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Biomolecular condensation involving the cytoskeleton

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ABSTRACT

Biomolecular condensation of proteins contributes to the organization of the cytoplasm and nucleoplasm. A number of condensation processes appear to be directly involved in regulating the structure, function and dynamics of the cytoskeleton. Liquid-liquid phase separation of cytoskeleton proteins, together with polymerization modulators, promotes cytoskeletal fiber nucleation and branching. Furthermore, the attachment of protein condensates to the cytoskeleton can contribute to cytoskeleton stability and organization, regulate transport, create patterns of functional reaction containers, and connect the cytoskeleton with membranes. Surface-bound condensates can exert and buffer mechanical forces that give stability and flexibility to the cytoskeleton, thus, may play a large role in cell biology. In this review, we introduce the concept and role of cellular biomolecular condensation, explain its special function on cytoskeletal fiber surfaces, and point out potential definition and experimental caveats. We review the current literature on protein condensation processes related to the actin, tubulin, and intermediate filament cytoskeleton, and discuss some of them in the context of neurobiology. In summary, we provide an overview about biomolecular condensation in relation to cytoskeleton structure and function, which offers a base for the exploration and interpretation of cytoskeletal condensates in neurobiology.

1. Introduction

The cellular interior, separated from the extracellular space by the lipid bilayer of the plasma membrane, is a crowded environment, in which a plethora of interactions and reactions have to happen in parallel, precisely controlled in time and space. Cytosolic compartmentalization through persistent lipid membrane-bound cellular organelles and conditional transient biomolecular condensates enables cells to organize molecular processes and adapt to conditions. In addition, cells (or parts of them) have to adopt certain shapes and change their location, which implies the necessity to exert and resist mechanical forces. These features are realized by the cytoskeleton, a meshwork of dynamically polymerizing, mechanically stable cytoskeletal fibers, and, to some extent, the plasma membrane.

In recent years, the contribution of liquid-like biomolecular protein condensates to a number of cellular processes has been discovered (Alberti et al., 2019), including an emerging role of condensate interactions with the cytoskeleton (Wiegand and Hyman, 2020) and plasma and organelle membranes (Zhao and Zhang, 2020). Through their unique biochemical and liquid-like material properties, protein condensates can nucleate (Woodruff et al., 2017; Hernández-Vega et al., 2017) and organize (Woodruff et al., 2017) cytoskeletal fibers, initiate fiber branching (Li et al., 2012; King and Petry, 2020), regulate cargo transport (Siahaan et al., 2019; Tan et al., 2019), and form functional protein degradation and synthesis "containers" on fibers and membranes (Ma and Mayr, 2018; Carrettiero et al., 2022). Disturbance of condensate biology that impairs these essential interactions, for example in disease, could lead to aberrant cytoskeleton dynamics and stability that result in the dysregulation of various molecular processes.

In this review, we introduce the concept of liquid-liquid phase separation (LLPS) that underlies the formation of liquid-like protein condensates, summarize the literature on interactions of protein condensates with the cytoskeleton, and discuss some implications for neurobiology and potential roles in neurodegeneration.

Abbreviations: LLPS, Liquid-like phase separation; 1, 6-HD, 1,6-hexanediol; GFAP, Glial fibrillary acidic protein; PSD, Postsynaptic density; NMDAR, N-Methyl-D-aspartate receptor; AMPAR, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor; nNOS, neuronal nitric oxide synthase; CAMKII, Ca2 + /calmodulin-dependent protein kinase II; SV, Synaptic vesicles; MT, microtubule.

