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Review Article

Drops and fibers — how biomolecular condensates and cytoskeletal filaments influence each other

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The cellular cytoskeleton self-organizes by specific monomer–monomer interactions resulting in the polymerization of filaments. While we have long thought about the role of polymerization in cytoskeleton formation, we have only begun to consider the role of condensation in cytoskeletal organization. In this review, we highlight how the interplay between polymerization and condensation leads to the formation of the cytoskeleton.

Introduction

The cellular cytoskeleton is a dynamic structure consisting of filaments that constantly exchange monomers with the cytoplasm by nucleation, polymerization and depolymerization. Despite extensive studies over the past 100 years, our understanding of the dynamic nature of the cytoskeleton and its regulation in cells and tissues is far from complete. Recent work has implicated biomolecular condensates in the regulation of the cytoskeleton [1–3]. For instance, the increased concentration of cytoskeletal monomers and control over the stoichiometry of regulators can affect the local rates of nucleation and growth of filaments [1,2,4–6]. Unlike polymers, whose formation is promoted by well-defined interactions of components, the formation of condensates is promoted by ensembles of configurations and variability in possible interactions (Box 1). The interaction between condensates and filaments opens up a multitude of questions. How does condensation facilitate the self-organization of filamentous networks at the right time at the right place? How do filament networks control the spatial organization of condensates? What effects do mechanical forces exerted by filaments and condensates have on each other? How do polymerization reactions of cytoskeletal filaments regulate the dynamic assembly and disassembly of condensates? In this review, we describe recent experiments and theory that show how the combined features of structural properties of filaments and conformational flexibility of condensates, provide new insights into the regulation of the cytoskeleton.

Condensates serve as reaction containers for nucleation and growth of cytoskeletal filaments

In this section, we describe how concepts inherent to condensation provide potential mechanisms to modulate the biochemistry of cytoskeletal systems. *In vitro*, high concentrations of cytoskeletal monomers and dimers alone are sufficient to spontaneously assemble cytoskeletal filaments (Figure 1a). Because condensates locally increase protein concentration, one possibility is that nucleation and growth of filaments could be driven by selective recruitment and concentration of monomers in condensates [40]. A classic example of condensate-mediated fiber formation is the condensation of tropoelastin, which precedes the assembly of elastin as part of the extracellular matrix fibers [41]. The formation of actin filaments [42] and microtubules [6] can be driven inside the condensates of actin-associated and microtubule-associated proteins (Figure 1b,c). However, condensates not only modulate the concentration of monomers, but also change the local environment. For instance, other solutes, the different stoichiometry of regulators or sorting of factors can have an impact on the polymerization dynamics of polymers inside condensates [2,5]. A key feature of condensates is their

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Box 1. Material properties of cytoskeletal filaments versus condensates

	Cytoskeletal filaments	Condensates
Molecular components	Actin monomers and αβ-tubulin are structured, globular proteins; intermediate filaments are α-helical rods with disordered tail domains.	Biomolecular condensates are multicomponent structures of various proteins and nucleic acids. Many of the proteins are long, contain disordered domains and feature multivalent binding, such as the cytoskeletal regulators WASP, tau or filaggrin [7].
Intermolecular interactions	The molecular interactions between the monomeric, structured proteins are strong (binding constants in the μM–nM range [8]) and of specific orientation.	The macromolecules comprising a condensate form multivalent and often transient or weak interactions (e.g. electrostatic or cation-π) [9,10]. The affinity of a molecule towards a condensate is, therefore, expressed in terms of partition coefficients that are in the order of 10–1000 (concentration inside versus outside condensed phase) rather than by monovalent binding constants.
Assembly	The monomeric proteins self-assemble via polarized growth in one dimension. Nucleation and growth of globular actin and αβ-tubulin into filaments is an active process consuming ATP/GTP, while the assembly of intermediate filaments is passive. *In vivo*, assembly of cytoskeletal filaments is controlled by regulators that limit or facilitate the nucleation of new filaments. Their length results from the on- and off-binding kinetics and capping structures that terminate filament growth.	*In vitro* and in initial descriptions of cellular phenomena [11], the formation of condensates has been explored analogous to passive phase separation, which does not require the consumption of a chemical energy source such as ATP. However, active processes triggering the formation and alternative condensation mechanisms, e.g. on intracellular surfaces receive more and more attention [12–15].
Higher order structure	Cytoskeletal filaments with periodic lattices may be several micrometers long and are discerned by their diameter into actin filaments ($d = 7$ nm), intermediate filaments ($d = 8$–12 nm) and microtubules ($d = 25$ nm). Despite similar dimensions the filaments show striking differences in flexibility, which is described by the persistence length L_P. Filamentous actin ($L_P \sim 10$ μm) [16] and intermediate filaments ($L_P \sim 1$ μm) [17] can be deformed in the size range of organelles. Microtubules on the other hand are remarkably stiff ($L_P \sim 5$ mm) and individual filaments can span throughout the cytoplasm and cellular protrusions [18]. Higher order structures such as the mitotic spindle [19] and cytoskeletal networks emerge through cross-linking and bundling of filaments, even between different types [20], via regulators and molecular motors.	Many condensates do not display a dominant structure, i.e. are amorphous [21,22]. They are rather comprised of a dynamic rearrangement of macromolecules with short persistence lengths of ∼0.6–0.9 nm for proteins with disordered domains [23] and ∼50/70 nm for double-stranded DNA/RNA [24]. Thus, the structural unit of a condensate is purely defined by the increased concentration of a respective molecule above the dilute surrounding. *In vitro*, the enrichment of a protein or nucleic acid is associated with a higher density. In the complex environment of the cytosol or nucleosol, however, the local density of total macromolecules does not necessarily change through the redistribution of certain molecular species [25]. Nonetheless, exclusion of particles bigger than a few nanometers from cellular condensates [26] and gravitational effects of condensates in the nucleosol of big oocytes [27] provide *in vivo* examples of dense structures compared with the surrounding.
Turnover dynamics	Despite the strong interactions between the monomers, cytoskeletal filaments are dynamic, i.e. they constantly assemble and disassemble, e.g. in the actin cortex the half-time of actin turnover is in the order of 10 s [28].	The dynamic interactions between the molecules of the dense phase define their diffusion within the condensate as well as the exchange rate with molecules in the surrounding dilute phase. Depending on the viscosity, half-times from 2.5 s to >100 s have been reported for the turnover of molecules in cellular condensates [1,29].

