Trends in Cell Biology

CellPress REVIEWS

Opinion

Phase Separation and the Centrosome: A *Fait Accompli*?

Jordan W. Raff [1,*]

There is currently intense interest in the idea that many membraneless organelles might assemble through phase separation of their constituent molecules into biomolecular 'condensates' that have liquid-like properties. This idea is intuitively appealing, especially for complex organelles such as centrosomes, where a liquid-like structure would allow the many constituent molecules to diffuse and interact with one another efficiently. I discuss here recent studies that either support the concept of a liquid-like centrosome or suggest that centrosomes are assembled upon a more solid, stable scaffold. I suggest that it may be difficult to distinguish between these possibilities. I argue that the concept of biomolecular condensates is an important advance in cell biology, with potentially wide-ranging implications, but it seems premature to conclude that centrosomes, and perhaps other membraneless organelles, are necessarily best described as liquid-like phase-separated condensates.

Highlights

Mitotic centrosomes are structurally complex membraneless organelles that recruit hundreds of proteins and perform many important functions in cells.

Mitotic centrosomes are formed when a highly structured centriole recruits a more amorphous pericentriolar matrix (PCM) around itself; the biophysical nature of the PCM is unclear.

Some recent studies indicate that the mitotic PCM is best described as a liquid-like condensate, whereas others suggest that the mitotic PCM is recruited to a more solid-like scaffold that assembles around the centriole.

It may be very difficult to experimentally distinguish between these alternative models of PCM structure.

The Cell Biology of Liquid–Liquid Phase Separation (LLPS)

In cell biology, there has been great interest recently in the idea that many membraneless organelles may be formed by phase separation of their constituent molecules into droplets – more recently termed biomolecular 'condensates' – that have gel- or liquid-like properties [1]. The assembly of these condensates is usually driven by proteins, often in association with nucleic acids, that have intrinsically disordered regions (IDRs) and/or low complexity domains (LCDs), or multiple copies of domains that interact with one another with relatively low affinity [2,3]. This emerging field has been reviewed extensively [4–7].

Although most cell biologists have an intuitive understanding of the differences between a solid and a liquid, few have the grounding in physical chemistry or the physics of soft matter to understand the nuances of these differences when applied to cells. The physics that describes the behaviour of an ideal solid, liquid, or gas is well understood, but few cell structures behave like any of these ideal states: they often exhibit viscoelastic behaviours, meaning that under different conditions they can be more liquid-like or more solid-like, and trying to understand the difference can often seem semantic [8]. Nevertheless, it is easy to see why the phase separation concept is proving so popular with cell biologists. The idea that membraneless organelles and intracellular assemblies might de-mix from the cytoplasm or nucleoplasm in much the same way that vinegar can de-mix from oil – a simple analogy that is often used to illustrate the concept of LLPS – is very attractive. Concentrating the different components of a membraneless organelle in a small volume of liquid would allow the constituent molecules to diffuse and interact with one another at high concentrations, thereby increasing the speed and efficiency of the relevant biological reactions. Alternatively, the ability of intracellular molecules to form a separate phase may be important for sequestering them away from the cytosol, either to keep them in an inert storage form or to protect them from unfavourable cytosolic conditions or inappropriate reactions, as appears to be the case with stress granules [9]. It is no surprise that the utility of the phase

[1]Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

*Correspondence: jordan.raff@path.ox.ac.uk (J.W. Raff).

separation concept is now being explored for many membraneless organelles and protein/nucleic acid assemblies.

In this review I discuss how the concept of phase separation can be applied to the centrosome, one of the best-studied membraneless organelles that has many important biological functions [10–12]. Recent experiments, primarily in flies and worms, are starting to shed light on the biophysical nature of the centrosome, but, in some cases, they have come to different conclusions about whether the centrosome is fundamentally liquid- or solid-like [13,14]. Several recent reviews have included the centrosome in the expanding list of membraneless organelles that are formed by LLPS [15–18]. I argue that this conclusion is premature, largely because of the difficulties in testing it rigorously – a problem that might also apply to other membraneless organelles and complex intracellular structures.

The Mysterious Centrosome

Centrosomes have long been among the most enigmatic of organelles. Early cell biologists marvelled at how this single tiny structure duplicates precisely to generate two centrosomes that then organise the two poles of the mitotic spindle, which then accurately segregates two copies of each chromosome. Although we now have a reasonably clear molecular understanding of how centrosomes duplicate [19–21] and how they nucleate microtubules (MTs) [22,23], several aspects of centrosome biology remain mysterious – including the biophysical nature of the centrosome itself.