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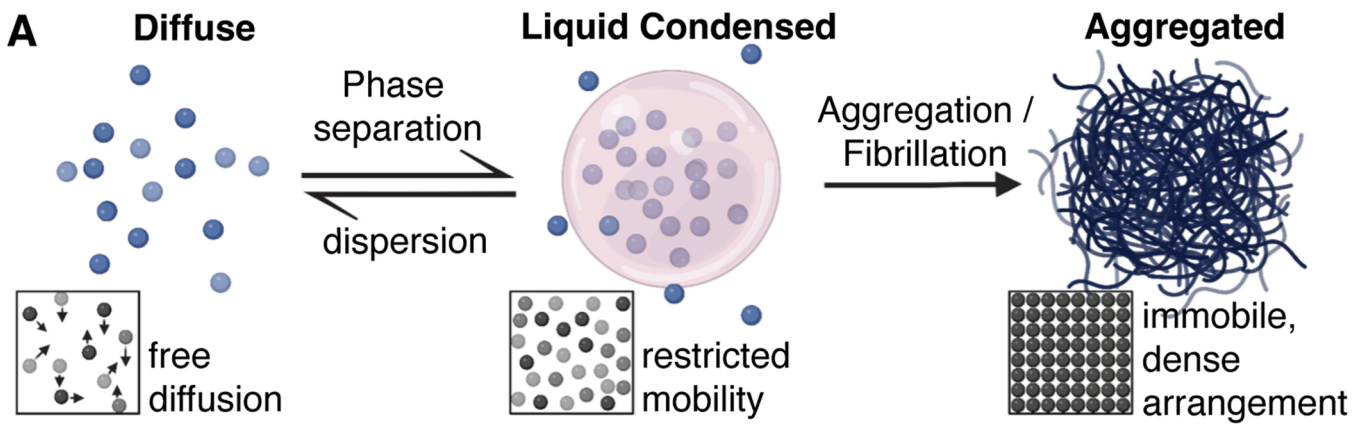