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Box 1. Material properties of cytoskeletal filaments versus condensates

	Cytoskeletal filaments	Condensates
Response to force	The rheological response of the cytoskeleton depends on the frequency at which it is probed. On very short timescales (<ms) the viscous response of individual filament dynamics and surrounding cytoplasm dominate [30]. On short timescales (~s) highly cross-linked networks, such as the actin cortex, are mainly elastic. This provides mechanical support for cells as they resist applied forces. On longer scales in the order of bond lifetimes of cross-linkers and turnover of the filaments (~min) the networks can rearrange and viscous properties emerge [31]. Thereby they either adopt the imposed shapes or enforce the network through extra connections. Furthermore, many biopolymer networks exhibit the property of strain-stiffening, a stiffness that increases non-linearly for large strains; this may also protect the integrity of the polymer network from assault. The absolute strength of the cytoskeletal structure of interest highly depends on the adaptor proteins and degree of cross-linking. While the storage and loss moduli of cytosolic networks have been quantified in the 10^1–10^4 Pa range [31,32], nuclear actin appears much softer ~10^{-1} Pa [33].	Initially, biomolecular condensates have been described to behave as liquids with features known from everyday life such as dripping or fusion of drops [11]. From a physical point of view, the simplest type of liquid is that of a Newtonian fluid, in which the force required to deform the material depends linearly on the deformation rate (viscous). Protein condensates, however, do not behave purely viscous but also exhibit elastic properties. For a completely elastic material, the force required to deform the material depends on the deformation not on the rate of deformation. The mechanical response of *in vitro* protein droplets has been found to be predominantly viscous, but dominated by elastic response at short interaction times (<0.1 s) with loss moduli and storage moduli in the order of 10^0–10^1 Pa, respectively [34]. Furthermore, the attractive interactions between the molecules holding the drops together cause a surface tension. As the system tries to minimize its energy arising from these forces, it will reduce its surface area and create spherical condensates. When an entire condensate, including its surface, is deformed, surface tension can lead to an apparent elastic response at long time scales.
Complex material properties	Additional to the passive and active response to forces the polymerizing filaments and network contractions through motor proteins, such as myosin or kinesin, apply forces themselves. Biomolecules or whole organelles are transported along the filaments and deformation of the networks induces cell shape changes. Taking the complex mechanical behavior together the cytoskeleton has been described as active gel with viscoelastic behavior [35].	The material properties of cellular condensates vary widely and *in vitro* the properties have been found to depend on the composition, temperature, salt concentration and age [9,34]. Hardened condensates can withstand higher forces, e.g. those applied by spindle microtubules during mitotic chromosome segregation [36] and could protect biochemical components under stress conditions [37]. However, the liquid-to-solid transition is also often associated with gelation [38] or aggregation of proteins, which results in pathological fiber formation as seen, e.g. for FUS [39].

switch-like formation above a saturation concentration. This allows for biological information processing and filters noise, e.g. originating from varying expression levels [43], which could help to reliably trigger the polymerization of cytoskeletal filaments in space and time (Figure 1d).

Actin dynamics can be modulated by condensates

Recent work suggests that actin dynamics are likely modulated by the interaction of actin and its regulators with condensates. A good example is the role of neuronal Wiskott–Aldrich syndrome protein (N-WASP), its adaptor protein Nck, and the transmembrane protein nephrin that selectively bind and thereby control local arp2/3-mediated actin assembly in kidney cells [45–47]. In their landmark study Li et al. [2] showcased how such multivalent signaling proteins form phase-separated compartments depending on the phosphorylation state of nephrin. Above the saturation concentration for condensation, the activity of regulators increases sharply and actin polymerization is nonlinearly enhanced. Clusters of Nck on membranes in living cells have been related to localized actin polymerization [48,49]. Following up on this observation, Case et al. [5] revealed that the stoichiometry of N-WASP/Nck/nephrin, which can be independently controlled inside condensates, defines the dwell times of regulators and thereby increases the actin assembly kinetics. Other examples of

