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Phase Transitioning the Centrosome into a Microtubule Nucleator

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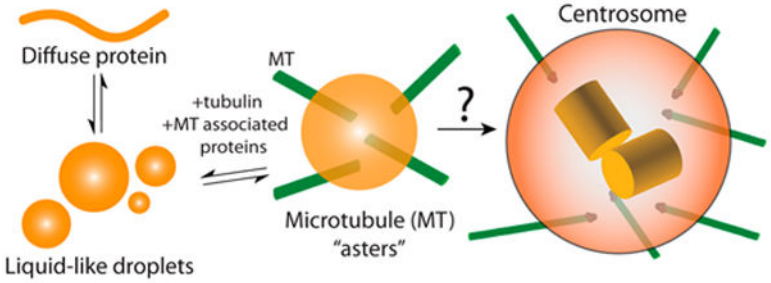
Abstract

Centrosomes are self-assembling, micron-scale, nonmembrane bound organelles that nucleate microtubules (MTs) and organize the microtubule cytoskeleton of the cell. They orchestrate critical cellular processes such as ciliary-based motility, vesicle trafficking, and cell division. Much is known about the role of the centrosome in these contexts, but we have a less comprehensive understanding of how the centrosome assembles and generates microtubules. Studies over the past 10 years have fundamentally shifted our view of these processes. Subdiffraction imaging has probed the amorphous haze of material surrounding the core of the centrosome revealing a complex, hierarchically organized structure whose composition and size changes profoundly during the transition from interphase to mitosis. New biophysical insights into protein phase transitions, where a diffuse protein spontaneously separates into a locally concentrated, nonmembrane bounded compartment, have provided a fresh perspective into how the centrosome might rapidly condense from diffuse cytoplasmic components. In this Perspective, we focus on recent findings that identify several centrosomal proteins that undergo phase transitions. We discuss how to reconcile these results with the current model of the underlying organization of proteins in the centrosome. Furthermore, we reflect on how these findings impact our understanding of how the centrosome undergoes self-assembly and promotes MT nucleation.

Graphical Abstract



The centrosome is the major microtubule organizing center (MTOC) of the cell. Most microtubules (MTs) originate from the centrosome in many cell types, and it consequently

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provides a central reference point that organizes the cellular interior. The interphase centrosome directs the MT cytoske-leton to provide structural support, cell shape, polarity, and the internal highways on which vesicular cargos are transported (Figure 1A). Prior to mitosis, the centrosome duplicates, with each copy migrating to form the two opposing poles of the mitotic spindle (Figure 1A). From this location, centrosomes provide critical positioning information that allows correct chromosomal segregation into the two daughter cells. Dysregulation of centrosomal assembly, duplication, and MT nucleation ability can result in aberrant spindle pole number or spindle disorganization, and has been implicated in increased genomic instability and uncontrolled cell division in cancer. While much is known about the role of the centrosome in these contexts, we lack a fundamental understanding of how the centrosome generates microtubules. In this Perspective, we focus on how the discovery of protein phase transitions within the centrosome affects our understanding of centrosome architecture, assembly, and ability to nucleate MTs as a major MTOC of the cell.

The Centrosome as an MTOC and the Gamma-Tubulin Ring Complex.

The centrosome is a micron-sized organelle that is not enclosed by a membrane. It is composed of two core cylindrical MT structures, or centrioles, surrounded by a dense shell of proteins recruited from the cytoplasm, termed pericentriolar material (PCM). The two centrioles are hollow structures with a 9-fold symmetric arrangement of MT triplets, which are organized by an internal cartwheel-like scaffold. Centrioles are arranged in a characteristic orthogonal orientation toward each other (Figure 1B). During the transition from interphase to mitosis, the composition and size of the PCM changes profoundly, concurrent with an increase in the MT nucleating ability of the centrosome.[1] Although initially believed to be an amorphous structure, subdiffraction imaging of the PCM has revealed a complex, hierarchically organized structure, as discussed below.

Initial electron microscopy (EM) studies of the PCM established that centrosomal MTs originate in the PCM, identifying this region as the site of MT nucleation.[2,3] Further EM studies revealed the existence of ~25 nm diameter, ringshaped complexes distributed throughout the PCM, coinciding with both the 25 nm diameter of MTs and location of MT minus ends, suggesting these structures play a role in MT nucleation.[3] This, coupled with the identification of γ-tubulin, and its essential role in MT nucleation,[4,5] led to the discovery of the main MT nucleating complex in the cell, the γ-tubulin ring complex (γTuRC) (Figure 1B).[6,7] This complex of γ-tubulin and γ-tubulin complex proteins (GCPs 2 through 6) was structurally characterized via low resolution EM tomography as a cone-shaped complex.[8] It has a ring of 13 γ-tubulin molecules, corresponding to the 13 protofilaments in MTs, that interface with alpha tubulin at the MT minus end.[8,9] This ring of γ-tubulin is proposed to be a standardizing template for MT nucleation.[9] γ-Tubulin is considered the universal MT nucleator in the cell because its depletion results in loss of MT nucleation in most organisms. A few centrosomal factors that recruit and activate γTuRC have been described, although additional centrosomal factors might also be involved.[10,11] What these additional factors are, and how they are organized and regulated at the centrosome to promote MT nucleation, remains a key question in understanding how the centrosome acts as a MTOC.