Two barrel-shaped structures, the centrioles, form the core of the centrosome. Centrioles are structurally complex, ninefold radial symmetric structures that are usually organised in pairs – an older mother and a younger daughter, arranged at right angles to each other (Figure 1A). When centrioles form centrosomes (Figure 1B) they do so by recruiting pericentriolar material/matrix (PCM) around the mother centriole [24–26], and it is the PCM that nucleates MTs [27]. During interphase, centrioles organise little PCM and, although the classical textbook view usually depicts the centrosome as the major MT-organising centre (MTOC) in the interphase cell, the centrosome often plays a relatively minor part in organising interphase MT arrays in many cell types *in vivo* [28–30]. Indeed, in most terminally differentiated cells in humans, centrioles do not form a prominent centrosome at all, but instead migrate to the cell cortex to form a primary cilium, itself a complex MT-based structure that has many important functions (Figure 1C) [20,31].

As cells prepare to enter mitosis, however, there is a dramatic and rapid increase in the amount of PCM organised around the mother centriole – a process termed centrosome maturation [25,32]. This rapid assembly of the mitotic PCM is a remarkable feat of bioengineering because this PCM is thought to contain several hundred proteins [33] – many of which help to nucleate and organise MTs, but many others are involved in processes such as cell-cycle regulation and cell signalling [34,35]. The organising principles that allow such a complex protein machine to be assembled, and later disassembled, so quickly are still poorly understood.

Importantly, the mechanisms underlying mitotic centrosome assembly somehow ensure that the two spatially separated centrosomes usually grow to the same size, and thus organise two equally sized mitotic spindle poles. This equal sizing is challenging for the cell because even a small difference in the initial size of the two centrosomes would be expected to be amplified as the centrosomes grow – because the larger centrosome would outcompete the smaller centrosome for new components [36] (Figure 2). This problem would be especially challenging if the PCM behaves as a phase-separated condensate because new PCM proteins would need to incorporate at the phase boundary between the cytoplasm and the PCM, and the surface area