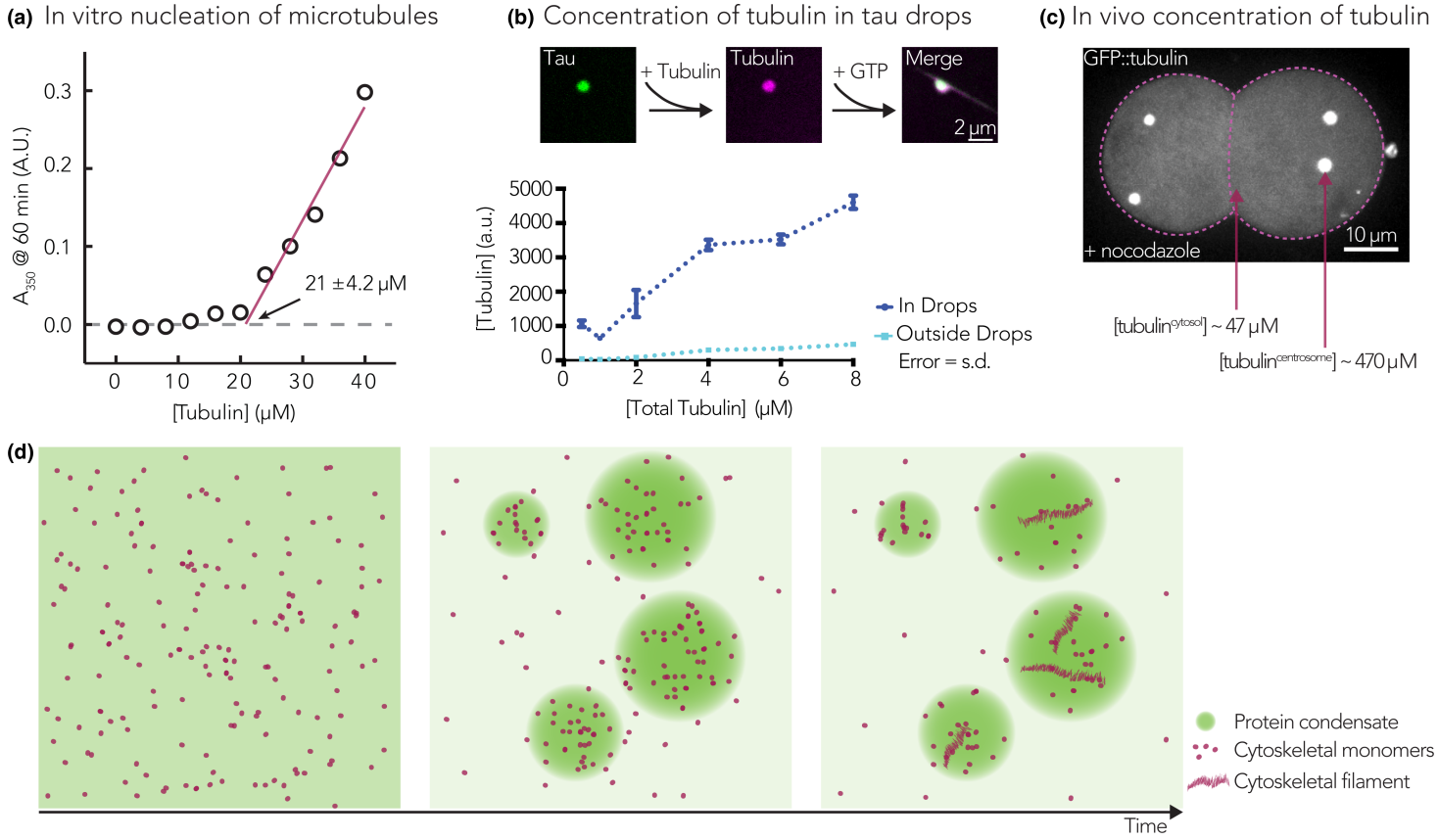

Figure 1. Nucleation of cytoskeletal filaments can be triggered by condensates.

(**a**) The critical concentration for spontaneous nucleation of microtubules from purified tubulin is ~21 μM, determined by increased absorbance at 350 nm. Modified with permission from [44]. (**b**) *In vitro* condensates of tau concentrate tubulin ~10-fold. When GTP is added, this triggers the nucleation of microtubules, which grow out and deform the tau drops. Modified with permission from [6] and images kindly provided by Amayra Hernández-Vega. (**c**) In *C. elegans* embryos the cytosolic tubulin concentration is ~47 μM, but the growth of microtubules is hindered by regulatory proteins. In centrosomes soluble tubulin is concentrated to peak values of 470 μM, which may partly explain the nucleation of spindle microtubules. Modified with permission from [1]. (**d**) In a homogeneous cytoplasm, cytoskeletal monomers or dimers (magenta dots) are randomly distributed. Upon condensation of cytoskeletal regulators (green), the monomers get concentrated in the droplets. The higher local concentration may be sufficient to exceed the critical concentration for nucleation. Note that the emerging filaments are another molecular species with distinct partitioning coefficients. Condensates could thus act as sink for monomers.

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condensates enhancing actin polymerization include activated T-cell receptors [3] and postsynaptic densities found in neurons. *In vitro* reconstituted postsynaptic densities enrich the actin regulatory protein cortactin and actin monomers, which promotes actin filament assembly and bundle formation [50]. Sequestration of regulators upon condensation can further result in the reduction in actin polymerization, which was observed for actin nucleation factors in yeast. Under stress conditions, these factors assemble in transient condensates, that inhibit their interaction with regulatory proteins at the tip-localized polarisome complex [51]. Thereby the normal assembly of actin cables is halted, which is necessary for polarized yeast growth.

Taken together, ample evidence is accumulating that condensates can regulate the biochemistry of actin polymerization by increasing the monomer concentration, defining the stoichiometry of regulators and sequestering regulators. More generally, the actin cytoskeleton provides a good example of how the weak multivalent interactions that drive condensate formation, collaborate with higher affinity interactions and defined binding constants, to spatio-temporally modulate biochemistry.

Microtubule dynamics can be modulated by condensates

Studies on the regulation of microtubule nucleation provide important evidence for nucleation driven by condensate formation. The classical viewpoint suggests that microtubule nucleation is mainly mediated by the gamma-tubulin ring complex (γ-TuRC) [52] and the polymerase XMAP215 [53]. The nucleators are further recruited to specific microtubule-organizing centers, e.g. centrosomes at distinct cellular locations. However, genetic evidence for the roles of these nucleators, especially in mitosis, are unclear. For instance, depletion of γ-tubulin in *Caenorhabditis elegans* embryos by RNAi does not prevent the formation of a robust microtubule aster [54]. Furthermore, the mechanisms by which cells that lack centrosomes can locally nucleate microtubules remain an open question. One idea would be a gradient of regulating proteins, but this requires constant energy and could be difficult to maintain in a stirred system [55–57].

