The Centrosome, a Multitalented Renaissance Organelle

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SUMMARY

The centrosome acts as a microtubule-organizing center (MTOC) from the G_1 to G_2 phases of the cell cycle; it can mature into a spindle pole during mitosis and/or transition into a cilium by elongating microtubules (MTs) from the basal body on cell differentiation or cell cycle arrest. New studies hint that the centrosome functions in more than MT organization. For instance, it has recently been shown that a specific substructure of the centrosome—the mother centriole appendages—are required for the recycling of endosomes back to the plasma membrane. This alone could have important implications for a renaissance in our understanding of the development of primary cilia, endosome recycling, and the immune response. Here, we review newly identified roles for the centrosome in directing membrane traffic, the immunological synapse, and the stress response.

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Outline

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1 INTRODUCTION: CENTROSOMES—SMALL BUT NOT SIMPLE

Theodore Boveri published his seminal work in 1888, describing the origin of the centrosome from the sperm centriole after fertilization (Scheer 2014). This initial centriole will become the mother centriole and will duplicate to form a daughter centriole. Another round of duplication is required to make two mitotic spindle poles, each containing two centrioles. These two duplication cycles are required to form the first mitotic spindle, thus initiating the process of development and growth of an embryo.

The centrosome has been visualized by light microscopy since the 1880s and, subsequently, by transmission electron microscopy (TEM), which revealed the two barrel-shaped centrioles surrounded by pericentriolar material (PCM). The centriole itself contains substructures such as a cartwheel-like structure and two sets of appendages at the distal end of the oldest centriole (a.k.a. "mother centriole"; Fig. 1). The cartwheel-like structure serves as a platform to assemble microtubule (MT) triplets arranged with ninefold symmetry. It is argued that the assembly of this structure de novo involves a complex of proteins (Kitagawa et al. 2011), which are regulated by a polo-like kinase—the serine/threonine-protein kinase PLK4 (centriole assembly has been reviewed elsewhere; Brito et al. 2012). However, the role of appendages in centrosome function remains more enigmatic. We know that the oldest centriole (the mother) is structurally distinct from the younger centriole (the "daughter") in that it contains distal appendages and subdistal appendages (Fig. 1). The proteins that comprise these appendages are enriched specifically at the mother centriole, whereas the daughter centriole possesses its own specific molecular components (e.g., centrobin—a protein required to ensure the length of the newly formed centrioles; Zou et al. 2005; Gudi et al. 2015). The current proposed role for distal appendages is in docking the centrosome to the plasma membrane during the formation of cilia (Tanos et al. 2013), whereas the subdistal appendages are proposed to act in MT anchoring in interphase cells (Delgehyr et al. 2005). More recent evidence indicates that these appendage proteins are also important for ensuring symmetric division and membrane trafficking (see below).

Another important structure of the centrosome is the PCM that surrounds the two centrioles. It was originally speculated that the PCM was a disorganized meshwork of proteins (as reviewed elsewhere; Mennella et al. 2014). With the advent of superresolution microscopy and deconvolution, the PCM appears to have a lattice-like organization (Dictenberg et al. 1998) and ring-like arrangements (Lawo et al. 2012). Superresolution microscopy also revealed molecular components of the PCM (e.g., pericentrin, γ-tubulin), which were detected at a resolution of <200 nm. These studies allowed the precise modeling of different PCM components within the lattice (Fu and Glover 2012; Lawo et al. 2012; Mennella et al. 2012; Sonnen et al. 2012). The first striking observation was that pericentrin is organized with its carboxyl terminus closer to the centriole wall, and the amino terminus of the protein extends toward the outer layer of the lattice (Mennella et al. 2012). γ-tubulin is found in a more peripheral layer. Pericentrin displays a structure similar to "arms" reaching across and, potentially, securing the lattice network (Lawo et al. 2012), arguing for its importance in lattice stability (Mennella et al. 2012). This topology might explain why γ-tubulin is lost so readily in cells lacking pericentrin (Zimmerman et al. 2004; Chen et al. 2014).

2 THE CONSTANTLY EVOLVING ROLE OF THE CENTROSOME THROUGHOUT THE CELL CYCLE

The dynamic, yet organized, centrosome provides a platform for multiple functions. As the cell enters mitosis, the ability of the centrosome to nucleate MTs increases, concomitant with recruitment of additional PCM and signaling components, thus transforming the once PCM-poor interphase centrosome into a mature PCM-rich spindle pole. Once division is completed and the cell commits to differentiate, the once-again PCM-poor centrosome can move toward the plasma membrane and function as a basal body for cilia formation (Fig. 2). Besides these classical functions of the centrosome, its recently appreciated roles in membrane trafficking and formation of immunological synapses (Fig. 2) direct a fast-burgeoning area of research, driven in part by the identification of more than 100 different centrosome proteins and an open-access centrosome proteome to peruse (Andersen et al. 2003; Jakobsen et al. 2011). However, the framework for how these proteins are organized, the dynamics of their localization to the centrosome throughout the cell cycle, and their organization have not been fully elucidated. With the ability to use live-cell imaging and superresolution microscopy, we envision that these questions will quickly be resolved.