The New Frontier: Phase Transitions.

A new, unexpected development in the centrosome field is the discovery that protein phase transitions may play a role in centrosomal assembly and MT nucleation. A general example of a protein phase transition is the spontaneous separation of a previously diffuse protein into a locally concentrated, nonmembrane bounded compartment (Figure 1C).[12] This biophysical behavior provides an additional means for the cell to organize the cytoplasm into discrete regions in order to segregate specific functions. Phase transitioning proteins have been described in nonmembrane bounded organelles, including the nucleolus, Cajal bodies, the pyrenoid, and germ granules in *Caenorhabditis elegans* development.[13,14] Proteins undergoing phase transitions can exist in several different configurations, dependent on their innate properties and interactions with other external factors (Figure 1D).[13] Two of these possible configurations are of great interest when considering centrosomal assembly and organization. For instance, a phase transitioning protein can enter a liquid-like droplet state that separates from the bulk cytoplasm (Figure 1C). In this state, dynamic exchange with the surrounding cytoplasm is maintained, despite the formation of an interphase boundary. Furthermore, a phase transitioning protein can also form a solid, or insoluble, aggregate (Figure 1C) where exchange with the cytoplasm is limited.[15] Transitions between phases can be regulated, as evidenced by proteins that require phosphorylation to form a liquid-like droplet (Figure 1D).[16] Of special interest is the finding that different phase-transitioning proteins can reside within the same compartment, forming phases within phases.[17] This mechanism allows a nonmembrane bounded compartment to be further subdivided. In the following sections, we will discuss aspects of centrosome structure and function, and how the discovery of phase transitions changes our understanding of those aspects.

■ HOW IS THE CENTROSOME ORGANIZED?

Centrioles and the Pericentriolar Material (PCM).

At the core of the centrosome, two centrioles are stereotypically oriented at 90 deg with respect to each other[2,18,19] During centrosomal duplication and maturation in S and G2 phases, each centriole duplicates, producing two mother-daughter centriole pairs that each form a new centrosome. The two centrosomes then separate and help assemble the bipolar mitotic spindle.[18,19] In vertebrates, a single centriole is made of nine groups of three MTs, or MT triplets, that are arranged around a central hub, much like the spokes in a wheel (Figure 2A).[20,21] Each MT triplet forms a blade at the end of a spoke composed of a centriolar assembly protein, SAS6.[22,23] Connecting each SAS6 spoke to each MT triplet is CEP135 (centrosomal protein of 135 kDa).[22] Other factors like CPAP, STIL, centrin, and CEP120 also form part of the centriole body or material surrounding the MT blades.[22,24] In invertebrates such as *Drosophila*, each centriole is formed from MT doublets, instead of MT triplets, but centrioles are otherwise highly conserved in composition and architecture.

The PCM surrounding centrioles consists of hundreds of different protein components based on proteomic studies (Figure 2B).[25,26] Initially, the PCM was assumed to have no underlying organization as it presents a hazy, electron-dense appearance under electron microscopy (EM). The first hints of an internal PCM structure were uncovered in biochemical assays exploring the MT nucleation ability of *Drosophila* centrosomes. Treating

isolated centrosomes with high molar potassium iodide exposed an insoluble scaffold, or "centromatrix."[27] This scaffold was capable of recruiting soluble factors from *Drosophila* embryo extract and restoring MT nucleation ability.[27] Recent advances in subdiffraction imaging finally managed to pierce the PCM haze, revealing a complex, hierarchical internal structure. This structure undergoes profound changes in composition and organization during the transition from interphase to mitosis.[28]