Recent evidence suggests that the concentration of tubulin in condensates, could provide a mechanism for microtubule nucleation. Pioneering work from Jiang et al. [4] found the evolutionarily conserved *Xenopus* lamin-B spindle matrix protein BuGZ can form liquid condensates *in vitro*, which concentrate tubulin. Further evidence for nucleation of microtubules by concentration in condensates came from studying model condensates formed by the microtubule-associated protein tau. *In vitro*, tau phase separates under crowding conditions and concentrates tubulin, which can thereby surpass the nucleation threshold and polymerize microtubule bundles [6] (Figure 1b). Studies in *C. elegans* provide strong *in vivo* support for the role of condensates in centrosome nucleation (see review [36] for details). The *C. elegans* centrosome scaffold protein SPD-5 was shown to phase separate *in vitro* and recruits clients like microtubule polymerases and stabilizing proteins (e.g. homologs of XMAP215, TPX2 and Polo Kinase) [1]. Tubulin was concentrated 4-fold in this recombinant pericentriolar material, which is sufficient to nucleate microtubule asters that extrude out of the condensates. Supporting evidence comes from work *in vivo* showing that soluble tubulin concentrates ∼10 fold with peak values of 470 μM in *C. elegans* centrosomes over the surrounding cytosolic concentration (Figure 1c). This is about an order of magnitude higher than the concentration required for spontaneous nucleation of tubulin in a test tube, providing strong evidence for concentration-enhanced microtubule nucleation at centrosomes [58]. In acentrosomal oocytes, the regulatory kinase aurora A (AURA), its substrate TACC3 and the clathrin heavy chain CHC17 (both binding microtubules) have been identified to form a liquid-like meiotic spindle domain [59]. However, in contrast with centrosomes, tubulin is not significantly enriched within these condensates. Nonetheless the concentration of microtubule regulatory factors enhances the microtubule growth rates and ensures proper spindle assembly.

More recent work has shown that microtubule-dependent nucleation of new filaments (known as branching) can also be condensate driven. The spindle microtubule regulator TPX2 and tubulin co-condense *in vitro*, which promotes branching microtubule polymerization [60]. TPX2 condensates preferentially form on existing microtubules (as we will discuss in section 'condensates of regulators can wet cytoskeletal filaments'), which opens up the exciting idea that regulation of condensate formation might play a key role in spindle assembly.

It is important to point out that it has long been known that acentrosomal spindles have liquid-like mesoscale properties, as shown with the fusion of two *ex vivo* assembled spindles [61]. Relaxation of their shape thereby depends on the dynamic turnover of microtubules aligning along a common axis [36], which can be theoretically described in the framework of liquid crystals [62].

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Condensates can interact with other cytoskeletal proteins

As it emerges that condensation can serve as general regulator for biochemical reactions it is not surprising to find further examples of 'condensate assisted' cytoskeletal assembly. For instance, the bacterial tubulin homolog FtsZ with its multivalent interaction partners SlmASBS undergoes phase separation *in vitro* and FtsZ filaments emerge from the dynamic condensates upon GTP addition [63].

We speculate that likewise intermediate filaments could be regulated from condensates. Cytoplasmic intermediate filaments are comprised of central alpha-helices and a head region, that form the filaments, and disordered tail domains, that mediate protein–protein interactions. Toxic proline–arginine poly-dipeptides, which are expanded in amyothrophic lateral sclerosis (ALS) patients, were found to bind the tail regions of vimentin and condense along the filaments [64].

Mutations in the disordered regions of intermediate filaments have been associated with cellular pathologies [65] and neuronal filaments have been found abnormally accumulated in the cytoplasm and axons of motor neurons in neurodegenerative diseases such as Alzheimers [66,67]. Also, overexpression of the neuronal filament peripherin leads to spheroids in axons [68]. Hence, like other pathological amyloids such as tau and FUS [69,70], aberrant phase transitions of intermediate filaments might be linked to an increased risk of aggregation.

Biophysical interactions with cytoskeletal filaments can influence condensate dynamics

On a larger scale, beyond individual biochemical reactions, the physical interactions of cytoskeletal filaments and condensates can influence each other's dynamics, size and shape. Furthermore, the combination of these cellular entities can give rise to such complex higher-order structures as the mitotic spindle. In the following paragraph, we describe how condensates and the cytoskeleton could arrange themselves in the spatially limited cytosol and what effects their mutual interactions can have.

Material properties of condensates define their response to cytoskeletal forces

While liquid-like condensates are often associated with fast diffusion, allowing unhindered biochemical reactions, some condensates have slow internal dynamics, and are better described as glasses or gels. They have been suggested to emerge from liquid-like condensates through hardening [90]. It is likely that the regulation of the material properties of condensates plays an important role in the organization of the cytoskeleton. Indeed, *in vitro* condensates of pericentriolar material solidify rapidly and no longer possess internal rearrangements [1], like centrosomes in metaphase-arrested *C. elegans* embryos [91]. This might hamper microtubule growth inside the pericentriolar material at later stages, and the solidifying matrix pushes longer filaments outward. Consistently, a combination of fluorescence microscopy and electron tomography revealed only short microtubule filaments in the center of mitotic centrosomes [58], while in the periphery longer filaments originate that protrude out of the pericentriolar material [54,92]. Hardening was also considered important for the mechanical stability of the centrosomes to anchor microtubules and withstand their pulling forces [93]. Inactivation of the stabilizing proteins during mitotic exit then weakens the centrosome again, enabling its force-mediated disassembly [73]. We anticipate that many aspects of condensate/cytoskeletal interaction will depend on the material properties of condensates (Figure 2a).

