered in the worm *C. elegans*, and the protein is required for centriole assembly. The centrioles within the single-cell *C. elegans* embryos lack a prominent hub and spoke configuration as described above, but they do contain a larger diameter tube (approx. 60 nm) that is seen early in assembly before the appearance of the singlet microtubules [34]. This tube may be formed from an interaction between Sas6 and Sas5 [54] and may assemble in a spiral [55]. Other variations on hub and spoke organization have been observed [18]. Another interesting feature of cartwheels is that they appear dynamic and are not necessarily permanent in the centriole. While critical to assembly, the structure is lost from centrioles as they proceed through the cell cycle in vertebrate cells. This can be observed in the electron microscope or by tracking the presence of Sas6 at centrioles (reviewed by [50]). Basal bodies, on the other hand, appear to maintain their cartwheels after assembly. In *Chlamydomonas*, the length of cartwheels in forming basal bodies is longer than that of the mother basal bodies, suggesting a supportive role during new basal body assembly and shortening as the basal body matures [53]. This maintenance of the cartwheel may reflect their importance in providing structural support to basal bodies that are stressed by the beating of the cilia they anchor [56]. In centrioles, this kind of mechanical stress may be less of an issue. Centriole age is important in cells, and the lack of a cartwheel may be a mark of centriole maturity. Alternatively, the loss of cartwheels could be a side effect of the tight cell cycle control under which cartwheel components are kept. In mitotic cells, cartwheel component levels are low, which help prevent inappropriate duplication.

Unlike the cartwheel, the lumen of the centriole distal to the cartwheel is poorly understood. It is a separate compartment that is known to contain centrin, Poc5 and several other molecules [50]. Correspondingly, imaging demonstrates that centriole lumens are free of ribosomes, but can contain an amorphous mass of protein. Cryoelectron tomography revealed that the distal region of the centriole lumen contains a 45 nm, periodic stack of rings forming a scaffold within the centriole lumen [57]. These are probably analogous to the luminal discs and A-tubule feet observed previously in centrioles and basal bodies, respectively [58,59]. This distal scaffold may serve to organize the distal, luminal proteins. In addition, defective basal bodies in several *Tetrahymena* mutants affecting the stability of the structure show ribosome infiltration [47]. The fact that the lumen is cleared of ribosomes and some proteins are deposited in the lumen suggests a function for this compartment that is yet to be discovered.

5. Outside the microtubule scaffold

Centrioles have an important function in organizing the PCM on the outside of the microtubule scaffold as well as connectors between the two centrioles, and the distal and subdistal appendages, marks of maturation. Both the appendages and the PCM are critical to the function of centrioles and centrosomes as microtubule organizing centres and as signalling platforms. The topics of PCM assembly and function [60] and the centrosome as a signalling platform [61] are treated elsewhere in this volume.

The two centrioles in the centrosome are tethered to each other at their proximal ends. The older centriole organizes the site of new centriole assembly, and this is where the centrioles are adhered to each other. However, both the site of centriole assembly and the connection are rather amorphous, and little structural information is available. Isolated centriole pairs from human lymphoblastoma cells contain a filamentous structure between the two centrioles [62]. Upon duplication, the resulting two pairs of centrioles are also tethered to each other via a link between the mother (pre-existing) centrioles. The potentially filamentous protein C-Nap1 was localized to this structure. C-Nap1 was found to be a substrate of the Nek2 kinase [63], and Nek2 phosphorylation is thought to dissociate C-Nap1 from the centrioles [64]. C-Nap1 interacts with rootelin and LRRC45 [65], potentially forming the fibrous bundle linking the centrioles. The regulation of centriole cohesion is an important issue in licensing centriole duplication and in spindle formation (see [66,67]).

Another centrosomal cell cycle event is maturation—the addition of PCM that increases microtubule nucleation capacity as the cell prepares to assemble a mitotic spindle (see Hyman *et al.* [60]). Microtubule nucleation occurs at the PCM, as does the accumulation of important cell cycle regulators. From a structural point of view the PCM is a rather poorly defined material around the centrioles. Many components of the PCM have been identified, and there is growing information about molecular interactions. The introduction of super-resolution microscopy has allowed for greatly improved imaging of the PCM and provided evidence for a higher degree of ordered structure than previously understood [68].

Finally, older centrioles are recognizable by additional structures, the distal and the subdistal appendages (figure 3*a*). These structures are added to existing centrioles that have already completed a cell cycle and as such are markers of maturation [58,71]. The addition of the appendages makes it possible for the centriole to be converted to a basal body (well reviewed in Jana *et al.* [72]). The distal appendages are fibrous extensions radiating out from the distal end of the centriole [58,71]. Several centriole components, CEP164, CCDC123/CEP89/CEP123, CCDC41, SCLT1 and FBF1 are found in the distal appendages [72]. Functional analysis of these genes has determined that they function in primary cilia formation. Proximal to the distal appendages are the subdistal appendages that often appear as distinct triangular structures attached laterally to the sides of the microtubule scaffold (figure 3*a*). In some cell