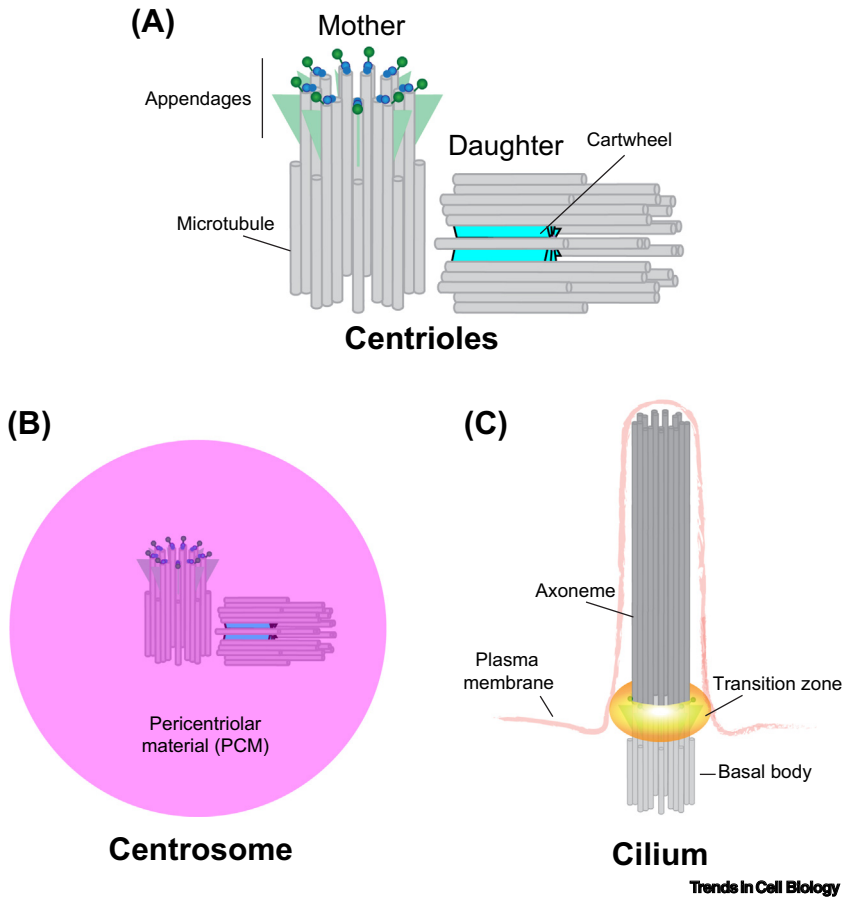

Figure 1. Centrioles Organise Centrosomes and Cilia. (A) Most animal cells are born with a single pair of centrioles, comprising an older mother and younger daughter. The centriole pair duplicates precisely once during the cell cycle, when the mother and daughter centrioles separate and a new daughter centriole assembles around a central cartwheel structure that forms on the side of the two pre-existing centrioles. Mother centrioles are often decorated by distal and subdistal appendages. (B) Centrosomes form when a mother centriole recruits pericentriolar material/matrix (PCM) around itself. In interphase, the mother centriole organises only a small torus of PCM [19,75,86–88] (not shown), but, as illustrated here, the PCM expands dramatically as cells prepare to enter mitosis. (C) Cilia form at the cell membrane when an axoneme of MTs extends from the distal end of the mother centriole (now termed a basal body). A transition zone links the distal end of the basal body to the plasma membrane. Cilia have many important functions, as reviewed extensively elsewhere [89–91].

of this boundary will always be larger at larger centrosomes – and the larger centrosome will therefore incorporate new components faster than the smaller one.

Early Evidence for a Solid-Like Mitotic Centrosome Scaffold

Classical electron microscopy (EM) studies have revealed that, in contrast to the highly structured centrioles at the centre of the mitotic centrosome, the surrounding mitotic PCM is electron-dense but generally amorphous [37,38]. Electron tomography (ET) of purified mitotic centrosomes from the early embryos of clams and flies revealed a fibrous matrix surrounding the centriole that contains numerous channels that are much larger than the underlying scaffold structure, indicating that this matrix is likely to be highly permeable to the surrounding cytosol [39,40]. Ring-like structures were dispersed throughout the PCM, and subsequent analyses revealed that these rings were γ-tubulin ring complexes (γ-TuRCs) [41]. Treatment of purified mitotic centrosomes with high salt concentrations largely removes γ-tubulin (and many other proteins) from

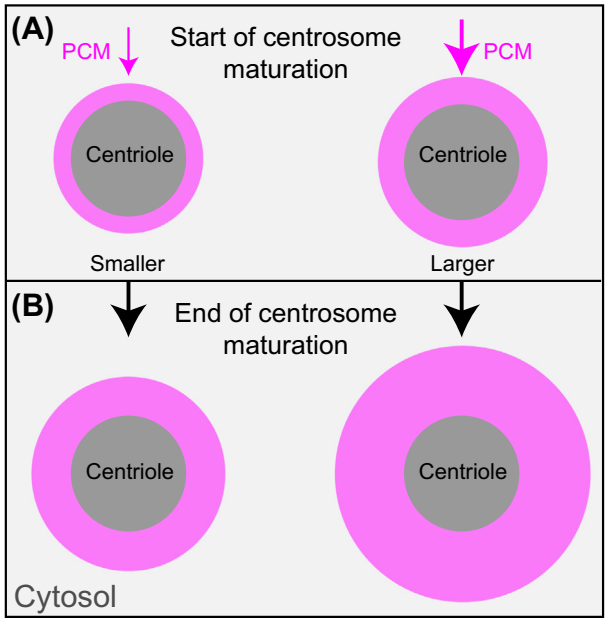

Figure 2. The Problem of Mitotic Centrosome Size Regulation. (A) In cells preparing to enter mitosis, the two maturing centrosomes are spatially separated but start to recruit mitotic pericentriolar material/matrix (PCM; magenta) around their mother centrioles (grey) at the same time. By stochastic variation, one would expect that the two maturing centrosomes are initially unlikely to be identical in size (the one on the right is slightly larger in this illustration), and in some cells – such as the *Drosophila* neuroblast [92] – the two centrosomes are initially highly asymmetric in size. The larger centrosome will have a greater surface area, and therefore should incorporate new PCM components at a faster rate (indicated by the size of the magenta arrows). (B) As a result, even a small difference in initial centrosome size would be expected to be amplified during the growth process such that the two mature centrosomes would be of very different sizes (as illustrated here). This is not, however, what is observed in cells, and even in *Drosophila* neuroblasts the initially asymmetrically sized centrosomes ultimately grow to similar sizes. It is unclear how this equal sizing is achieved, although the observation that a key mitotic PCM scaffolding protein in flies (Spd-2) is only incorporated into centrosomes at the centriole surface may provide an answer (see text and Figure 3).

the centrosomes, and these 'salt-stripped' centrosomes no longer nucleate MTs, although a fibrous electron-dense 'centromatrix' remains, at least in clam centrosomes [39,42]. When salt-stripped centrosomes are mixed with cytoplasmic embryo extracts, they regain their ability to bind γ-tubulin and to nucleate MTs; when the extracts are first depleted of γ-tubulin, they no longer restore the ability to nucleate MTs. These findings support the strong evidence that centrosomes largely nucleate MTs through γ-TuRCs recruited to the PCM matrix [43–47].