Proteins that make up the proteasome were one of many interesting centrosome candidates that have been identified (Badano et al. 2005; Wigley et al. 1999), but their specific subcentrosome localization is still unknown. In eukaryotes, proteasomes drive the selective degradation of protein substrates containing covalently linked ubiquitin chains. Although proteasomes are distributed throughout the cell, their localization at the centrosome argues for a specific biological function at this distinct subcellular site. For instance, studies linking the proteasome to the centrosome show a role for centrosome localization in neuronal func-

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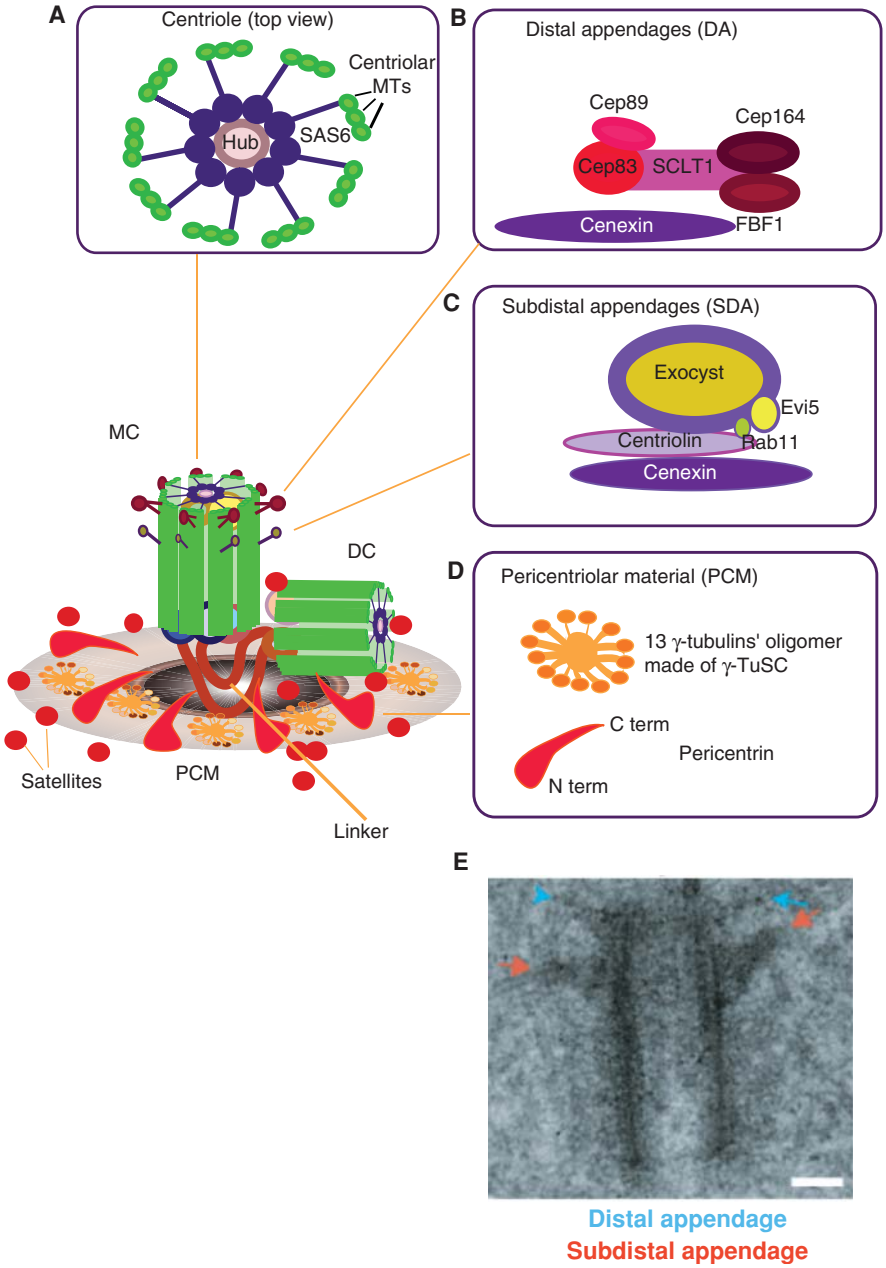

Figure 1. Centrosome substructures. A model highlighting the centrosome and its specific substructures, namely, (*A*) the centrioles, (*B*) distal appendages, (*C*) subdistal appendages, and (*D*) pericentriolar material (PCM). The centrosome comprises the mother centriole (MC), daughter centriole (DC), tethered together by "linker" structures, PCM, and satellites. The "linker" ensures centriole engagement and timely linker degradation that licenses centriole duplication during S phase. The protein composition of the "linker" remains elusive, but the involvement of pericentrin, Cep215, and Cep68 in centriole connections has recently been shown (Pagan et al. 2015). (*A*) Illustration showing the centriole barrel (in top view). Depicted is a central hub, a rod-shaped structure of SAS6 homodimers that form oligomers (Kitagawa et al. 2011; Guichard et al. 2013), from which nine spokes of SAS6 homodimers emanate, which each radiate toward a microtubule (MT) triplet. (*B*) Relative organization of molecular players forming the distal appendages (DAs), with Cep83 being in closest proximity to the centriole. (*C*) A model in which it is proposed that cenexin/Odf2 is responsible for the integrity of the subdistal appendages (SDAs) and for connecting them to the MC. (*D*) Organization of the PCM into 13 protofilament oligomers that contain γ-tubulin in the outermost layer; the carboxyl terminus (C term) of pericentrin is located close to the centriole barrel, whereas the amino terminus (A term) is oriented toward the outer lattice layer. (*E*) Electron micrograph of a mother centriole with two sets of appendages; DAs are highlighted by blue arrows and SDAs are highlighted by red arrows. Scale bar, 0.1 μm. (*E*, Reproduced from Hung et al. 2016, with permission from Elsevier.)