When liquid condensates of proteins susceptible to aggregation harden, irreversible protein aggregates may be formed, which could be associated with cellular pathologies. This transition could be induced and accelerated by shear forces (on the order of the forces acting during axonal transport) acting on the condensates [74]. This is because the shear forces provide energy, allowing molecule rearrangements. However, it is early days for the exploration of liquid-to-solid transitions and further work is needed to distinguish what drives these changes in material properties.

Condensates are embedded in viscoelastic cytoskeletal networks

Condensates with an increased density over their surrounding can be prevented from gravitational sedimentation by cytoskeletal networks. Due to the small dimensions of cells, however, gravitational effects only come to play for the largest entities. In big oocytes, such as the >1 mm *Xenopus laevis* eggs, the nuclear actin scaffold was found to keep nucleoli from sinking due to gravity and coalescing [27]. While the actin network shows the

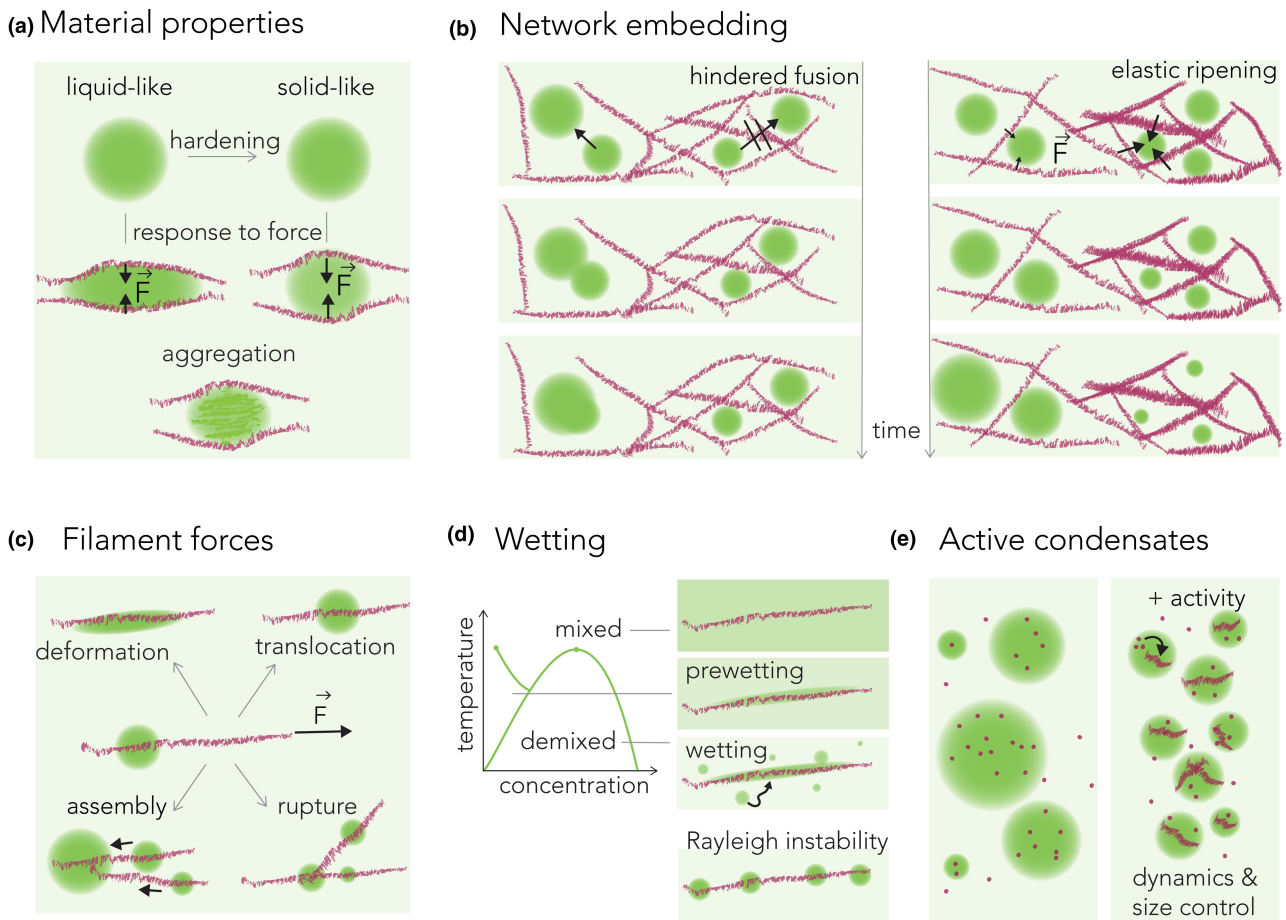

Figure 2. Biophysical interactions of condensates and cytoskeletal filaments.