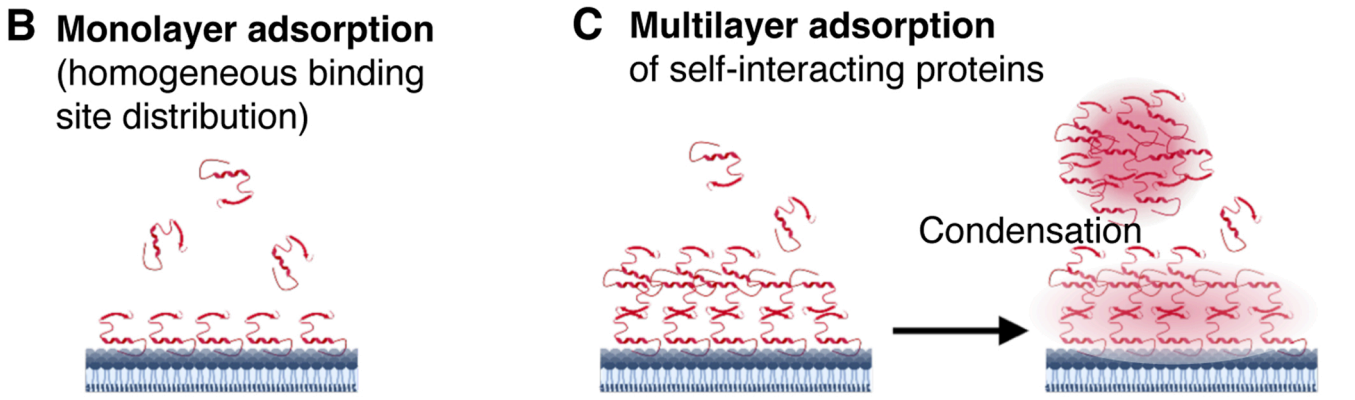

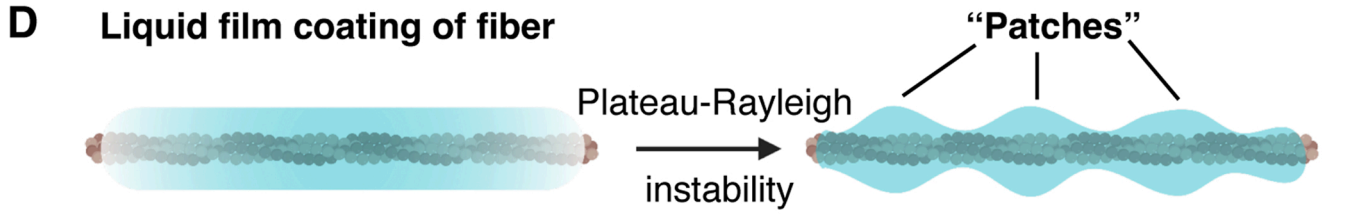

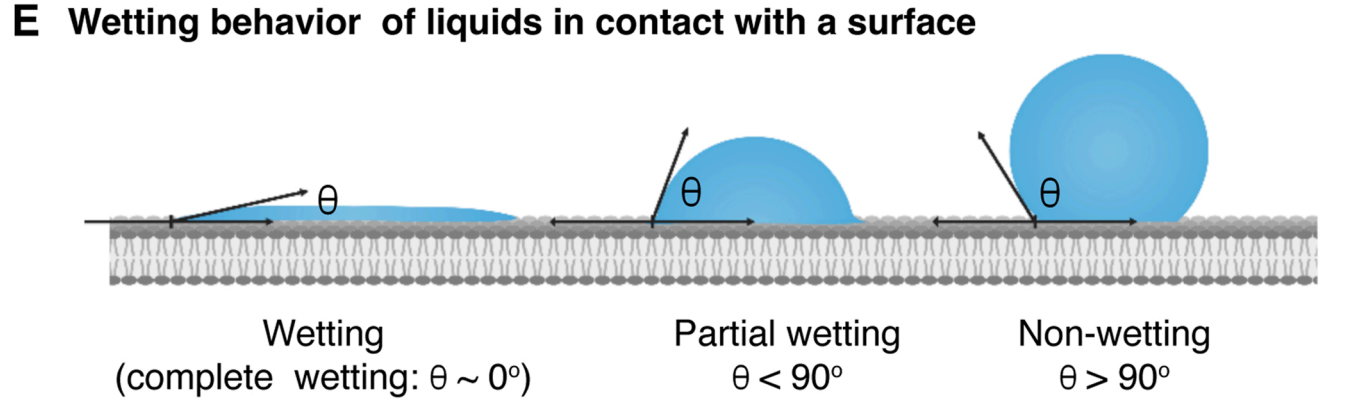

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Fig. 1. Basic principles of biomolecular condensate formation and surface interactions. A) Formation and Maturation of biomolecular condensates. LLPS drives the de-mixing of diffuse proteins in a solution into liquid condensates that have an increased protein density and restricted protein diffusion. Under certain conditions, such as disease mutation or certain PTMs, liquid-like condensates of aggregating proteins can trigger an irreversible transition into protein aggregation, i.e., the formation of β-sheet rich fibrillary structure characterized by high protein density and order. B) Monolayer adsorption of proteins on surfaces with homogenous binding site distribution described by Langmuir isotherm. C) Langmuir isotherm adsorption of self-associating proteins results in multilayer adsorption, which can give rise to phase separated condensates or aggregates. D) Principle of Plateau-Rayleigh instability: condensates wetting a cytoskeleton fiber form a cylindrical liquid coat surrounding the fiber surface. Fluctuations along the cylinder cause instability of the thin liquid coat and breaks the liquid-like cylinder into condensate patches. The Plateau-Rayleigh instability ensures that all fluid cylinders ultimately disintegrate (Xuan & Biggins, 2017). E) Wetting describes how a liquid interacts with a surface and is a measurement for surface tension. The contact angle, θ, between the contact point tangential of the liquid phase boundary and the surface is a measurement of wettability. Smaller contact angles are a result of strong adhesive forces between the liquid and the surface, whereas strong cohesive forces between molecules inside the liquid result in large contact angles and lead to non-wetting behavior.