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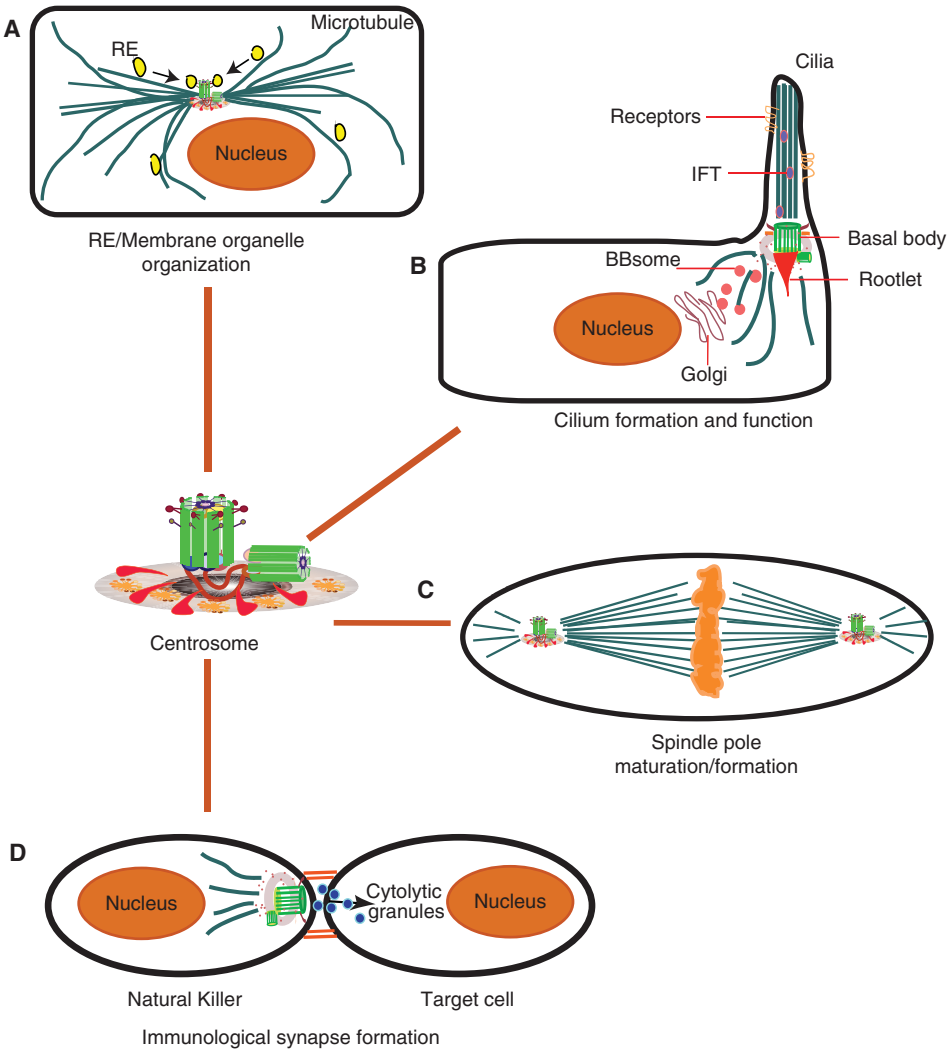

Figure 2. The various functions of the centrosome. The figure summarizes the main activities of the centrosome, including (*A*) organization of membrane organelles (e.g., recycling endosome); (*B*) cilium formation and function; (*C*) spindle pole maturation during mitosis; and (*D*) formation of an immunological synapse, in which a natural killer cell recognizes an infected cell. IFT, intraflagellar transport; RE, recycling endosome.

tion. Specifically, an E3-ubiquitin ligase linked to Parkinson's disease is enriched at the centrosome (Zhao et al. 2003). These E3 ligases are enzymes that recognize targets for degradation by tagging them with ubiquitin. This work suggests that the centrosomal localization of this E3 ligase provides a subcellular site to specifically ubiquitinate and degrade protein aggregates that are crucially involved in the pathogenesis of Parkinson's disease. A centrosomal localization of proteasome components was also proposed to be important for the development of dendrites. This was tested by developing a tool to inhibit proteasome function specifically at the centrosome (Puram et al. 2013). The proteasomal subunit S5a/Rpn10 was identified as an essential component for proteasomal activity specifically at the centrosome in neurons to promote dendrite arbor elaboration. A deeper understanding of the molecular relationship between the centrosome and the proteasome could elucidate potential therapeutic targets in Parkinson's disease progression and, thus, expand our understanding of neuronal development.