The picture that emerges from these studies is of a fibrous, porous, mitotic centrosome 'scaffold' that binds centrosomal proteins such as γ-tubulin from the cytosol permeating the scaffold, thereby concentrating these proteins within the mitotic PCM.

Spd-2, Polo, and Cnn Cooperate to Assemble a Mitotic Centrosome Scaffold in Flies

A genome-wide RNAi screen for genes required for mitotic PCM assembly in *Drosophila* cultured cells identified *polo*, *centrosomin* (*cnn*), and *Spindle-defective-2* (*Spd-2*) as the strongest hits [48]. Flies mutant for both *cnn* and *Spd-2* can organise interphase PCM, but centrosomes fail to mature when cells enter mitosis, indicating that Cnn and Spd-2 proteins are strong candidates for components of the mitotic centrosome scaffold [25]. Spd-2 in flies exhibits an unusual dynamic behaviour at mitotic centrosomes. Most individual centrosome proteins turn over

throughout the volume of the mitotic PCM, indicating that they are constantly binding to, and being released from, an underlying scaffold structure that permeates the PCM [25]. By contrast, Spd-2 is only recruited into the centrosome at the surface of the mother centriole, where it appears to assemble into a fibrous-like scaffold that fluxes outwards, away from the mother centriole [25] (Figure 3A). Thus, Spd-2 does not incorporate into the PCM by binding to an underlying scaffold structure that is spread throughout the PCM volume, but instead appears to form a scaffold structure that assembles at the centriole surface and then spreads outwards. Spd-2 can recruit both Polo kinase [49] and Cnn [50] to the mitotic PCM. Polo then phosphorylates Cnn, allowing Cnn to assemble into its own scaffold that interacts with and helps to maintain the Spd-2 scaffold [25,51] (Figure 3A,B). In fly embryos, but not in fly somatic cells, the Cnn scaffold itself fluxes outwards along the centrosomal MTs, allowing it to extend beyond the Spd-2/Polo scaffold [25,51] (Figure 3A).

The dynamic behaviour of Spd-2 could help to explain how two spatially separated centrosomes grow to the same size. Even if cells initially enter mitosis with centrosomes of different sizes, both centrosomes can eventually reach the same steady-state size when the rate of addition of Spd-2 at the surface of the mother centriole (which is independent of the initial size of the centrosome – unbroken red arrow in Figure 3C) is balanced by the rate of Spd-2 loss from the periphery (which would be expected to increase as the surface area of the centrosome increases – broken red arrow in Figure 3C). In such a scenario it is the equal sizing of the mother centrioles that ultimately ensures the equal sizing of the mitotic centrosomes, as has been observed in early *C. elegans* embryos [52]. Although this model is attractive, it is important to stress that the apparent outward flux of Spd-2 from the mother centriole has so far only been observed in *Drosophila* embryos and cells, and the analysis of Spd-2 dynamics is complicated by the presence of potentially distinct pools at centrioles and in the PCM [53,54].

Molecular Components of the Mitotic Centrosome Scaffold Appear to Have Been Conserved in Evolution from Worms to Humans

Genetic approaches have identified a similar set of proteins that are required for mitotic PCM assembly in *C. elegans* [55–59]. As in flies, the centriole/centrosome protein SPD-2 recruits PLK-1 (the worm homologue of Polo) and a protein called SPD-5. Homologues of SPD-5 have not been identified outside of worm species but, although Cnn and SPD-5 exhibit little amino acid sequence homology, they are both large proteins with several predicted coiled-coil regions, and they appear to be functional homologues. As with fly Polo and Cnn, worm PLK-1 phosphorylates SPD-5 at multiple sites, which allows SPD-5 to assemble into a scaffold structure that is required for centrosome maturation [13,60,61]. SPD-5 appears to be the major component of the mitotic centrosome scaffold in worms, and centrosome maturation is essentially abolished in the absence of either SPD-2, PLK-1, or SPD-5.