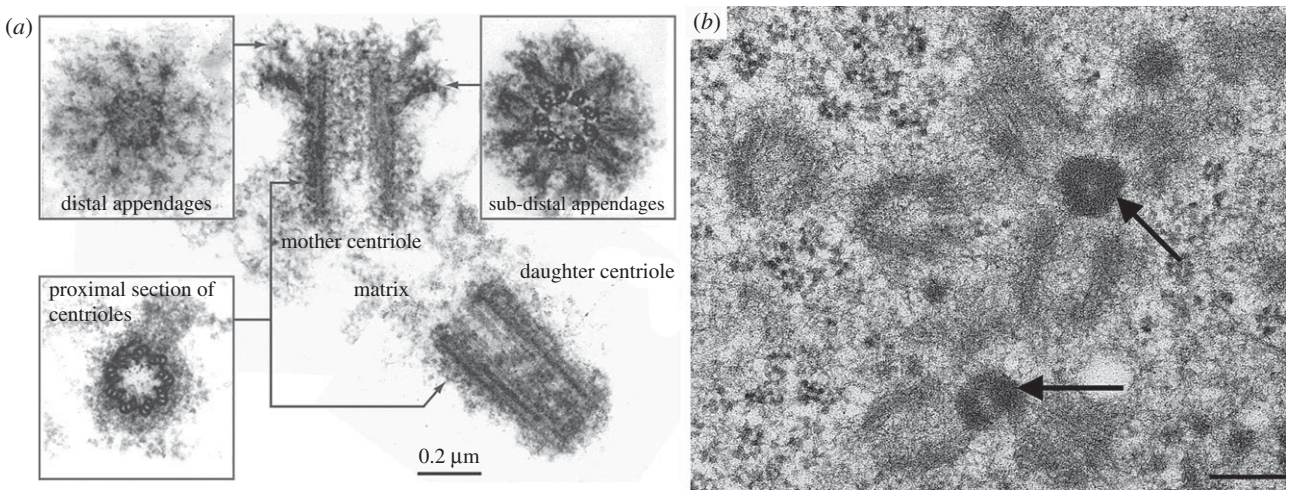

Figure 3. Structures outside the microtubule triplets of the centriole. (*a*) Electron micrographs of centrioles in isolated centrosomes shown in longitudinal section and informative cross sections highlighting the distal and subdistal appendages. Reprinted from [69] based on original images in [58]. (*b*) Deuterosomes (arrows) organizing the formation of basal bodies in mouse tracheal epithelium cells as in [70]. Bar, 200 nm.

types, such as KE37, there is a transitional zone of triplet to doublet microtubules to which appendages assemble [58]. Appendages have also been reported on triplet microtubules in other cultured mammalian cells [71] and basal bodies [59]. The subdistal appendage contains ninein, centriolin, ε-tubulin and CEP170 [72]. The subdistal appendages are involved in microtubule anchoring and require microtubule-associated proteins for their organization [73]. Odf2/cenexin, first identified as a sperm tail component has been found at both structures and is required for their assembly [74]. Structures homologous to the distal and subdistal appendages can also be found on basal bodies, and are referred to as basal feet and transitional fibres, respectively [74].

6. Deuterosomes

Deuterosomes promote de novo formation of centrioles destined to become basal bodies as part of the developmental programme to produce multi-ciliated cells [75]. Vertebrate cells that execute this programme have been used to identify centriolar components based on their transcriptional induction [76]. The deuterosome appears as a non-descript cytoplasmic density that can have a fibrous nature (figure 3*b*) although recently components of the deuterosome have been identified [70,77]. Notably, the core components of centriole assembly, such as Sas6, have been implicated in deutersome-dependent centriole assembly, indicating that a shared assembly mechanism is used by this pathway.

7. Future prospects

It is a pleasure to study a cellular structure that can be directly observed with the appropriate microscope and imaging tools. The morphological work reviewed here is rapidly advancing owing to improved imaging, and structural biology approaches coupled with an increasing knowledge of centriolar components. The field is generating significant interest and has been expertly reviewed by Gonczy [50] and Jana *et al.* [72]. However, observing the centriole or basal body structural complexity does remind one of the work left to accomplish. In the future, we must continue to define the proteins present at centrioles and basal bodies and apply ever improving labelling and imaging technologies to these structures. Beyond completing the list of conserved components, the function or structural contributions of these proteins will need to be determined, some of which will be challenging such as the role of the minor tubulin isoforms.

Cryoelectron tomography and subvolume averaging studies have provided important insights into the structural details of centrioles, and it will be necessary to continue and refine their use in the field. Beyond better imaging to reveal ultrastructural detail, we also need to map proteins into those structures. We have surprisingly little information on the exact locations of components within the centrioles and basal bodies. This task will benefit from super-resolution microscopy that allows for an initial pass at the localization maps [68]. Finally, live cell microscopic techniques will allow for the analysis of dynamic behaviour of centriolar components during assembly and maintenance. This will be needed to fully understand how centrioles are assembled, maintained and serve to organize the PCM or cilia.

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