3 MT-ORGANIZING CENTER AND SPINDLE POLES

The centrosome is most commonly known as a microtubule-organizing center (MTOC). MTs appear to be organized at the centrosome in three different ways. They can be nucleated at this site from/by γ-tubulin ring complexes (γ-TuRCs) located in the PCM (Fig. 1). γ-TuRCs consist of γ-tubulin small complexes (γ-TuSCs) and accessory proteins (Doxsey 2001). Unlike MT growth in vitro, in which

variable numbers of protofilaments are formed, centrosome-nucleated MTs typically comprise 13 protofilaments (reviewed in Doxsey 2001). γ-TuRCs form oligomers that have a slight helical geometry similar to the helical turns of the MT itself and are consistent with a role for the γ-TuRCs templating the assembly of 13-fold microtubule protofilaments to form an MT (Kollman et al. 2010). MTs (apparently centrosome-nucleated) can also be anchored at subdistal appendages of the centrosome in interphase cells, but little is known about how these MTs arise and anchor, other than them requiring ninein for their anchoring (Delgehyr et al. 2005). MTs can also be elongated from the older centriole during ciliogenesis.

The centrosome function expands when the interphase centrosome matures into a mitotic spindle pole. Centrosome maturation occurs every cell cycle and involves recruitment of signaling molecules to initiate centrosome separation and maturation, followed by the addition of PCM components to increase MT nucleation. The role of centrioles in mitotic spindle assembly appears to be dispensable during *Drosophila* development (Basto et al. 2006)—in this case, the cells rely on acentriolar MTOCs. However, in chicken DT40 cells, centrioles are required for timely spindle assembly and chromosome stability (Sir et al. 2013). In developing mouse embryos, acentriolar mitosis causes early embryonic lethality, arguing for an essential role for centrioles in mammalian development (Bazzi and Anderson 2014). To initiate spindle maturation in either *Drosophila* or mammalian cells, two well-studied kinases—Aurora A and Plk1—must be recruited to the spindle poles (reviewed in Barr and Gergely 2007), where they play crucial roles in spindle assembly and mitotic progression. Interestingly, Aurora A modifies several important cell cycle–related proteins that include CPEB (cytoplasmic polyadenylation element binding) to regulate translation of cyclin-B–Cdk1 for mitotic entry (Mendez and Richter 2001; Sasayama et al. 2005); Eg5, LATS2, and NDEL1, which are required for centrosome separation and maturation (Toji et al. 2004); and TACC (transforming acid coiled coil) for astral MT stability (Conte et al. 2003; Barros et al. 2005). Therefore, it is not surprising that defects in Aurora A signaling ultimately delay mitotic entry, induce monopolar spindle formation, and cause misaligned chromosomes (Hochegger et al. 2013). Plk1 seems to be more specific and directly phosphorylates substrates required for centrosome maturation and spindle assembly proteins (reviewed by Lens et al. 2010).

4 BASAL BODY

When a cell exits the cell cycle, during differentiation or because of a lack of nutrients (Goto et al. 2013), the centrosome relinquishes its main role as an MTOC and relocates from its perinuclear site to the apical plasma membrane where one of the two centrioles, the mother centriole, serves as a basal body for ciliogenesis (Fig. 2) (reviewed by Kobayashi and Dynlacht 2011; Hehnly and Doxsey 2012). The basal body can revert back to an MTOC and reenter the cell cycle by reabsorbing the cilia and moving away from the apical membrane.

As the centrosome is at the base of the primary cilium and is required for anchoring the cilium at the plasma membrane, it is not surprising that many centrosome proteins were found to be crucial for ciliogenesis (Graser et al. 2007; Mikule et al. 2007; Nigg and Raff 2009). The mother centriole appendages comprise a handful of centrosome proteins that have been implicated in the formation of cilia (e.g., Cep164, ODF2/Cenexin, Cep83, and centriolin; Gromley et al. 2003; Ishikawa et al. 2005; Graser et al. 2007; Chang et al. 2013; Tanos et al. 2013). The distal appendage molecular components, Cep164 and Cep83, are thought to be important for docking of the mother centriole at the plasma membrane during ciliogenesis. The role of the subdistal appendages is less defined. Interestingly, ODF2/cenexin localizes to both subdistal and distal appendages and contributes to the formation of both (Ishikawa et al. 2005; Chang et al. 2013). One interesting role for subdistal appendages is that they might act as a site to assemble a ciliary vesicle (Sorokin 1962), which is a membranous capsule proposed to be required for fusion of the cilium with the plasma membrane (Hehnly et al. 2013). Another recent study showed that ciliary components, including membrane vesicles, remain associated with the mother centriole during mitosis (Paridaen et al. 2013). Furthermore, it seems that the daughter cell that inherits the oldest centriole will be the first to form a primary cilium (Paridaen et al. 2013).

Besides the formation of the ciliary vesicle, the centrosome undergoes additional changes during ciliogenesis that include the establishment of a ciliary rootlet (Yang et al. 2002). During assembly of cilia, the centrosome (basal body) facilitates the formation of the ciliary rootlet. Rootletin—a structural component of the ciliary rootlet (Yang et al. 2002)—is also a component of the centrosome during G_1–S cell cycle stages and is required for centrosome cohesion (Bahe et al. 2005). Interestingly, a lack of rootletin does not affect initial cilia development, but is implicated in long-term cilia stability in photoreceptors and, when lost, leads to retinal degeneration (Yang et al. 2005).