2. Concept of biomolecular condensation

2.1. Relevance of cellular biomolecular condensates

Biomolecular condensates enriched in specific proteins and nucleic acids give cells the possibility for fast and reversible formation of reaction "containers" and structural entities. First discovered as liquid-like protein-RNA granules, P-granules, established in germ cells of *C. elegans* (Brangwynne et al., 2009), nowadays several cellular processes are known to involve biomolecular condensates. Some of the best studied examples of cytosolic condensation include the reversible, stress-induced formation of stress granules (SGs) containing distinct RNA-binding proteins (RBPs) and RNA (Wheeler et al., 2016), the condensate-induced aggregation of proteins associated with neurodegenerative diseases, such as FUS, TDP-43, and hnRNPA in amyotrophic lateral sclerosis (Patel et al., 2015; Molliex et al., 2015) and Tau in Alzheimer's disease and frontotemporal dementia (Wegmann et al., 2018), and the condensation of cytoskeleton binding proteins on F-actin (Balaban et al., 2021; Banjade and Rosen, 2014; Ditlev et al., 2019; Graham et al., 2022; Feng et al., 2022b; Su et al., 2016; Yan et al., 2022) and microtubules (MTs) (Hernández-Vega et al., 2017; Siahaan et al., 2019; Tan et al., 2019; Zhang et al., 2020). In the nucleus, condensation of histones (Gibson et al., 2019) and histone-associated proteins (Larson et al., 2017) together with DNA coordinate chromatin condensation (Antonin and Neumann, 2016; Hansen et al., 2021), and the condensation of nucleolar proteins and rRNA are essential for the formation and function of the nucleolus (Brangwynne et al., 2011; Feric et al., 2016; Lafontaine et al., 2020). At the interphase between cyto- and nucleoplasm, in the nuclear envelope, the central pore of nuclear pore complexes is filled with unstructured, phase separating peptide chains of nucleoporins (Schmidt and Görlich, 2015, 2016). All these cellular condensation processes also play a role in the central nervous system, and many of them have been shown to encode neurotoxicity when dysregulated.

2.2. Biophysical phenomenon of liquid-liquid phase separation

Although the principle of condensation applies to some extent to the formation of any macromolecular complex (also see *2.5. Notes on terminology of biomolecular condensates*), the definition of *biomolecular condensation* is limited to condensates that are formed through liquid-liquid phase separation (LLPS), which describes the de-mixing of a solution into two or more liquid-like phases of distinct molecular composition and fluid properties. *Biomolecular condensation* is thus reminiscent to the transition of proteins from a diffuse dilute to a liquid condensed state of matter. Once in the liquid state, proteins can either return into the diffuse state (=dispersion of condensates), or transition into a solid-like state of aggregation (Fig. 1A).

What defines a protein phase as liquid? – Similar to water or other macroscopic liquids, biomolecular condensates exhibit physical properties characteristic of liquids, i.e., they form spherical droplets in solution to minimizes interface energies, they can fuse to form larger droplets, and they show wetting behavior on surfaces. Molecularly, condensates are dense in proteins, and sometimes nucleic acids, that interact with each other based on many, rather weak interactions. This multitude (=multivalency) of interactions allows the contained molecules to remain mobile, yet at a reduced degree of conformational and diffusional freedom, which provides the base for the liquid behavior of condensates. In contrast, the presence of a few specific and strong interactions is usually needed to encode protein structure and specific protein-protein interactions, e.g. in macromolecular protein complexes.

A general problem with studying condensation processes in small confined cellular spaces – e.g. in spines, axons, and dendrites – is the resolution limit for optical detection of the liquid state, which relies on monitoring of dynamic processes such as molecular diffusion (e.g. by FRAP or single particle imaging) and liquid behavior of condensates (e.g. droplet fusion and wetting). Furthermore, some defined fluid dynamic and fluid mechanic laws may not apply at the small scale of cellular processes. Consequently, many condensation processes envisioned in small cellular sub-compartments can only be approximated from reconstituted systems (e.g., using purified recombinant proteins or overexpression in cells), where observations are made at a magnitude of size larger than they exist in the physiological cellular scenario. Such systems can be useful to assess the potential role of condensation in a cellular process; however, they still model only aspects of it, often with a bias towards the envisioned role of condensation, and need thorough validation in the respective cellular system. In fact, up to now, most studies on cellular condensation rely on overexpression of proteins (parts) fused to fluorescent proteins. Whereas such systems were useful to establish the role of biomolecular condensation in cell biology, following studies should aim at reducing overexpression and fusion protein artifacts by using endogenous protein tagging (i.e., using CRISPR, (Willems et al., 2020) or knock-in strategies, preferentially utilizing small, inactive tags that enable fluorescent labeling in living cells (e.g., SNAP or CLIP (Provost and Sun, 2010), or unnatural amino acids (Reinkemeier et al., 2019; Arsić et al., 2022).