Within the interphase PCM, factors are separated into nested concentric layers, anchored by a series of spokes.[24,29] Much like the SAS6 cartwheel spokes within the core centrioles, these PCM spokes are a radial arrangement of a centrosomal protein: pericentrin (PCNT) in vertebrates, or pericentrin-like protein (PLP) in *Drosophila* (Figure 2B).[24,29] Each pericentrin/PLP spoke is an elongated, coiled-coil protein with its C-terminus docked in the interior of the centrosome near the centrioles.[24,29] The C-terminus contains a centrosomal targeting domain known as a PACT domain (pericentrin-AKAP450 centrosomal targeting), which appears to be necessary for this localization.[30] Pericentrin's N-terminus points directly away from the interior, defining the outer boundary of the PCM during interphase.[24] At this stage, the PCM is about 100 nm in radius, with one PCNT spoke spanning this length.[24] It is unclear if other coiled-coil proteins, like AKAP450 which shares the PACT domain, also form spokes within the PCM.[30] Besides spokes, PCM proteins are arranged in nested layers. Going from the core to the outside, these layers are composed of CEP192, then CEP152, CDK5RAP2, NEDD1, and γ-tubulin (TUBG1) (see Figure 2)[24] These last three layers, comprising the CDK5RAP2, NEDD1, and γ-tubulin regions, are the site of MT nucleation and anchoring within the interphase centrosome (Figure 2B).

During the transition from interphase to mitosis, the size and MT nucleation ability of the PCM increase in a process termed centrosome maturation.[1] The architecture of the mitotic PCM is not as well-defined as the interphase PCM, appearing as an extended cloud of material under subdiffraction imaging.[24,29] This cloud of material is primarily composed of CDK5RAP2 (Cnn in *Drosophila)* and also contains NEDD1 and γTuRC, the universal MT nucleator. It remains unclear if this change in organization of the centrosome at the onset of mitosis is required for the increase in MT nucleating ability, and furthermore what factors mediate this reorganization. A potential aspect of interest is that this reorganization may reflect a change in the phase-transitioning properties of specific proteins within the centrosome.

Centrosomal Factors That Recruit and Activate γTuRC.

In both the interphase and mitotic versions of the PCM, γTuRC is recruited from the cytoplasm in order to nucleate and anchor MTs. Genome-wide RNAi screens of *Drosophila* and human cells have identified several centrosomal factors affecting either γTuRC activation, localization, or both.[34,38] Table 1 lists a few of these known γTuRC factors. The outer three layers of the interphase PCM contain two known direct interaction partners of γTuRC: CDK5RAP2 and NEDD1.[32,33,39] Because CDK5RAP2 interacts with, localizes, and activates γTuRC,[10,32] it is an interesting candidate as a key driver of the centrosome's expansion and 3–5-fold increased MT nucleation ability at the onset of mitosis.[1]In *vitro*, CDK5RAP2 can increase γTuRC MT nucleation 7-fold.[10] NEDD1 (previously known as

GCP-WD) directly interacts with γTuRC and is involved in targeting of the complex to the centrosome.[33,39] The N-terminus of NEDD1 is required for localization to the centrosome, while the C-terminus interacts with the fully assembled γTuRC.[11,33] Mitotic targeting of NEDD1 to the centrosome is promoted by phosphorylation via NEK9, a kinase downstream of PLK1, which will be discussed in the context of centrosomal assembly below.[37,40] In addition to NEDD1, the MOZART proteins (Table 1), which have recently been purified as components of γTuRC itself, are necessary for MT nucleation and γTuRC targeting to the centrosome.[35] Other components of the PCM, such as pericentrin and CEP192, are also important of γTuRC recruitment, and additionally bind to CDK5RAP2 and NEDD1, suggesting these lie upstream in the binding hierarchy within the centrosome.[37]

■ CENTROSOMAL SELF-ASSEMBLY: GETTING ALL THE COMPONENTS INTO ONE PLACE

Centriolar Assembly.

The centrosome self-assembles from at least 100 unique nanometer-sized protein components that scale into a spherical organelle roughly one micron in diameter.[24,25,41] In a general sense, this self-assembly process involves the assembly of centrioles, followed by recruitment of the surrounding pericentriolar material (PCM) from the cytoplasm. The assembly and duplication of the core centrioles have been well-characterized (see recent reviews[42,43]). Briefly, the process of centriolar assembly appears to involve a relatively small cast of conserved proteins, regulated by cell-cycle dependent kinases such as CDK1.[44,45] PLK4 (or SAK) is a protein kinase that initiates centriolar assembly triggering the recruitment of centriolar components like STIL and SAS6.[46] A protein phosphatase, PP2A, aids in centrosomal targeting of SAS6 and SAS5 (*C. elegans* homologue of STIL), and in restricting centrosome number[47,48] SAS6 homo-oligomerizes, forming the central hub and spokes of the centriolar cartwheel (Figure 2A).[23,49,50] SAS6 and STIL recruit factors such as CPAP/Sas-4, which bind α/βtubulin heterodimers.[22,51] These α/β tubulin heterodimers polymerize into the centriolar MTs and are joined into MT triplets or doublets, which make up the blades of the SAS6 cartwheel (Figure 2A). Interestingly, *C. elegans* centrioles are built with only single MTs. Regardless, the centriolar MTs are joined to the SAS6 spokes by factors like CEP135.[22] CEP152 (Asterless; Asl in *D. melanogaster)* is also required for this process and is recruited to the centriolar [22,52] wall.