PCM components of the centrosome, such as pericentrin, are also implicated in ciliogenesis (for further details, see Delaval and Doxsey 2010). One possible explanation for the perturbation of ciliogenesis that accompanies the loss of pericentrin is that pericentrin interacts with components

of the intraflagellar transport (IFT) system, IFT20 and IFT88 (Jurczyk et al. 2004). IFT is a cargo-trafficking pathway that functions inside cilia and contributes to their assembly and the delivery of signaling proteins (e.g., sonic hedgehog; reviewed by Sung and Leroux 2013). The IFT system efficiently delivers cargo to the cilium in a timely manner, which is important as the cilium itself lacks translational machinery. The cilium consists of two protein subcomplexes, IFT-A and IFT-B, which use the motor proteins kinesin 2 and dynein to move bidirectionally along MTs in the cilium.

An unexpected recent finding showed that formation of the cilium involves autophagy-regulated degradation of centrosome satellite proteins. Centriolar satellites (Fig. 1) are cytoplasmic granules that have been proposed to function in centrosome protein targeting and exchange, as well as communication between centrosomes and the cytoplasm. More specifically, the centriolar satellite protein oral-facial-digital syndrome 1 protein (OFD1) is specifically degraded by autophagy, and this degradation is important for ciliogenesis (Tang et al. 2013). The interaction of OFD1 with the autophagic protein LC3 is enhanced by another satellite protein, PCM1. It has also been shown that there is a mutual interdependence between ciliogenesis and autophagy, favoring a model in which autophagy influences ciliogenesis (Pampliega et al. 2013). Other support for this comes from the observation that cigarette smoke causes the loss or shortening of cilia, and this is correlated with an overall increase in autophagy (Lam et al. 2013). Inhibition of autophagy prevents cilia shortening in response to cigarette smoke, thus supporting the notion regarding an inhibitory effect of autophagy on ciliogenesis.

Most cell types contain primary cilia, implicating their importance in cellular function. One particularly interesting observation is the presence of signaling receptors at the primary cilium, suggesting a role as a specialized signaling "antenna." Proteome analyses of nonmotile cilia have identified signaling components such as sonic hedgehog, smoothened and Wnt (Liu et al. 2007; Mayer et al. 2009; Ishikawa et al. 2012). These cilia-localized signaling cascades have been modeled to influence neuronal migration and cerebral cortical development (reviewed by Guemez-Gamboa et al. 2014). One proposed mechanism for how cilia might regulate neuronal migration is that they could act as an antenna sensing hormonal changes in the extracellular environment. Strikingly, a recent study revealed a branched cilium in neurons of the nematode *Caenorhabditis elegans*, suggesting that there could be some diversity in the range of morphology of primary cilia (Doroquez et al. 2014). Another example of the diversity of cilia comes from comparative proteomic studies (Liu et al. 2007). When compared with proteome analysis between photoreceptor sensory cilia and other cilia proteomes, a higher number of transport and light-perception components, such as photoreceptors and photoadaptor proteins, were detected in photoreceptor cilia. This specialized organization of distinct signaling components suggests that the cilium is an organelle that is "tunable" to specific cell types.

5 LOSS OF CENTROSOME OR CILIA FUNCTION LEADS TO CILIOPATHIES

Dysfunction of primary (sensory) cilia is associated with several human disorders, collectively termed ciliopathies. The primary cilium was thought to be the reason behind the syndromic phenotypes associated with their disruption, including obesity, cystic kidneys, polydactyly, *situs inversus*, and encephalocele, to name just a few. One recent example linking primary cilia with obesity is a study on the obesity-linked hormone leptin. Leptin is produced by adipocytes and was shown to stimulate elongation of cilia in the hypothalamus neurons in adulthood (Han et al. 2014). These findings suggested that leptin governs cilia length, possibly to increase the sensitivity of hypothalamic neurons to metabolic signals.

One specific ciliopathy, Bardet–Biedl syndrome (BBS), has at least nine associated "ciliopathy" phenotypes that include retinopathy, polydactyly, *situs inversus*, and developmental delays such as a hypoplastic (underdeveloped) cerebellum and obesity. The multiple phenotypes of BBS make it difficult to determine whether loss of cilia is the sole defect in this disorder. BBS can result from mutations in at least 14 different genes, some of which are involved in a protein complex called the BBSome that contributes to cilia structure, formation, and function. However, some BBS proteins have been implicated in cellular structures outside the cilium (reviewed by Vertii et al. 2015a). For instance, BBS4 and BBS6 both localize to the centrosome/basal body during interphase, when a cilium is emanating from the basal body, and during mitotic progression, when the cilium is not present (Kim et al. 2004, 2005). Based on these known BBS protein localizations to the centrosome, we propose that defects in centrosome function, leading to mitotic or cilia defects, might cocontribute to the etiology of ciliopathies.