(**a**) The material properties of condensates influence their physical interactions with cytoskeletal filaments. While liquid-like condensates can be easily deformed and dragged by filaments [34,71,72], more solid-like condensates withstand higher forces ($\vec{F}$) [73]. Such forces exerted by the cytoskeleton could further lead to internal rearrangements and promote the aggregation of proteins [74]. (**b**) A dense cytoskeletal network can restrict the movement of embedded condensates and thereby hindering their fusion [27,75]. Furthermore, the growth of condensates can be controlled via elastic ripening, which is the growth of condensates in a soft environment on the expense of condensates in stiff networks [76–78]. (**c**) Directed polymerization and contraction of filaments can deform [71], drag [79,80], assemble [81,82] or disrupt interacting condensates [72] and thereby control their spatiotemporal dynamics. (**d**) Wetting phenomena can arise when proteins condense at a filament, which can occur below the saturation concentration of bulk phase separation (prewetting) or above (wetting) [14,15,83–85]. Surface tension can further drive pearling of a viscous fluid bound to a filament as described by the Rayleigh–Plateau instability. The resulting droplets, e.g. bound to microtubules [86], appear like water dewdrops on a spider web. (**e**) Polymerization of actin and microtubule filaments is a chemical reaction consuming ATP/GTP. This drives the system out of equilibrium, which could impact the dynamic assembly and disassembly of condensates, e.g. limiting their size [87–89].

viscoelastic response to force, beyond timescales where polymerization/depolymerization take place it reacts predominantly viscous (Box 1). In fact, older oocytes (several weeks) show larger and fewer nucleoli as the actin network creeps and makes room for their fusion, which seems to limit the overall lifetime of oocytes [33]. Also, at shorter time and length scales cytoskeletal networks can stabilize condensates and keep them from fusing by caging or fencing individual drops apart from each other (Figure 2b). This was recently shown for keratohyalin granules embedded in a dense keratin network in keratinocytes [75]. These observations strongly suggest that the cytoskeleton suppresses the coalescence of condensates by providing a spatial separation.

However, trapped condensates that cannot fuse can still grow through Ostwald ripening. This thermodynamically driven process describes the growth of bigger condensates on the expense of smaller ones via the diffusive exchange of material [94]. It turns out, that the elastic properties of a surrounding network, such as the

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cytoskeleton, can further regulate ripening. When condensates grow, they can push the network outwards, which in turn applies a counterforce on the drops. This limits their growth and thereby narrows the size distribution of condensates [76]. Furthermore, the elastic energy can tune the nucleation and ripening of condensates. Since it is energetically favorable for condensates to grow in areas of low elastic counterforce, the stiffness of cytoskeletal networks can regulate condensate formation. So-called 'elastic ripening' has been observed in polymer networks where condensates shrink in areas of high stiffness at the expense of growth in areas of low stiffness [78]. This effect opposes Ostwald ripening and controls the dynamics of condensates by the stiffness of the local environment [77]. Similar to the observations that nuclear condensates grow preferentially in low-density euchromatic regions [95], growth of condensates in the cytosol are likely to be affected by the mechanical resistance of cytoskeletal networks, e.g. cellular pressure waves exerted by cortical actin have recently been correlated with decreased levels of nuclear condensates [96]. Together, membraneless compartments interspersed with cytoskeletal filaments can considerably crowd the cytoplasm and thus exert mechanical pressure on other organelles, the consequences of which are still to be investigated.

Cytoskeletal forces can deform and rupture condensates

As we know from *in vitro* experiments, protein condensates can be deformed or even dissolved by externally applied forces such as shear forces from harsh pipetting of a phase-separated protein solution [25,72] or optomechanical deformation of condensates by optical tweezers [34].

Such forces can also be transduced by the cytoskeleton and are not, as discussed above, limited to the elastic energy of the network, but can be actively generated from inside the condensates. The contraction and oriented polymerization of cytoskeletal filaments generates mechanical forces that can actively deform and translocate condensates (Figure 2c). In fact, it has been found that condensates of the actin regulators nephrin/NCK/N-WASP deform upon actin polymerization *in vitro* on supported lipid bilayers [71], as well as in HeLa cells, where they move rapidly across the basal membrane as the actomyosin network contracts [79]. Another example is the elongated shape of the mitotic centrosomes in *C. elegans*, which was found to be induced by microtubule-based forces, since treatment with the microtubule polymerization inhibitor nocodazole relaxed the elongated shape [72]. The polymerization and pulling forces induced by the cytoskeletal filaments could thereby serve as negative feedback mechanism to dynamically disassemble the condensates, e.g. for centrosomes during mitotic exit [72]. To further investigate how condensates are deformed by internal forces, a novel tool called 'ActuAtor' can help, which triggers localized actin polymerization in cells in response to chemical or optical stimuli [97]. Actin localized to stress granule proteins polymerizes within these stress granules and disperses the anchor proteins of the 'ActuAtor'. Until now, it has been difficult to discern the effect of actin polymerization forces on the dynamics of condensates *in vivo*, e.g. the disruption of actin filaments by small molecule inhibitors observed within nucleoli leads to their fusion, but also influences the scaffolding function of actin networks [98]. Thus, this method can be of great value to follow the effect of actin forces on preformed condensates.

The assembly (not only deformation/disruption) of condensates can also be driven by the dynamics and forces of the cytoskeleton. Tight junctions assemble through phase separation of zona occludens proteins in the cytosol and their recruitment to the membrane via a retrograde flow driven by the actomyosin cortex [81]. Actin filaments are enriched within the condensates and transduce cortical tension on the tight junctions, presumably controlling their distribution along the cell membrane. After depolymerization of actin in MDCK-II monolayers, the tight junction layer was disrupted and buckled up into individual droplets [82]. It has also been suggested that the formation of micrometric stress granules depends on cytoskeletal dynamics to overcome the time constraints of hindered diffusion. Microtubules thereby promote the coalescence of small stress granules via pushing, pulling or sliding along the filaments [80] (Figure 2c).