Pericentriolar Material (PCM) Assembly.

Unlike centriole duplication, our understanding of PCM assembly has only recently begun to take shape. The first stage of PCM assembly occurs in interphase and includes protein recruitment around centrioles and formation of the nested layers discussed above. The second PCM assembly stage occurs during the transition into mitosis, where the PCM incorporates new material, increasing in both size and MT nucleation capacity. Subdiffraction limit imaging of this mitotic PCM suggests that this expansion process results in a more amorphous and disorganized architecture when compared to interphase $_{PCM}$.[24,29]

Genome-wide RNAi studies of *Drosophila* and human cell lines have identified a core set of factors that are required for robust PCM assembly.[34,38,53] Among these are the PCM

components described previously, namely, pericentrin (PCNT), PLKl, CEP152, CEP192, and CDK5RAP2.[53,54] Beginning in interphase, a centriolar component, CPAP/Sas-4 provides a scaffold for these components, linking the PCM to the centrioles.[54] In *Drosophila*, homologues of PCNT, CDK5RAP2, and CEP152 interact to form cytoplasmic complexes that are then integrated into the interphase PCM at the centrioles via binding to CPAP.[54,55] The interactions between CDK5RAP2 and CEP192 with PCNT, which forms the spokes of the PCM, may segregate these components into the nested layers observed in the interphase PCM (Figure 2B).[24,29]

In mitotic PCM expansion, the kinase PLK1 *(Drosophila* Polo) initiates PCM expansion and its continuous activity is required to maintain the mitotic PCM around the centrioles.[56] PLK1/Polo phosphorylates PCNT/PLP, which then promotes recruitment of CEP192, NEDD1, γ-tubulin, and the major mitotic kinase Aurora A.[57] Like PLK1, the continuous presence of PCNT/PLP is critical for maintaining PCM integrity, with depletion of PCNT/PLP by RNAi resulting in dispersal of the PCM.[24,29,24]At this stage, the PCM begins to expand beyond the bounds of the PCNT/PLP spokes, via a mechanism that is still not fully understood. What is known is that PCM expansion is dependent on CEP152/*Asl* for recruitment of two key PCM components: CEP192/dSpd-2 and CDK5RAP2/Cnn.[58] The *Drosophila* homologues of CEP192 and CDK5RAP2 form large scaffolds that extend beyond the PCNT spokes and recruit factors that make up most of the mitotic PCM[58]

Overexpression of either CEP192 or CDK5RAP2 is sufficient to form ectopic PCM-like scaffolds.[32,59] In *Drosophila* embryos, the centrosomal scaffold appears to be built in an “inside-out” manner, with incorporation of new material occurring only around the core centrioles.[55,58] Thus, in *Drosophila* embryos, PCM expansion during the transition into mitosis is thought to be driven by the rate of incorporation of new material inside the centrosome and an overall flux of CDK5RAP2/Cnn outward. This is in contrast to how PCM expansion in *C. elegans* is thought to occur. In *C. elegans* embryos, fluorescence recovery after photobleaching (FRAP) studies of mitotic centrosomes have revealed that SPD-5 (*C. elegans* functional homologue of CDK5RAP2) similarly forms a scaffold within the centrosome.[60] However, unlike in *Drosophila* embryos, new SPD-5 molecules incorporate into the expanding scaffold throughout the volume of the PCM and are not restricted to the area around the core centrioles.[60] Therefore, it seems that at least two different mechanisms for PCM assembly exist between *C. elegans* and *Drosophila*. To further complicate matters, new data from *Drosophila* brain cells suggest that PCM assembly might occur via another mechanism that lacks the outward Cnn flux described in *Drosophila* embryos.[61] This raises the intriguing possibility that in addition to changes in protein dynamics at the centrosome seen over the course of the cell cycle, differences in protein behavior may also depend on cell type and function.

Despite these differences, it is clear that PCM expansion in both *C. elegans* and *Drosophila* relies on the ability of CDK5RAP2 homologues (Cnn and SPD-5) to form micron scale centrosomal structures. This ability is of particular interest in light of the recent discovery of protein phase transitions within the centrosome and will be the focus of the discussion in the next section.

■ PHASE TRANSITIONS: IMPACT ON CENTROSOMAL ASSEMBLY AND FUNCTION

Phase Transitions in PCM Assembly.