6 THE ENDOCYTIC RECYCLING MACHINERY INTERACTS DIRECTLY WITH THE CENTROSOME AND PLAYS A ROLE IN CILIA FORMATION AND THE CELL CYCLE

Intercellular trafficking is dependent on centrosome structure (Fig. 2). One example of this is the recycling endosome and its interaction with mother centriole appendages

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(Hehnly et al. 2012). The appendages are required for the assembly of the ciliary vesicle, which forms at the mother centriole before the centriole docks to the plasma membrane from which the primary cilium emerges (Sorokin 1962). The recycling endosome contains two GTPases—Rab11 and Rab8—that regulate cargo recycling in and out of this organelle. Recently, these GTPases were shown to interact directly with a mother centriole appendage protein, cenexin (Hehnly et al. 2012, 2013; Chang et al. 2013). In addition, a Rab11–Rab8 GTPase cascade was shown to be required for formation of cilia, in which active Rab11 recruits the Rab8 GDP–GTP exchange factor (GEF) Rabin8 to activate Rab8 and, thus, induce cilia formation (Knödler et al. 2010; Westlake et al. 2011). Rab8 and Rab11 also interact with several well-defined proteins required for ciliogenesis such as the BBSome, the vesicle tethering complex known as the exocyst, and the mother centriole appendage protein cenexin (Fielding et al. 2005; Wu et al. 2005; Nachury et al. 2007; Hehnly et al. 2012, 2013; Chang et al. 2013). However, the molecular mechanism underlying the interplay between these molecular components during ciliogenesis is unknown. Early steps in ciliogenesis require the Rab8–Rab11 cascade and the membrane-shaping proteins EHD1 and EHD3 (Lu et al. 2015). We speculate that the interaction between the recycling endosome and its machinery with mother centriole appendages facilitates the organization of the Rab11–Rab8 GTPase cascade, ensuring the initiation of the formation of cilia at the appropriate time during the cell cycle.

Strikingly, a recent study implicated Rab11 and its role in dynein-based endosome motility in maturation of spindle poles and mitotic progression. Time-lapse imaging showed Rab11-associated endosomes organized around interphase centrosomes, mitotic spindles, and mitotic spindle poles (Hehnly and Doxsey 2014). The Rab11-decorated endosomes contained γ-tubulin and dynein, suggesting that endosomes might act as carriers for localizing MT-nucleating and spindle pole proteins to the mitotic spindle poles. Taken together, the results show that Rab11-associated endosomes are important in centrosome maturation (Hehnly and Doxsey 2014) and ciliogenesis (Knödler et al. 2010; Westlake et al. 2011), thus showing their role in centrosome function.

7 THE CENTROSOME AS A STRESS SENSOR

Signaling by centrosomes is crucial for cell cycle progression (Doxsey et al. 2005). Cyclin-dependent kinases (CDKs) are well-known regulators of cell cycle progression, and so it is not surprising that two cyclins—cyclin E and cyclin A—physically interact with and activate CDKs, including Cdk2 and Cdk1, and activate these kinases. Interestingly, cyclins E and A both display centrosome localization and are required not only for centriole duplication but also for DNA replication. Each of these cyclins, cyclin E and A, contain their own unique centrosome-localization signals, which are thought to spatially activate CDKs (Matsumoto and Maller 2004; Pascreau et al. 2010). Cyclin B also localizes to the centrosome. However, in this case, the sequestration at the centrosome serves to prevent cyclin B from conducting its nuclear function (Krämer et al. 2004) and does not directly affect the centrosome.

On induction of DNA damage, the pathway involving the proteins ATM, ATR, and Chk1 regulates the localization of cyclin B at centrosomes. Normal entry into mitosis occurs after activation of Cdk1, resulting in chromosome condensation in the nucleus and centrosome separation, as well as increased MT-nucleation activity in the cytoplasm. The centrosome-associated serine/threonine protein kinase Chk1 is proposed to colocalize with, and thus shield, centrosome-localized Cdk1 from unscheduled activation by the cytoplasmic tyrosine-protein phosphatase Cdc25B, thereby contributing to the accurate timing of the initial steps of cell division, including mitotic spindle formation (Krämer et al. 2004). However, on DNA damage, a Chk1-mediated checkpoint induces excessive formation of centriolar satellites that constitute assembly platforms for centrosomal proteins, which subsequently leads to centrosome amplification (Löffler et al. 2013). This proposed centrosome-inactivation checkpoint comprising centrosome amplification might lead to the elimination of cells by mitotic catastrophe in response to DNA damage. Furthermore, following UV-induced DNA damage, Chk1 first accumulates, and then becomes phosphorylated, at the centrosome (Löffler et al. 2007). The specific phosphorylation of Chk1 is a prerequisite for its degradation by the proteasome (Zhang et al. 2005). One interesting possibility is that, on DNA damage, the nuclear fraction of Chk1 accumulates at the centrosome, where it is then spatially, and in a timely manner, modified for degradation. This would be one of several examples showing that signaling activities involving centrosomes and nuclear events are linked in the response to cellular stress. For example, it appears that the disruption of centrosome structure results in a G_1 arrest through a p38–p53 stress pathway (Mikule et al. 2007). Similarly, increasing p38 or p53 expression modulates PLK4 activity, thereby regulating centriole duplication (Nakamura et al. 2013).