Vertebrate homologues of Spd-2/SPD-2, Polo/PLK-1, and Cnn (Cep192, Plk1, and Cep215/Cdk5Rap2, respectively) all have important roles in mitotic centrosome assembly, indicating that this mitotic PCM assembly pathway is likely to have been conserved in evolution [62–68]. In vertebrates, Cep192/Spd-2 not only recruits Plk1 to centrosomes but also recruits Aurora A, another protein kinase that has a major role in orchestrating mitotic events at the centrosome [69]. There appears to be an intricate interplay between Cep192/Spd-2, Aurora A, and Plk1 in vertebrates, with Cep192/Spd-2 helping to activate Aurora A, which in turn phosphorylates Cep192/Spd-2 to allow it to recruit Plk1 [69–71]. It is not yet clear if the details of this pathway are conserved in flies or worms, but Aurora A also plays an important part in regulating mitotic centrosome assembly in both species [72,73]. In vertebrate cells, another large centriole and PCM protein, pericentrin, also has an important role in mitotic centrosome assembly, which is dependent on its phosphorylation by Plk1 [64,74]. In flies, the pericentrin-like-protein (PLP) has

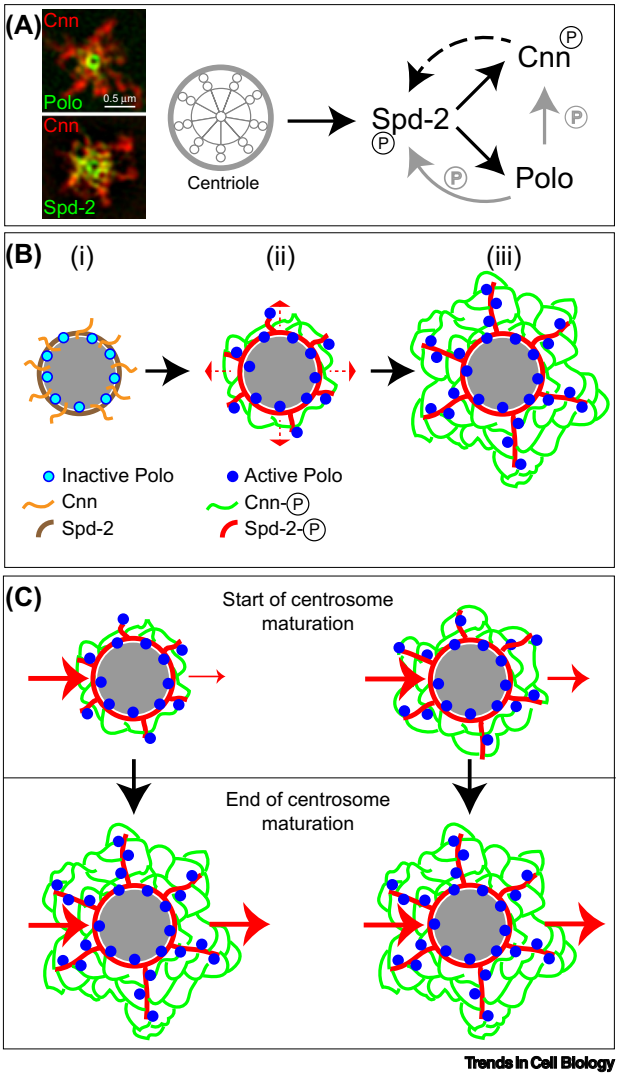

Figure 3. Spd-2, Polo, and Cnn Cooperate To Assemble the Mitotic Centrosome Scaffold in Flies. (A) Micrographs show a 3D-structured illumination (3D-SIM) image of centrosomes in living fly embryos illustrating the scaffold-like structures formed by Spd-2, Polo, and Cnn around the mother centriole (viewed here down the barrel of the mother). The schematic depicts the putative pathway of scaffold assembly in flies: black arrows indicate recruitment, grey arrows indicate phosphorylation (P), and the black broken arrow indicates that Cnn helps to maintain the Spd-2 scaffold, but does not recruit Spd-2 into the scaffold. (B) The schematic illustrates how this pathway may form a positive feedback loop to drive the expansion of the mitotic pericentriolar material/matrix (PCM) around the mother centriole (grey). (i) During interphase, Polo, Spd-2, and Cnn are organised in a torus around the mother centriole [75,93]: Polo is inactive, Spd-2 and Cnn are not phosphorylated, and therefore no scaffold assembles. (ii) As cells prepare for mitosis, Polo (and other mitotic kinases) are activated: Spd-2 is phosphorylated and assembles into a scaffold that fluxes away from the centriole (indicated by red broken arrows) [25]. The phosphorylated Spd-2 scaffold recruits Polo and Cnn [49,50]; Polo then phosphorylates Cnn, allowing it also to assemble into a scaffold [14,51]. (iii) The Cnn scaffold helps to maintain the Spd-2 scaffold, allowing it to expand further and thus recruit more Polo and Cnn – thereby creating a positive feedback loop. [25,49]. (C) The schematic illustrates how this mechanism may ensure that two maturing centrosomes of initially unequal size (top panels) ultimately grow to the same size (bottom panels), where the rate of addition of Spd-2 at the centriole surface (red arrows into the centriole) equals the rate of Spd-2 loss at the centrosome periphery (red arrows out of the PCM). In this schema the rate of Spd-2 incorporation is dependent on the size of the mother centriole (which remains constant), whereas the rate of Spd-2 loss at the PCM periphery increases as PCM size increases; this is a reasonable assumption but has not been proven experimentally.