Phase transitioning proteins have been described in a number of nonmembrane bounded organelles and can exist in a diffuse state or, dependent on external factors, spontaneously separate into a locally concentrated, nonmembrane bounded compartment.[13,14] There is now evidence that CDK5RAP2 homologues, *C. elegans* SPD-5 and *Drosophila* Cnn, are phase transitioning proteins, capable of assuming a solid aggregate or a liquid-like droplet form (Figure 3A).[15,31,62]

Two studies by Woodruff and colleagues[31,62] found that SPD-5 forms solid aggregates or liquid-like droplets *in vitro* under specific conditions (e.g., level of crowding and presence of other factors; Figure 3B). *In vitro,* SPD-5 protein alone can form a solid network, or insoluble phase.[62] In contrast, with the addition of crowding agents, SPD-5 forms liquid-like droplets that mimic the spherical appearance of the *C. elegans* mitotic PCM (Figure 3B).[31] Like the mitotic PCM, SPD-5 droplet formation can be initiated, or enhanced, by the presence of known centrosomal assembly factors such as PLK1 and the *C. elegans* homologue of CEP192, SPD-2.[31] Specifically, the kinase activity of PLK1 promotes droplet formation even at low concentrations of SPD-5.[31]

Similar to the observed isotropic incorporation of new SPD-5 molecules throughout the PCM in *C. elegans* embryos,[60] SPD-5 phase droplets isotropically incorporate new material throughout their volume.[31] Furthermore, SPD-5 droplets older than 30 seconds do not exchange SPD-5 molecules with the surrounding solution.[31] This is in agreement with previous FRAP data from *C. elegans* embryos which suggests that SPD-5 forms a long-lived scaffold within the PCM.[60] Interestingly, FRAP experiments show that SPD-5 droplets are able to expand and incorporate new monomers at early time points (<10 min), but lose this ability at later time points.[31] Woodruff and colleagues propose that these “aged” droplets (>10 min old) harden into a glass or gel-like state.[31] They further propose that the ability of SPD-5 droplets to rapidly expand at early time points, and then harden, allows the *C. elegans* PCM to quickly expand and then eventually harden to provide resistance against the pulling forces generated during mitosis.[31]

Functionally, these hardened SPD-5 droplets (or condensates) can recruit factors like the MT polymerase ZYG-9 (*C. elegans* homologue of human ch-TOG and *Xenopus* XMAP215) and the MT nucleation effector TPXL-1 (functional homologue of *Xenopus* TPX2).[31]The presence of ZYG-9 and TPXL-1 lowers the initial threshold for SPD-5 to form a liquid-like droplet, allowing droplet formation at low SPD-5 concentrations.[31] Remarkably, the presence of ZYG-9, which can interact with tubulin heterodimers, and TPXL-1 in SPD-5 condensates is sufficient to concentrate tubulin and nucleate MTs independently of γTuRC (Figure 3B).[31] These MT nucleating SPD-5 condensates mimic the appearance of centrosomal asters. Depolymerization of MTs into free tubulin heterodimers by nocodazole treatment of *C. elegans* embryos reveals that the *in vivo* centrosome similarly concentrates tubulin.[31]

Finally, FRAP experiments show that despite being in a hardened condensate, ZYG-9 and TPXL-1 are in free exchange with the surrounding solution, lending support to the hypothesis that SPD-5 condensates are porous, gel-like scaffolds.[31] By extension, this hypothesis suggests that the SPD-5 scaffold in *C. elegans* embryos is likely also a gel-like scaffold, able to selectively concentrate some factors, while retaining others for longer time scales.

The *Drosophila* homologue of CDK5RAP2, Centrosomin (Cnn), has also been observed forming large micron-scale scaffolds similar to those first reported with SPD-5 (Figure 3C).[15,62] Feng and colleagues,[15] investigating the molecular underpinnings of this property, identified and purified two conserved domains of Cnn: a phosphoregulated-multimerization domain, or PReM (a region within a leucine zipper domain phosphorylated by PLK1/Polo) and a C-terminal domain necessary for centrosomal enrichment of Cnn, CM2. Together, the PReM and CM2 domains form a 2:2 tetramer complex that alone is sufficient to generate micron-scale scaffolds *in vitro*.[15] Like SPD-5, FRAP experiments of these PReM:CM2 scaffolds show that scaffold components are not exchanged with the surrounding solution.[15] These solid networks are long-lived, with no exchange occurring over 20 min.[15] This is in agreement with previous FRAP studies in *Drosophila* embryos which show that there are few internal rearrangements of Cnn within the centrosome *in vivo*.[63]

Like SPD-5, the formation of these micron-scale Cnn (PReM:CM2) structures is enhanced by PLK1, which specifically phosphorylates residues in the PReM domain.[15] Mutagenesis of these PReM residues indicates that they are critical for scaffold formation both *in vitro* and *in vivo*.[15] This suggests a mechanism for cell-cycle control of Cnn-driven PCM expansion in *Drosophila.* Additionally, the identification of PReM and CM2 as domains sufficient to drive Cnn into a solid phase is a novel insight, as this implies that multivalent and variable affinity interactions between Cnn molecules are one of the key drivers of PCM assembly in flies.