A further aspect of how forces can promote the phase separation of membrane proteins was proposed via the membrane-coupled cytoskeleton [99]. As Veksler and Gov argue, the force balance in a passive system between the attractive forces of the molecules versus the entropic penalty for compositional heterogeneity introduced by phase separation and membrane curvature can be broken by actin polymerization forces. Indeed, the addition of actin can shift the condensation temperature and cause phase separation of membrane components in model lipid bilayers [100]. This effect is counteracted by the observation that a tightly membrane-bound actin network can prevent the formation of macroscopic phase separation in model membranes, as predicted by the 'picket fence' model [101]. It will, therefore, be interesting to distinguish the effects of mechanical forces

on membrane protein condensates in many *in vivo* scenarios such as the immune synapse, tight junctions or lamellipodia.

Condensates of regulators can wet cytoskeletal filaments

A physical phenomenon that has been observed or proposed to arise from attractive interactions between condensates and cytoskeletal filaments resembles similarities with the wetting of dewdrops on a spider web. Several studies suggest a form of surface-assisted condensation at intracellular interfaces [71,102] and cytoskeletal filaments [14,15,86].

One of the ways that could induce microphases on intracellular surfaces is by prewetting phenomena described in soft matter physics [83]. Binding of proteins on a filament locally increases their concentrations and a phase transition on the surface can take place below the protein's saturation concentration in bulk (Figure 2d). It has been reported that microtubules induce phase separation of components of the inner centromere under conditions where they alone are in a homogenous phase [85]. Another example could be tau 'islands' on microtubules [14,15]. The microtubule-bound condensates share similarities to phase-separated tau drops in solution such as fusion and dissolution. However, the tau islands do not grow in 3D, as expected from condensates as protein concentration increases, and might be explained by other phenomena such as cooperative binding. The role of tau-decorated microtubules could be to protect against severing factors, which were found to be excluded from the tau-coated areas. Interestingly, the interaction of microtubules with molecular motors can influence the attached tau islands and ultimately lead to their displacement [15]. Further work will be required to clarify the mechanisms of tau islands formation and to closely monitor the condensation of other regulators on cytoskeletal fibers.

In the saturated regime, where proteins phase separate in bulk, interactions with cytoskeletal filaments can lead to partial or complete wetting (Figure 2d). Depending on the surface tension, a condensed film on filaments can also drive the pearling of drops, which is described by the Rayleigh–Plateau instability. The microtubule nucleation regulator TPX2 has recently been proposed to coat microtubules *in vitro* at physiological concentrations (0.1 μM) before breaking up into sub-diffractive condensates [86] (preprint). This hydrodynamic instability leads to confined regions along microtubules, which cluster regulators and γ-tubulin [60,103] and thereby facilitate nucleation of branched microtubules from existing filaments [104].

It remains unclear how e.g. posttranslational modifications of tubulin [105] can affect the condensation of regulators. Inhomogeneities along the filaments and the disordered domains in intermediate filaments could further serve as nucleation center of condensates [64]. To better understand the diverse modes of interaction of condensates and the cytoskeleton, it will be important in future studies to quantify protein concentration in the local cellular environment and the growth dynamics of the condensates.

Polymerization reactions of cytoskeletal proteins can influence condensate dynamics

The reduced complexity of *in vitro* systems tends to lack the dynamics of protein condensates observed *in vivo*. In cells, condensate assembly appears to be faster, the size and the distribution of condensates is often smaller and narrower and their material properties are notoriously difficult to assess *in vivo* [106]. In addition, condensates also disassemble under physiological conditions, which is rarely achieved *in vitro* without the usage of disruptive solvents. Multiple components such as the complexity of protein mixtures, different isoforms and phosphorylation states [107] or small hydrotope-like molecules such as ATP [108] certainly play a role. But, most importantly, *in vivo* biochemical reactions drive the condensates away from thermodynamic equilibrium. Reactions that change the solubility of a protein can actively regulate the growth and size of the condensates [89,109] (Figure 2e). For instance, the growth of bigger condensates through Ostwald ripening can be suppressed as predicted for first-order reactions [87].

Polymerization and cross-linking of cytoskeletal proteins consuming ATP/GTP can be such a reaction, which renders the condensates 'active' [88]. The conversion of monomers to filaments within condensates can further stimulate an influx of monomers through concentration-dependent fluxes [40]. This gradually changes the composition, which in turn can affect condensate growth and disassembly rates. Viscoelastic networks additionally prevent unhindered diffusion and fusion of condensates within cells (as discussed in 'condensates are embedded in viscoelastic cytoskeletal networks' section). Thus, the cytoskeleton could effectively prevent condensates from coarsening and counteract the thermodynamic equilibrium of one big drop as observed *in vitro*.

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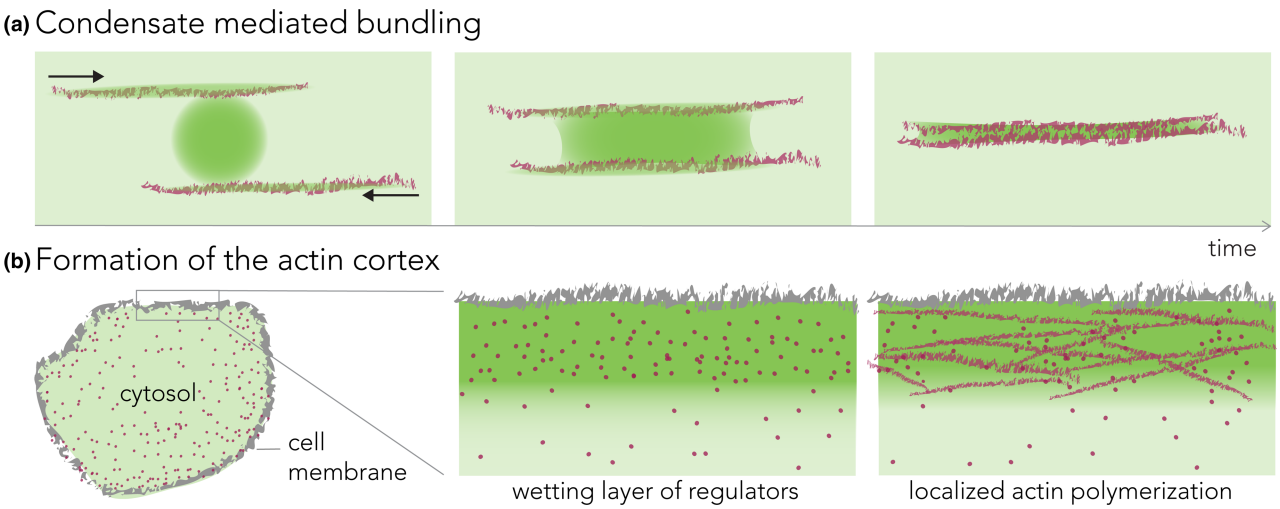