8 CAN THE CENTROSOME DISCRIMINATE BETWEEN DIFFERENT TYPES OF STRESS?

Heat stress, like DNA damage, can have a deleterious effect on centrosome function. For instance, cells either under-

going heat stress, such as fever, or incurring DNA damage have a higher propensity to form a primary cilium (Villumsen et al. 2013), suggesting that multiple cellular stresses can cause cells to exit the cell cycle and remain in G_0. The centrosome seems to be sensitive to stress. When comparing the DNA damage response in *Drosophila* embryos (Sibon et al. 2000) with the heat-stress response of mammalian cells (Vidair et al. 1993), both undergo dramatic but somewhat opposite changes in centrosome structure. For example, although UV stress causes centriolar satellite amplification and centrosome overduplication, heat stress in some cases causes disassembly of the PCM (Vidair et al. 1993; Brown et al. 1996b; Löffler et al. 2013). There have been several discrepancies between studies on changes in centrosome function that might have resulted from differences in heat-stress intensity and/or duration (Vidair et al. 1993; Brown et al. 1996b; Villumsen et al. 2013). Based on these differences, it is important to define the physiological relevance of heat stress in vivo on centrosome structure/function and correlate these phenotypes to what has been reported in the literature. Analysis of leukocytes from patients with febrile condition revealed decreased centrosome integrity compared with that of healthy controls (Vertii et al. 2015b). These data are supported by experiments in vitro with fever-mimicking temperature regimens, indicating that the centrosome is a heat-stress-sensitive organelle. Moreover, this sensitivity is unique to the centrosome, as other nonmembranous organelles, the midbody, and kinetochore appear to be heat-stress-tolerant (Vertii et al. 2015b). The heat-stress-related defects in centrosome integrity resulted in functional consequences for the centrosome, such as its ability to nucleate MTs and polarize during formation of the immunological synapse (IS; see below).

9 CENTROSOMES IN THE IMMUNE RESPONSE

Recent studies have shown that centrosome reorientation occurs during formation of the IS. IS formation is a necessary step within the immune response that includes membrane rearrangement at the sites where a T cell contacts an antigen-presenting cell (APC), resulting in activation of the T cell (Kloc et al. 2013). Cytotoxic T lymphocyte (CTL) or natural killer (NK) cells form one type of IS that can eliminate infected or tumorigenic cells by releasing cytolytic granules. Another type of IS occurs when T helper cells recognize an antigen presented by the APC through its T-cell receptor (TCR). The IS then assembles into a highly organized structure that contains a peripheral and central supramolecular activation complex (pSMAC and cSMAC, respectively) (Dustin et al. 2010; Thauland and Parker 2010). Once formation of the IS is initiated, the centrosome reorients toward the cSMAC within the IS and proceeds to dock at the plasma membrane where it can control secretion of lytic granules (Fig. 2) (Stinchcombe et al. 2006). At the same time that the centrosome docks at the plasma membrane, secretory granules move in a dynein-dependent manner toward the centrosome (Mentlik et al. 2010). Centrosome reorientation and movement toward the IS depends on several molecular factors that include MTs; MT motors such as dynein and kinesin; membrane components such as diacylglycerol; and signaling components that include protein kinase C (PKC), casein kinase 1 delta, Lck, Fyn, and Zap-70 (Knox et al. 1993; Kuhné et al. 2003; Combs et al. 2006; Martín-Cófreces et al. 2006; Quann et al. 2009; Bertrand et al. 2010; Tsun et al. 2011; Zyss et al. 2011; Liu et al. 2013; Jenkins et al. 2014). The molecular interplay between all these components has yet to be deciphered. However, recent evidence illustrates that MTs are required for centrosomes to orient themselves at the IS, and that this process is biphasic with centrosome polarization toward, and docking at, the IS (Yi et al. 2013). This work suggests an interesting model whereby centrosome reorientation might influence polarity formation in the IS. Future studies are required to investigate whether reorientation of the centrosome sets up T-cell polarity or whether T-cell polarity initiates centrosome reorientation.

Recently, similarities have been discerned between IS formation and cilia formation. For instance, in both cases, the centrosome is required to reorient toward a polarized membrane, either the apical membrane in the context of cilia formation or the IS in T cells. Both these processes involve hedgehog signaling either to function (Rohatgi et al. 2007; Tasouri and Tucker 2011) or to dock the centrosome at the plasma membrane (Stinchcombe et al. 2006; de la Roche et al. 2013). The similarities between IS formation and cilia formation provide a rationale to test whether proteins involved in cilia formation are also required in IS formation (e.g., IFT proteins, mother centriole appendage proteins). Along these lines, a recent finding has implicated an IFT protein—IFT20—in assembly of the IS in T cells (Finetti et al. 2009). Specifically, IFT20 is implicated as a central regulator of TCR recycling to the IS, possibly through an interplay between IFT20 and the Rab GTPase network that controls endosomal recycling (Finetti et al. 2014). Recycling endosomes, specifically ones containing either the Rab8 or Rab11 GTPases, have also been implicated in formation of cilia (Knödler et al. 2010). Therefore, we propose that the centrosome might be the control center for organizing the endosomal pathway containing IFT20 (Hehnly et al. 2012; Finetti et al. 2014) to initiate either cilia formation or IS formation at the right time and place.

10 MAINTAINING CENTROSOME INTEGRITY REQUIRES CHAPERONE MACHINERY

A high percentage of centrosomal proteins are >150 kDa in size and contain large hydrophobic coiled-coil motifs that are important in centrosome assembly. However, these characteristics imply a risk of rapid aggregation under cellular stress, suggesting that the centrosome is a highly unstable macromolecular complex. Maintaining centrosome integrity is essential for cell cycle progression and cilia formation. One proposed mechanism for maintaining centrosome integrity, and thus function, is through chaperone assistance. Chaperones are important components that aid folding of newly synthesized proteins, prevent aggregation of misfolded proteins, and target misfolded proteins for proteasome-mediated degradation (Horwich 2014). In mammalian cells, there are multiple chaperones, including heat-shock protein (Hsp) families: Hsp100, Hsp90, Hsp70, the chaperonin complex TRiC/CCT, and small chaperones such as crystallins (Kampinga et al. 2009). Some of these, such as Hsp70 and Hsp90, use ATP to prevent protein aggregation and facilitate proper folding of target proteins, whereas others, such as the small chaperones Hsp27 and αB crystallin, are ATP-independent but still efficient in their chaperone function.