a role in both interphase [75,76] and mitotic [77–79] PCM assembly, but its role in mitotic PCM assembly is relatively minor compared with Spd-2 and Cnn. Pericentrin-family proteins appear to interact directly with Cnn-family proteins in both vertebrates and flies [78,80].

Is the Mitotic PCM a Phase-Separated Biomolecular Condensate?

The observations described above have largely been interpreted as supporting a model in which Spd-2, Polo, and Cnn, and their homologues in other species, cooperate to assemble a fibrous, solid-like centrosome scaffold. Recent studies of the behaviour of the worm SPD-5 protein *in vitro*, however, have led to an alternative view of mitotic PCM assembly. Large coiled-coil proteins such as Cnn and SPD-5 are notoriously difficult to work with *in vitro*, but Woodruff *et al.* succeeded in purifying full-length recombinant SPD-5 from insect cells [60]. Purified SPD-5 self-assembles *in vitro* into large, micron-scale aggregates, catalysed by purified recombinant SPD-2 and PLK-1. These assemblies appear to function as *bona fide* scaffolds *in vitro* because they can specifically recruit SPD-2 and PLK-1, but not several other non-centrosomal proteins. Perhaps surprisingly, they do not recruit γ-tubulin.

Although the SPD-5 scaffolds formed *in vitro* appear to be solid-like, it was subsequently shown that in the presence of a molecular crowding agent (which reduces the volume of solvent available to other molecules, and so potentially better mimics the crowded environment of the cytosol), purified SPD-5 can form spherical condensates that now look more like centrosomes [13]. These SPD-5 condensates have transient liquid-like properties, and they can recruit ZYG-9 (the worm homologue of ch-TOG/XMAP215, a protein that promotes MT growth and stability) and TPXL-1 (the worm homologue of TPX-2, a protein that promotes MT stability). Moreover, the condensates formed by these three proteins can concentrate α/β-tubulin dimers ~fourfold over cytosolic levels and can nucleate MT asters. Based on these observations, the authors suggest that the mitotic PCM is best described as a 'selective phase' that concentrates MT regulators and α/β-tubulin, allowing the PCM to organise MT arrays. This interpretation is consistent with an earlier mathematical model that considered the centrosome as a liquid droplet [36].

Although the ability to reconstitute MT organisation from purified centrosomal proteins *in vitro* is an important achievement, it is not clear how closely this reconstituted system accurately mimics MT nucleation from centrosomes *in vivo*. Many purified proteins can form liquid-like condensates *in vitro*; indeed, such LLPS is the bane of the lives of many protein crystallographers because it often prevents proteins in solution from undergoing the liquid–solid phase separation that is required for crystal formation. At least two other MT-binding proteins, Tau [81] and the spindle matrix protein BugZ [82], can also form condensates that concentrate tubulin, and, in the case of Tau, the tubulin polymerises into long MT bundles within the condensate [81]. Moreover, as discussed earlier, centrosomes normally nucleate MTs by recruiting γ-TuRCs, and these are not present in the SPD-5/ZYG-9/TPXL-1/tubulin condensates. Although the major pathway of MT nucleation at centrosomes requires γ-tubulin, there is a less efficient pathway that allows centrosomes to organise MT arrays independently of γ-tubulin, at least in worm embryos and some fly cells [47,83]. Perhaps the SPD-5/ZYG-9/TPXL-1 condensates mimic this alternative pathway, although this pathway does not appear to require ZYG-9 in worm embryos.