Despite similar observations of phase transitions with SPD-5 and Cnn, there are some key distinctions, perhaps reflecting species-specific differences in PCM assembly between *C. elegans* and *Drosophila*. On the basis of SPD-5, Woodruff and colleagues suggest that the *C. elegans* centrosome is assembled as a liquid-like compartment that hardens into a porous gel. From the Cnn data, Feng and colleagues suggest that the *Drosophila* centrosome is a solid scaffold, built from the incorporation of new Cnn material around the centrioles. Furthermore, it is unclear, molecularly, what regions within SPD-5 mediate its phase transitions, while Feng et al. have shown that multivalent, intermolecular interactions between Cnn molecules are at least part of this behavior. So, is the centrosome built as a solid, insoluble scaffold, or is it a liquidlike droplet that transitions into a hardened gel-like state? There is currently insufficient data to support one model over the other, and these differences may ultimately be reflective of biological differences in centrosomal assembly in diverse cell types. It is possible that these findings could be reconciled in the future with further research into whether the full-length Cnn protein is capable of forming liquid-like droplets in crowded conditions, as was reported with SPD-5.

Ultimately, these findings in *C. elegans* and *Drosophila* are exciting and strongly suggest that vertebrate CDK5RAP2 might also phase transition. Answering the question of whether CDK5RAP2 behaves similarly will be a new unexplored avenue for investigating the assembly and function of the vertebrate centrosome.

Centrosomal Phase Transitions: Perspective on the Future.

The assembly of the PCM was originally thought to be completely orchestrated by the core centrioles, acting as a template that recruited and organized PCM components from the surrounding cytoplasm[64]. While centrioles are indeed important for efficient and robust PCM assembly, the recent discoveries of phase transitions by centrosomal proteins suggest an additional mechanism, which is particularly appealing in explaining the known aspects of centrosomal assembly and function. For instance, PCM expansion appears to be autocatalytic in nature, scaling up relatively quickly from nanometer sized components into the micron-scale centrosome present in mitosis.[65] Furthermore, this expansion is limited, stopping at a cell type-dependent maximum size that is roughly equal for both mitotic centrosomes.[55,66] Additionally, this selfassembly process is regulated by kinases like PLK1, which control the transition of the interphase PCM into its expanded and more potent form in mitosis.[55,57] Lastly, the centrosome is one of only a few known organelles lacking a membrane to separate its compartment from the cytoplasm. While it is too early to generalize the findings of phase separations by homologues of a single centrosomal protein, a PCM assembly mechanism incorporating protein phase transitions could address these known aspects of the centrosome. In addition, a phase transition based mechanism for centrosomal assembly could address the dynamic nature of the centrosome, where some components are selectively retained for long time scales, while others are allowed to rapidly exchange with the surrounding cytoplasm. Phase transitions could also provide a simple way to bring together over 100 unique centrosomal components into a local compartment without the use of a membrane.

At the same time, the discovery that centrosomal proteins undergo phase transitions raises many questions. What other centrosomal proteins undergo phase transitions and what is their binding hierarchy? Which centrosomal proteins are recruited into a phase compartment, while others are excluded? How is this selectivity achieved? How can phase transitions explain the internal architecture of the PCM, which has recently been established? More research will be necessary to delineate which centrosomal proteins are active phase transitioning proteins and how they interact with each other to contribute to the centrosome's structure and function.

One of the most pressing questions highlighted by the recent studies is the following: what is the role of γTuRC? SPD-5 phase droplets containing ZYG-9 and TPXL-1 concentrate tubulin and nucleate MTs independently of γTuRC.[31] This agrees with previous work that had established that γTuRC is dispensable during mitosis in *C. elegans*.[67,68] Perhaps γTuRC's role in a phase separated centrosome (as proposed for *C. elegans*) is as a stabilizer and anchor for MT minus ends, and as an additional platform for generating MTs. In contrast, γ-tubulin depletion from human and *Drosophila* centrosomes results in complete loss of MT nucleation,[27,69] indicating that γTuRC might have a more critical role in these

organisms. Most curiously, if a simple liquid droplet with no internal structure is capable of generating MTs, why would a structure containing hundreds of proteins and a complicated organization be necessary? Future research will have to illuminate how phase transitions drive centrosome architecture, assembly, and its ability to act as a major MTOC of the cell.