What drives LLPS of proteins? – The ability of a protein to establish multivalent interactions that enable LLPS is encoded in its amino acid sequence. Most interactions driving or contributing to LLPS involve charged protein domains (Boyko et al., 2020), structural units (e.g., PSD-95, (Zeng et al., 2018)) or individual residues (e.g., KXGS motifs of Tau, (Ambadipudi et al., 2019)) and are, thus, sensitive to shielding by counter ions. As a result, many condensation processes are highly sensitive to the ion composition of the buffer system used. This seems especially true for condensates driven by the co-condensation of cationic proteins (or protein domains) with RNA (or other polyanions); for example, Tau-RNA coacervation is extremely sensitive to salt ion concentrations (Zhang et al., 2017b; Lin et al., 2019; Hochmair et al., 2022). To which extent the conditional intracellular ion composition influences biomolecular condensation in living cells is hard to determine.

On the amino acid residue level, electrostatic interactions between charged residues (Glu/Asp-Arg/Lys) or Cation-pi interactions (Arg-Tyr) appear to be strong drivers of LLPS (Wang et al., 2018), whereas stretches of non-polar, non-charged residues (Ala, Gly, Ser) maintain the molecular flexibility in the condensed phase (Martin et al., 2020). Protein domains that are enriched in these residues and show an otherwise low amino acid complexity (= low complexity domains (LCDs); (Kato et al., 2012)), are in many LLPS proteins essential for their condensation.

Bioinformatics predictors of LLPS propensity (e.g. CatGranule, http://service.tartaglialab.com/update_submission/488527/7ceee71361) and FuzDrop, https://fuzdrop.bio.unipd.it/predictor) often use the abundance of these residues and the protein disorder prediction in their algorithms. Recently, it was suggested that also H-bonds between polypeptide backbone residues contribute to the protein phase separation (Zhou et al., 2022). The sensitivity of many in vitro and *in cellulo* condensates to the amphipathic alcohol 1,6-hexane diol (1,6-HD), which acts on hydrogen bonds, supports this finding. Notably, the use of 1,6-hexane diol (1,6-HD) to dissolve cellular LLPS is debated and should be interpreted with care because of averse cellular responses induced by the chemical, which may lead to misinterpretation of experimental results (Düster et al., 2021; Itoh et al., 2021).

In vitro, protein condensation can also be triggered by the presence of molecular crowding agents such as polyethylene glycol (PEG), Dextran (DEX), or Ficoll. These molecules exert excluded volume effects, which essentially reduces the volume available for other molecules and thereby increases their apparent concentration in a solution. This promotes self-interaction and condensation of proteins that can undergo LLPS. Notably, low amounts of these molecular crowders may mimic the crowded environment inside cells, however, they do not account for the plethora of specific and unspecific interactions encountered by cellular proteins that may "distract" or "encourage" their condensation under cellular conditions.

What modulates LLPS of proteins? - A way to modulate the electrostatic landscape, and thus the LLPS behavior, of proteins inside the cytoplasm are posttranslational modifications. For example, phosphorylation adds negative charges to Ser and Tyr residues, and acetylation and methylation remove positive charges on Lys and Arg residues, respectively. PTMs can thus modulate the charge of residues important for LLPS and thereby modulate the phase separation behavior of a protein, for example, methylation of Arg residues in FUS (RGG3 domain) (Qamar et al., 2018; Hofweber et al., 2018), phosphorylation of TDP-43 (Gruijs da Silva et al., 2022), and acetylation of Lys residues in Tau (Ukmar-Godec et al., 2019; Ferreon et al., 2018) inhibits LLPS, whereas phosphorylation of Tau differentially promotes LLPS (Wegmann et al., 2018; Kanaan et al., 2020; Hochmair et al., 2022). O-GlcNAcylation of SynGAP reduces its co-LLPS with PSD-95 (Lv et al., 2022). PTMs seem to be common regulatory checkpoint for condensation and specific to individual proteins, and dysregulated protein modification could thus promote aberrant phase transitions in disease.