Regardless of how these condensates organise MTs, it is striking that the spherical condensates have this ability, whereas the original, solid-like, condensates do not. This suggests that the formation of a condensate with liquid-like properties might be important to allow centrosome proteins to cooperate efficiently to organise MTs. The SPD-5 condensates formed *in vitro*, however, are only transiently liquid-like because they rapidly 'mature' into a more viscous-gel- or solid-like state [13]. Interestingly, such a gel- or solid-like state also appears to be observed for both SPD-5 and Cnn molecules at centrosomes *in vivo* because dynamic studies indicate that both molecules

have little ability to internally rearrange once they are incorporated into centrosomes in living fly or worm embryos [50,51,54]. These findings led to the suggestion that SPD-5 might initially form a liquid-like condensate that expands around the mother centriole and then hardens once the centrosome reaches its full size; such hardening could be important for strengthening the centrosome, allowing it to maintain its shape and resist the extensive physical forces it will experience during cell division [13]. Although this is an attractive idea, there is currently no direct evidence that the SPD-5 or Cnn centrosomal scaffolds ever exist in a liquid-like state *in vivo*.

Moreover, the evidence that SPD-5 condensates are transiently liquid-like *in vitro* is relatively weak: because these condensates harden rapidly *in vitro*, they cannot be analysed in the ways that have very convincingly demonstrated the liquid-like properties of several other biomolecular condensates – such as 'dripping' under shear stress or droplet fusion observed directly using time-lapse microscopy [84] – although the fusion of small 'immature' SPD-5 droplets was inferred from static EM images [13].

Could Other PCM Proteins Phase-Separate into a Liquid-Like State upon Recruitment onto a Solid-Like Scaffold?

Although a liquid-like centrosome scaffold has not yet been demonstrated *in vivo*, it is possible that the proteins that are recruited to the scaffold – often referred to as 'clients' to distinguish them from the underlying scaffold that they interact with – could condense into a liquid-like

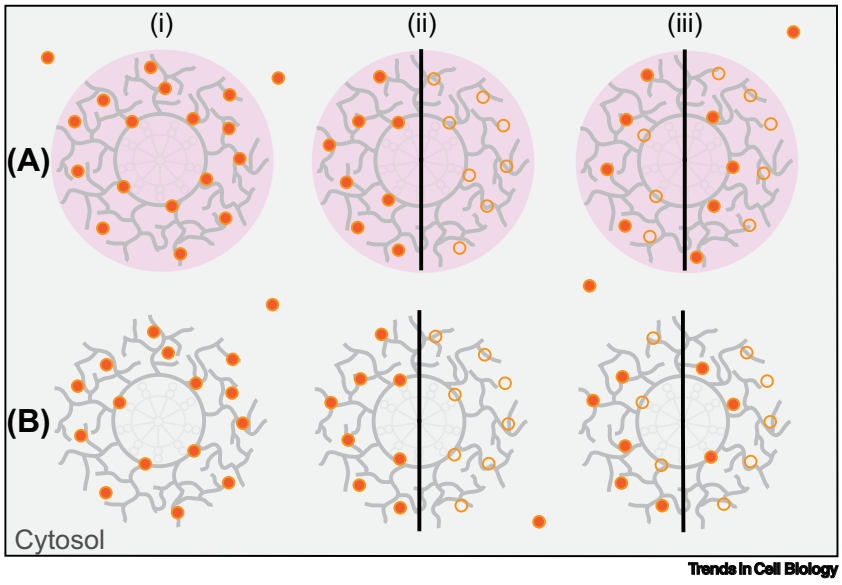

Figure 4. It May Be Difficult To Distinguish whether Client Molecules 'Phase Separate' Onto a Solid- or Gel-Like Scaffold, or Simply 'Bind' to the Scaffold. (A) (i) The schematic shows a mitotic centrosome organised by a gel-like or solid-like scaffold (grey) organising a liquid-like mitotic pericentriolar material/matrix (PCM, magenta) that has phase-separated from the cytosol. Fluorescently labelled client molecules (filled orange circles) are at a high concentration in the PCM phase and a low concentration in the cytosolic phase, and freely diffuse in both phases. (ii) If the client molecules in the right half of the PCM are photobleached (indicated by empty orange circles), fluorescence will rapidly recover in the bleached half (iii) as unbleached molecules diffuse in from the unbleached half (and vice versa). The movement of unbleached client molecules from the cytosolic phase into the PCM phase is negligible by comparison, owing to the low concentration of client proteins in the cytosol. Such behaviour is often taken as evidence that the client proteins are internally rearranging in a liquid-like phase. As illustrated in (B), however, this experiment could give a similar result even if the client molecules are not in a separate phase but are simply binding (and unbinding) to a solid-like scaffold from a cytosolic phase that permeates the scaffold. This is because the scaffold concentrates client proteins that can then diffuse within the PCM as they constantly bind to, and unbind from, the scaffold.

phase after recruitment to a scaffolded environment that has largely solid- or gel-like properties [13,85]. Experiments that are often performed to test this possibility are 'partial-bleach' analyses, in which fluorescently labelled client proteins are photo-bleached in only half of a condensate, and subsequent fluorescence recovery is monitored (Figure 4A). If fluorescent molecules move from the unbleached half into the bleached half of the condensate (and vice versa), this is taken to imply that the molecules can internally rearrange, suggesting they are in a liquid-like phase. Such an analysis was performed with several PCM clients that concentrated inside the SPD-5 condensates *in vitro*, and some of them rearranged in this way, suggesting that they had partitioned into a liquid-like phase organised by a solid- or gel-like SPD-5 scaffold [13].