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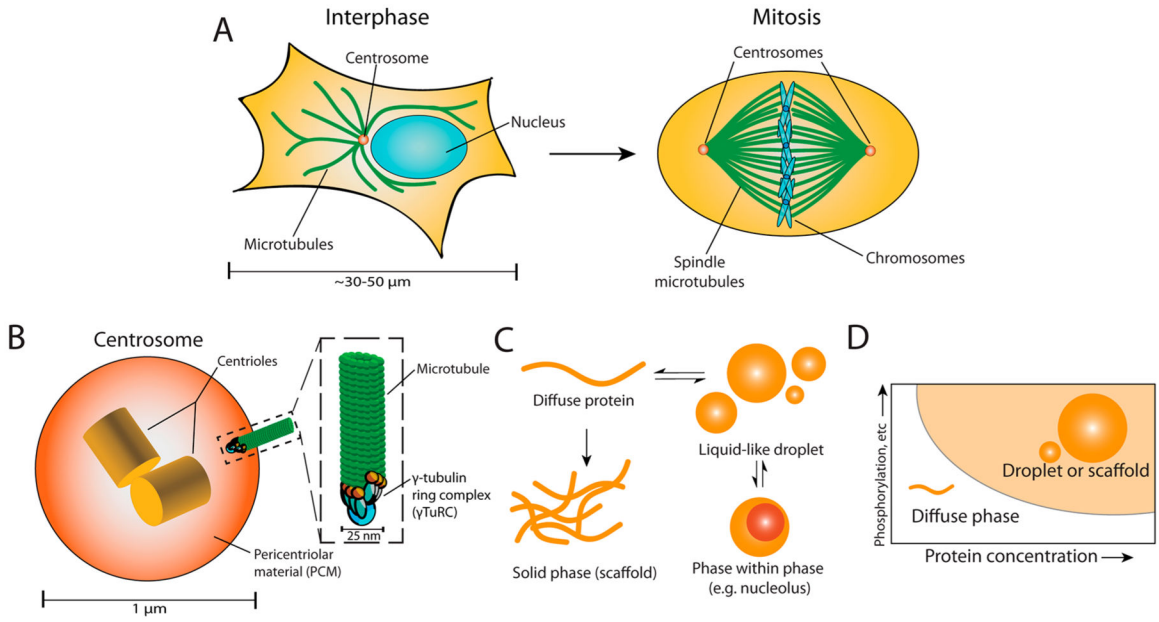

Figure 1.

Centrosome function, basic organization, and phase transitions. (A) Centrosomes are potent MT nucleators that organize the MT cytoskeleton which provides structural support, shape, cell polarity, and the highways on which vesicles are transported. During mitosis, the centrosome duplicates and forms the opposing poles of the mitotic spindle. (B) Simplified view of the main features of the centrosome. The centrosome is composed of two core centrioles oriented at 90° surrounded by a shell of proteins called the pericentriolar material (PCM). (C) A theoretical phase transitioning protein that can exist as either a diffuse protein, a liquid-like droplet, or a solid scaffold or network. In the nucleolus, proteins have been observed forming phase separations within the droplets of other proteins (ref 17). (D) A theoretical phase diagram; changes in protein concentration, presence of binding partners, or phosphorylation, among other factors, can affect whether a phase transitioning protein is in a diffuse or locally concentrated state (for an example, please see ref 16).