2.3. Surface-condensate interactions

In the macroscopic world, condensation of water molecules in the air on solid surfaces can be observed in daily life. In analogy to this gas-to-liquid transition, proteins transition from a diffuse into a liquid state during biomolecular condensation. In cells, the surfaces of cytoskeletal fibers and lipid membranes (plasma membrane and membrane around membrane-bound organelles) - can act as a platform for condensation processes. More and more examples for the link between condensates and the cytoskeleton and membranes are emerging, indicating the importance of the interplay between these cytosolic structures (Snead and Gladfelter, 2019; Zhao and Zhang, 2020; Wiegand and Hyman, 2020).

Physicochemical models are used to describe how proteins adsorb to the surface to form multi-molecular condensates, and how condensates interact with surfaces based on their liquid-like physical properties. The attachment of individual molecules to a multivalent surface reduces their degree of conformational freedom and concentrates them in a near 2-dimensional space. Since protein LLPS can be nucleated by small oligomeric assemblies of the respective condensing protein (Shin et al., 2017), focal surface binding of a few proteins, for example due to interaction with clustered receptors in the plasma membrane (Li et al., 2012; Case et al., 2019), can initiate phase separation on the at low solution concentrations. Accordingly, the protein concentration sufficient for surface condensation is usually lower than the saturation concentration, c_{sat}, of a protein needed to initiate phase transition in solution. Surfaces therefore can be catalyzers of protein condensation (Snead and Gladfelter, 2019), which has, for example, been shown for the tight junction protein ZO-1 (Beutel et al., 2019).

On surfaces with homogenous binding site distribution, after saturation of all binding sites on the surface described by Langmuir (Langmuir, 1918), proteins that tend to self-associate can form multi-layered condensates (Mitchison, 2020) giving rise to unsaturable binding (Brunauer-Emmett-Teller theory; (Brunauer et al., 1938)). An inhomogeneous distribution of binding sites on the surface can give rise to condensate "patches" on the surface (Fig. 1B,C). This model has been suggested for Tau's binding to MTs (Mitchison, 2020), which appears non-saturable by biochemistry (Ackmann et al., 2000), and shows the formation of condensed Tau patches on MTs (Siahaan et al., 2019; Tan et al., 2019). A similarly patchy distribution of proteins on cytoskeletal fibers could be produced by Plateau-Rayleigh instability of a thin liquid protein film on the surface of filaments (Haefner et al., 2015; Setru et al., 2021)(Fig. 1D).

The physical interaction of liquid-like condensates with surfaces can be described, based on their properties as a liquid, as wetting behavior (Alberti et al., 2018, 2019; De Gennes PG, 1985): *wetting* describes the coating of a surface with a thin liquid layer, *partial wetting* describes the attachment of a liquid condensates to a surface whereby the wetting angle determines the degree of wetting, and *non-wetting* is given in absence of condensate-surface interactions (Fig. 1E). The wetting behavior of a surface-condensate system is given by the physicochemical and material properties of both the surface and the condensate, which gives surface-condensate systems a unique balance between surface energies at the surface-condensate, surface-buffer, and condensate-buffer interphase. Attractive forces between the liquid condensate and the surface (adsorption) together with cohesive forces inside the condensate (surface tension and viscoelasticity) give rise to the wetting behavior, which can be described by the wetting angle, the contact angle between condensate and surface (Botterbusch and Baumgart, 2021). Any change in condensate and/or surface properties that give rise to changes in the aforementioned force balance will change the wetting scenario. Interestingly, flexible surfaces like lipid membranes can be bent upon binding of condensates (Kusumaatmaja et al., 2021) – convexly or concavely, depending on the surface tension of the system-, which is important for autophagosome formation and autophagic digestion of condensates (Agudo-Canalejo et al., 2021). Similarly, condensate mediated membrane bending could also be involved in the initial steps during focal membrane budding or membrane tube or filopodia formation. In the case of rather rigid, non-bendable (long persistence length) MTs, Tau condensates that attach to multiple MT bundles at a time influence the orientation of individual MT bundles (Hochmair et al., 2022), which may rely on the force balance between Tau condensate surface tension and cohesiveness. To which extent this observation contributes to MT bundling and orientation in cells and is influenced by physiological conditions, i.e., cytosol composition and Tau PTMs, remains to be investigated.