This experiment, however, cannot easily distinguish whether the client proteins are recruited into a liquid phase that is distinct from the cytosol (as oil is distinct from vinegar) (Figure 4A) or, instead, whether they are simply concentrated within a porous scaffold that is permeated by the cytosolic phase (Figure 4B). Thus, this 'partial-bleach' behaviour can be explained without invoking LLPS, and it seems difficult to differentiate between the two models illustrated in Figure 3. This problem could also apply to other membraneless organelles, such as the Balbiani body, in which an underlying scaffold appears to be largely solid- or gel-like, but where the client proteins they recruit appear to internally rearrange within the organelle [85].

Concluding Remarks

Phase separation is an emerging concept in cell biology that can help to explain the assembly of several membraneless organelles and protein/RNA assemblies. The key assembly proteins often have special motifs that allow them to undergo LLPS and maintain liquid-like properties in a condensed state, and there is increasing evidence that the ability of these proteins to phase-separate from the cytosol is important for their function. Nevertheless, as our appreciation of the significance of this phenomenon increases, it is important to keep in mind that the LLPS concept might not apply to all membraneless organelles or assemblies, and that it might be difficult to prove (or disprove) its role if the underlying scaffold exhibits solid- or viscous-gel-like properties (see Outstanding Questions).

I have illustrated this difficulty in the case of the mitotic centrosome. The key mitotic PCM scaffolding proteins in flies and worms, Cnn and SPD-5, do not seem to have any of the protein motifs so far associated with LLPS. Instead, they contain multiple extended regions that are predicted to form coiled-coils and, in the case of Cnn, the crystal structure of the interaction between two of these coiled-coil regions has been solved – with both coiled-coils partially unwinding at one end and entwining with each other to form a tetramer [14]. This relatively unusual coiled-coil interaction appears to be essential for mitotic PCM assembly in the fly, and the entwining of the two dimeric regions of Cnn is thought to alter the conformation of nearby regions in the dimer – allowing them to separate and interact with similar regions in other Cnn dimers. It will be important to test if any of the coiled-coil domains in SPD-5 also interact in this way, and to determine the nature of the other interactions that ultimately allow Cnn and SPD-5 to self-assemble into micron-scale scaffolds. Perhaps these other interactions will turn out to be high-affinity, stereotypical interactions such as those observed between the two coiled-coil regions of Cnn; alternatively, they might be multiple low-affinity interactions that initially allow the Cnn and SPD-5 to form liquid-like scaffolds, which eventually harden into more solid- or viscous gel-like structures.

Whatever the outcomes of these future experiments, it seems clear that the mitotic PCM scaffold in flies and worms is not liquid-like (in an oil/vinegar sense) for most of its lifetime. Historically, in such polarised controversies, both sides turn out to be partially right – and we should not have to wait long to find out, at least in the case of centrosomes.

Outstanding Questions

What is the nature of the intramolecular interactions that allow SPD-5 and Cnn to assemble into micron-scale structures? Are they specific, strong interactions that allow the assembly of a solid-like scaffold, or less-specific, weak interactions that are more compatible with LLPS?

How are these interactions regulated by phosphorylation to ensure that the scaffolds only assemble around the mother centriole, and only during mitosis?

Two dimeric coiled-coiled domains in fly Cnn interact to form a tetramer that is essential for Cnn scaffold assembly. These domains are not obviously conserved in worm SPD-5, but does SPD-5 scaffold assembly depend on structurally similar interactions? If so, it would suggest that the underlying scaffold structure is likely to be conserved.

Can Spd-2/Cep192 proteins form a scaffold, and does this scaffold flux outwards away from the mother centriole in other systems – as appears to be the case in flies?

Is the Spd-2/SPD-2, Polo/PLK-1, Cnn/SPD-5 pathway identified in flies and worms the dominant pathway of mitotic PCM assembly in more evolved eukaryotes? How does the pericentrin family of proteins fit into this pathway, and can pericentrin also form a mitotic PCM scaffold? If so, is this scaffold formed by LLPS?

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