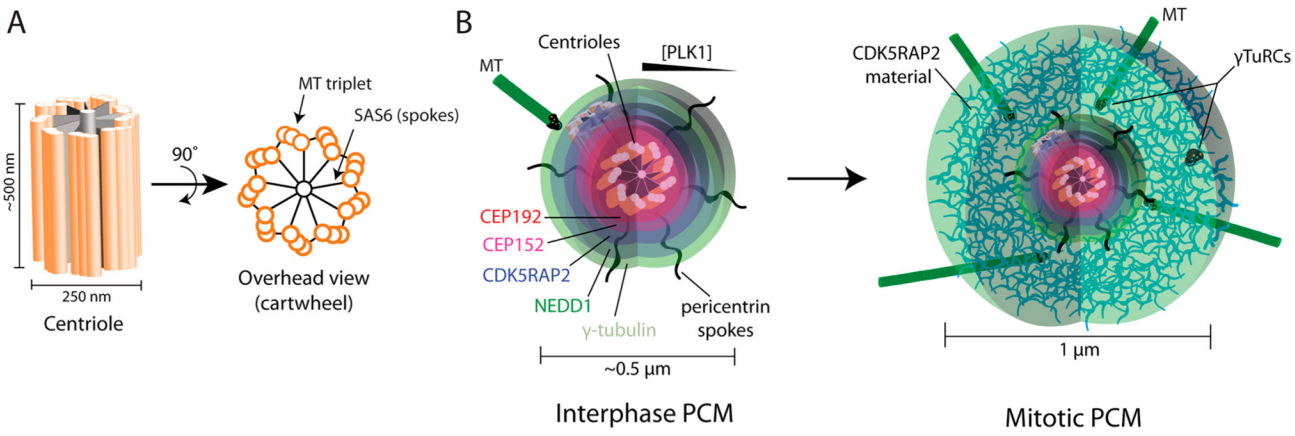

Figure 2.

Centrosome organization: centrioles and pericentriolar material (PCM). (A) A single centriole; side and overhead views. Two orthogonally oriented centrioles are at the core of the centrosome. In vertebrates, each centriole is made of MT triplets arranged in a "cartwheel" structure as shown. In invertebrates like Drosophila, these are instead made of MT doublets (not shown). (B) Diagram of centrosomal organization, specifically of the pericentriolar material (PCM) layers in interphase and mitosis. During interphase, PCM components are organized into nested concentric layers, as shown (refs 24 and 29). Prior to mitosis, the PCM expands and acquires factors, like NEDD1 and γTuRC that increase MT nucleation potency.

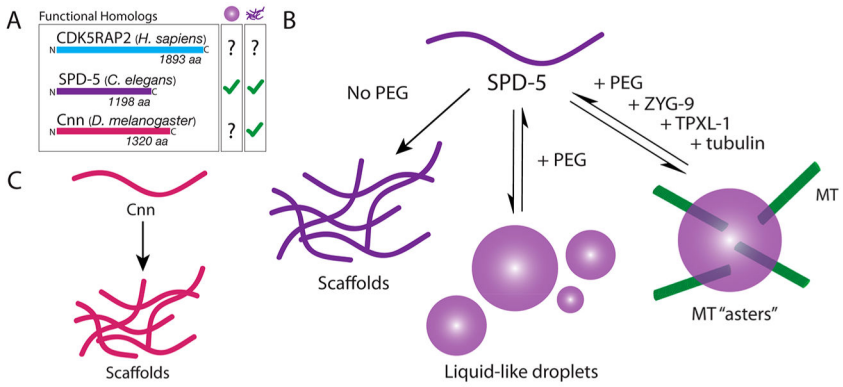

Figure 3.

Centrosomal protein phase transitions in C. *elegans* and *Drosophila.* (A) CDK5RAP2 (H. *sapiens)* is a vertebrate PCM compom Centrosomin (Cnn) is the *Drosophila* homologue of CDK5RAP2. SPD-5 appears to be the C. *elegans* functional homologue of CDK5RAP2, des] low sequence similarity. Both SPD-5 and Cnn form phase separated solid phases (scaffolds). SPD-5 is currently the only known homologue to fc liquid-like droplets. Whether CDK5RAP2 in vertebrates can phase transition is unknown. (B) SPD-5 can form solid phases (scaffolds) in the abse of a molecular crowding agent, poly(ethylene glycol), or PEG.[31,62] In the presence of PEG, SPD-5 can form liquid-like droplets. In the presence PEG, microtubule (MT) binding proteins (ZYG-9 and TPXL-1), and free tubulin heterodimers, SPD-5 droplets can nucleate MTs independently the gamma-tubulin ring complex, the universal MT nucleator in the cell.[29] (C) Centrosomin (Cnn) undergoes phase separation, forming s< scaffolds *in vitro* and *in vivo* (based on data from ref 15).

Table 1.

Selected Centrosome Factors Involved in γTuRC Recruitment or Activation

vertebrate name	homologues discussed in this review	γTuRC function
CDK5RAP2	centrosomin (Cnn; *D. melanogaster),*[15] SPD-5 (*C. elegans*; functional homologue)[31]	centrosomal recruitment,[32] γTuRC activator[10]
NEDD1	previously GCP-WD; xNEDD1 (*X. laevis)*	centrosomal recruitment[11,33]
MOZART1		mitotic centrosome targeting,[34] adaptor for γTuRC factors[35]
MOZART2A/B		interphase centrosome targeting,[36] adaptor for γTuRC factors
pericentrin	pericentrin-like protein (Plp; *D. melanogaster)*[29]	upstream recruitment factor[37]
CEP192	SPD-2 (*C. elegans),dSPD-2 (D. melanogaster)*	upstream recruitment factor[37]

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