The molecular chaperonin system differs dramatically from other chaperones (Kim et al. 2013). Chaperonins have been implicated in facilitating molecular components that assist in centrosome function. The chaperonin TRiC/CCT is responsible for folding tubulin and actin intermediates, as well as the PLK1 kinase (Llorca et al. 2000; Liu et al. 2005; Muñoz et al. 2011), and localizes to the centrosome (Brown et al. 1996a). Interestingly, CCT chaperonins have been identified as molecular components contributing to the onset of BBS (see Sec. 5 above). Two protein complexes implicated in the etiology of BBS consist of an octameric complex, the BBSome, and a handful of chaperonin-TRiC/CCT-like proteins (BBS6, BBS10, BBS12) (Nachury et al. 2007; Loktev et al. 2008; Jin et al. 2010; Wei et al. 2012). Any defect in protein complex assembly can result in BBS (Katsanis 2004; Yang et al. 2008; Billingsley et al. 2010). Many of the components that assist in formation of the BBSome localize to the centrosome and include BBS6 and the CCT proteins CCT1, CCT4, CCT5, and CCT8 (Kim et al. 2005; Seo et al. 2010; Zhang et al. 2012, 2013). Whether chaperonins require centrosome localization to interact with BBSome components is unknown. We speculate that the centrosome, which already contains many chaperone components, acts as a checkpoint to investigate the integrity of the BBSome complex.

Like the chaperonin system, the Hsp70–Hsp90 chaperone machinery localizes to the centrosome (Wigley et al. 1999). This localization suggests that Hsp70–Hsp90 might be involved in maintaining centrosome structure. In fact, when centrosome structure is compromised (by depletion of 13 specific centrosome proteins; Mikule et al. 2007), the cells arrest in G_1 phase. Strikingly, this same cell cycle arrest occurs when Hsp/Hsc70 is depleted (Powers et al. 2008). This finding suggests that the Hsp/Hsc70 depletion, like depletion of centrosome proteins, compromises centrosome structure and, thus, leads to cell cycle arrest. Such an intriguing correlation led to the hypothesis that chaperones have a bona fide role in centrosome structure/function, in addition to their role in being recruited to the centrosome under stress. For example, under heat stress, the PCM of the centrosome is disrupted (Vidair et al. 1993; Hut et al. 2005). This disruption occurs concomitant with Hsp70 recruitment to the centrosome, suggesting a role for this chaperone in centrosome protection. Furthermore, ectopic expression of Hsp70 protects the PCM when under heat stress (Brown et al. 1996a,b). However, as increased cellular levels of chaperones will protect multiple cellular organelles from stress-related defects, from these studies it is difficult to determine whether Hsp70 expression is directly protecting the centrosome or whether this effect is indirect. Specific targeting of Hsp70 to the centrosome rescues centrosomal defects during stress, most likely through its ability to nucleate MTs and to serve as a basal body for polarizing toward the IS (Vertii et al. 2015b).

11 CONCLUSION

The emerging roles of the centrosome in underpinning a myriad of cellular functions might be related to the possession by the centrosome of many unique structural components. For example, the centrosome nucleates most of its MTs from the PCM and anchors MTs at the subdistal appendages, which are also implicated in organizing recycling endosomes. Furthermore, the distal appendages are linked to a separate centrosome function that involves centriole docking to the plasma membrane for both cilia formation and the formation of the IS. Although these different centrosome substructures can be linked to different cellular processes, we still do not know whether there is cross talk between the substructures themselves. For instance, could the subdistal appendages regulate PCM function? Support for this idea comes from the observation that several subdistal appendage proteins become more PCM-like (e.g., ninein) during maturation of the spindle pole, suggesting that these molecular players "toggle" between their roles at the appendages and their roles at the PCM. However, the role of appendage proteins at the PCM is unclear, although we do know that the appendage protein ninein is required for symmetric cell division (Wang et al. 2009; Dauber et al.

2012). We propose that chaperone components guard these different centrosome structures and assist in the assembly and disassembly of the many substructures of centrosomes.

Centrosome multitasking involves dynamic molecular rearrangements and, sometimes, movement of the entire centrosome during cell migration or ciliogenesis. These changes are probably due to MT-based transport to and from the centrosome. However, an alternative method to reorganize centrosome structure could involve local degradation of protein pools through centrosome-localized proteasome activity. One can imagine that maintaining the centrosome requires dynamic transport of material to and from the centrosome, the initiation of newly synthesized material at the centrosome, and the degradation of material no longer needed. If any of these pathways are improperly regulated, the fate of the cell will ultimately be decided by the centrosome. Therefore, it will be important to gain a greater understanding of the molecular pathways that feed into the centrosome and how the centrosome ultimately influences them.

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