Figure 3. Possible effects of condensates on the organization of the cytoskeleton.

(**a**) Wetting of cytoskeletal fibers could promote sliding and bundle formation through the effect of surface tension. (**b**) Formation of the actin cortex could be partially driven by condensation of regulators on the plasma membrane. This would promote actin polymerization near the membrane and thereby control the spatial expansion of the cortex.

For example, the observation that actin polymerization reorganizes nephrin clusters [71] or lipid organization [110] in model membranes could be partly explained by chemical activity, in addition to the forces arising from the cytoskeletal assembly as discussed above (section 'cytoskeletal forces can deform and rupture condensates'). However, clear experimental evidence for the role of cytoskeletal reactions on the thermodynamics and thus on the regulation of growth and size of membraneless compartments is missing to date. Furthermore, biochemically active condensates are predicted to divide [111], which can also control their size. It will be interesting to see instances of cytoskeletal proteins controlling the assembly and disassembly of condensates in future studies.

Condensates can promote the formation of cytoskeletal structures

The question how condensation can contribute to the bundling of cytoskeletal filaments remains relatively unexplored. Bundling of individual filaments substantially increases the mechanical strength and controls various physiological processes, e.g. adhesion [112] and morphogenesis [113]. Similar to the pulling of DNA strands by surface-tension driven coalescence of light-induced CasDrops in the nucleus [114,115], cytoskeletal filaments could be mechanically brought together by the capillary forces of condensates [116] (Figure 3a). However, the persistence length of cytoskeletal filaments is orders of magnitude higher than that of nucleic acids (Box 1). Condensation-driven bundling would, therefore, only be effective if the sum of the low-affinity forces condensing the proteins exceeds the force required to deform the network. It might be thus limited to individual intermediate and actin filaments or short microtubules as these are more flexible than long and highly cross-linked structures [117]. Pulling of the filaments could be achieved through multivalent interactions of adaptor proteins with long disordered domains such as tau [20], CLIP1 or epsin [7]. Since the multivalent interactions confine the diffusion of the proteins, this can give rise to an entropic force that could further drive sliding of the filaments (as discussed in a recent opinion [118]). *In vitro*, it has been observed that BugZ and tau condensates promote the formation of microtubule bundles [4,6], *in vivo* to date such evidence is lacking.

Another process that could be explained by the physical chemistry of condensation is the assembly of entire cytoskeletal structures during development. While different isoforms and actin regulators are commonly discussed for the generation of different actin structures, little attention has been given to what limits their spatial expansion. We propose that the actin cortex, which is only a few nanometers in thickness [119], could be formed by condensation of actin regulators on the membrane (Figure 3b). Note, that the formation of the cortex has been suggested to be driven by the wetting of an active gel before, but neglecting the role of

cytoskeletal regulators [35,120]. Condensation of regulators can facilitate the formation of the cortex by localizing actin nucleation close to the membrane.

Conclusions and perspectives

The interactions between the cytoskeleton and condensates are numerous. While we currently have little evidence that cytoskeletal proteins themselves form biomolecular condensates, they can partition as clients into condensates that assemble from regulators, with striking consequences for cytoskeletal biochemistry. The concentration of cytoskeletal monomers and regulators through a jump-like onset of condensation provides an additional mechanism to regulate the assembly of cytoskeletal filaments besides biochemical pathways. As the field progresses, we will likely see more examples where cytoskeletal structures assemble or disassemble in response to stress or environmental changes that trigger the formation of condensates. Besides promoting the formation of cytoskeletal structures, filaments and networks formed by cytoskeletal proteins can directly impact the assembly, deformation and degradation of condensates. Although in its infancy, the idea that elastic properties of the surrounding networks can regulate and limit the dynamics of condensates presents a wide cell biological scope. Future work will reveal the generality of these interactions and their importance to life.

Summary

- Biomolecular condensates and cytoskeletal filaments regulate each other.
- Cytoskeletal monomers do not typically phase separate but many condensates concentrate cytoskeletal monomers and regulators.
- Thereby the dynamic assembly of cytoskeletal structures can be spatio-temporally regulated by means of condensation.
- Large scale interactions with the cytoskeleton can control the formation, shape and site of condensates.
- Non-trivial forms of condensation at intracellular surfaces such as cytoskeletal filaments could be a major route for the formation of condensates *in vivo*.

Competing Interests

A.A.H. is cofounder and member of the scientific advisory board of Dewpoint Therapeutics.

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Author contributions

Both authors conceived the ideas, T.W. wrote the original draft of the paper and incorporated insight and editing from A.A.H.

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Abbreviations

ALS, amyothrophic lateral sclerosis; N-WASP, Wiskott–Aldrich syndrome protein; γ-TuRC, gamma-tubulin ring complex.

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