Simultaneous wetting of liquid condensates on cytoskeletal fibers and membranes could provide a robust, yet flexible, connection between both these structures, and thereby enable a firm attachment or the gliding of the involved surfaces, depending on condensate properties. For example, it was suggested that a condensed synaptopodin phase may connect the synaptic smooth ER with actin filament in the spines to form and stabilize the spine apparatus (Falahati et al., 2021). Similarly, simultaneous interactions of the condensed postsynaptic density with F-actin and the dendritic spine membrane could contribute to spine stabilization.

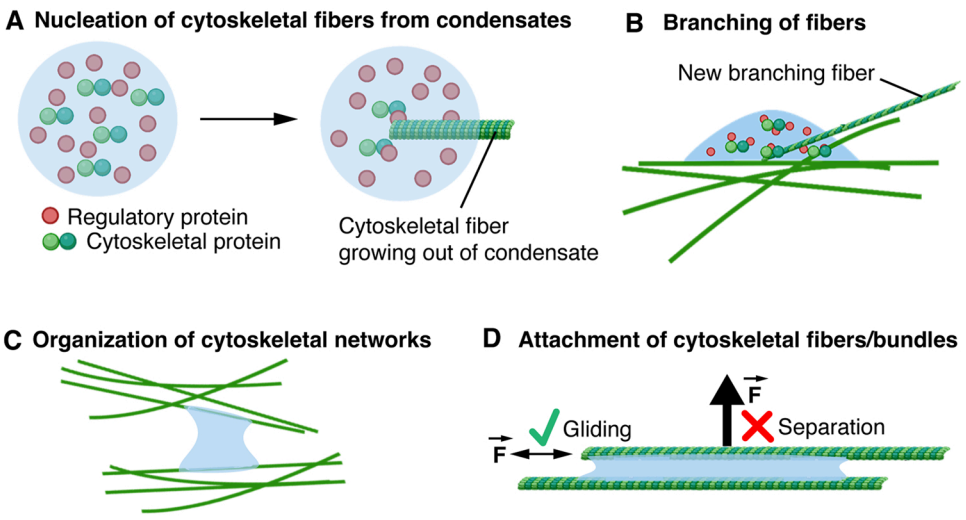

Fig. 2. Functional relevance of phase separation of and on cytoskeletal structures. A) Nucleation of cytoskeletal fibers from condensates of cytoskeletal proteins (green spheres) and regulators (red spheres). B) Condensation of cytoskeletal proteins (green spheres) with regulators (red spheres) on existing cytoskeletal fibers (green sticks) can nucleate new fibers (green striped stick) and thereby induce cytoskeletal branching. C) Condensates of cytoskeletal binding proteins that interact with multiple fibers or fiber bundles (of the same or different kind) at a time can help in the organization of cytoskeletal networks. D) A thin condensate film bridging individual cytoskeletal fibers/bundles provides strong forces against displacement (perpendicular to fiber surface) while allowing the gliding (parallel to fiber surface) of cytoskeletal fibers.

2.4. Notes on the terminology of biomolecular condensates

The term *biomolecular condensation* is used to describe the formation of protein-dense condensates with liquid-like properties via the process of LLPS. Biomolecular condensates (herein, *condensates*) contain multiple proteins of the same kind and are held together through weak multivalent interactions between molecules participating in the liquid phase. The formation of protein oligomers - e.g. of Tau, a-synuclein, or other aggregating proteins – and the assembly of macromolecular complexes such as ribosomes or nuclear pore complexes could also be interpreted as *condensation,* however, since these complexes are not liquid-like but structurally defined, one should rather use the term *assembling* to describe their formation. LLPS may well play a role to produce liquid intermediate states or parts of molecularly and structurally defined macromolecular complexes, i.e., the formation of pathological Tau oligomers from liquid condensates (Wegmann et al., 2018; Kanaan et al., 2020; Hochmair et al., 2022) or the gel-like phase separated FG-nucleoporins filling the pore of the structured nuclear pore complex ring (Frey et al., 2006). Because of the fine differences in terminology between assembling processes, and the experimentally hard to access physical state of liquid condensed matter at the subcellular scale (is it really liquid like?), the definition of molecular processes as *biomolecular condensation* should be considered with care. For a recent detailed discussion on the definition and characterization of biomolecular condensates see (Mittag and Pappu, 2022).

3. Phase separation processes involving cytoskeletal proteins

3.1. Condensation processes involving Tubulin

Microtubules are polymeric hollow rods formed by hetero-dimers of α-tubulin and β-tubulin that dynamically self-assemble in the presence of GTP. MTs are intrinsically polar (have a minus and a plus end) and continuously undergo cycles of assembly and disassembly at their plus end. MTs determine cell shape, are involved in cell movements, and facilitate the intracellular transport of organelles by motor proteins and the separation of chromosomes during mitosis. *De novo* assembly and growth of MTs follows the classic polymer model of nucleation and elongation characterized by two successive phases: slow αβ-tubulin oligomer formation (lag phase) is followed by rapid MT growth (log phase) that is initiated when these oligomers are large enough to overcome the kinetic barrier of self-assembly (Voter and Erickson, 1984). In cells, MT organizing centers (MTOCs) catalyze MT nucleation at tubulin concentrations too low to promote spontaneous nucleation. γ-tubulin, a minor species of the tubulin superfamily, at the MTOC seems critical for initiating MT assembly as immunodepletion of γ-tubulin blocks MT nucleation from the centrosome in higher eukaryote (Zheng et al., 1995). γ-tubulin is part of the γ-tubulin ring complex (γ-TuRC), a large ring-shaped cytoplasmic complex that contains at least six other proteins, including GCPs and MOZARTs. In eukaryotes, γ-TuRC localizes to both MTOC and non-MTOC sites where it helps MT nucleation by accelerating the MT lag phase through structural templating of MTs (Kollman et al., 2011; Mishra et al., 2010). Independent of γ-TuRcs, the MT polymerase XMAP215 is indispensable in several MT nucleation pathways, including branching/non-branching decisions (Alfaro-Aco et al., 2017; Thawani et al., 2018). In cells, MTs are covered by a number of MT associates proteins (MAPs) that regulate motor protein transport and MT stability. In differentiated neurons, some MAPs are localized specifically in axons (MAPT=Tau, Map4, Map7) or dendrites (MAP2), others are ubiquitous distributed (MAP1a/b, MAP6, CRMPs (Cabrales Fontela et al., 2017; Cuveillier et al., 2021). Notably, all MAPs are also binding the actin cytoskeleton (Sattilaro et al., 1981).

3.1.1. Nucleation of microtubules from spindle protein condensates

Several MAPs that undergo LLPS can recruit and concentrate tubulin in the condensed phase and thereby catalyze MT nucleation (Fig. 2 A) and related downstream functions. For example, the enrichment of tubulin in MAP condensates has been suggested as an initial step in MT assembly during centrosomal and acentrosomal spindle formation (Woodruff et al., 2017; Hernández-Vega et al., 2017; Jiang et al., 2015).

During mitotic spindle assembly, the spindle matrix protein BuGZ undergoes phase separation at the centrosome (=spindle pole) mediated by multivalent interactions of its C-terminal domain, and recruits tubulin into these phases through its N-terminal domain (Jiang et al., 2015). BuGZ phase separation is required for spindle matrix assembly and also enhances the activation of the regulatory kinase AuroraA (Jiang et al., 2015; Huang et al., 2018; Tiwary and Zheng, 2019). Depletion of BuGZ diminishes spindle size in Xenopus egg extracts and spindle MT density in mammalian cells (Jiang et al., 2015; Tiwary and Zheng, 2019). The pericentriolar scaffold protein SPD-5 has been shown to phase separate in presence of crowding agent in vitro and selectively recruit pericentriolar client proteins such as tubulin and MT regulator proteins, e.g. homologs of XMAP215, TPX2 and Polo Kinase (Woodruff et al., 2017). Such in vitro condensates were able to concentrate tubulin ~4-fold and were sufficient to organize radial MT arrays. Albeit the evidence that key centrosomal proteins form liquid-like condensates during spindle formation, the contribution of other assembly forms of these proteins, e.g. scaffolds, are discussed as well (Raff, 2019; Woodruff, 2018). For instance, SPD-2 assembles into a fibrous, outward growing scaffold at the surface of centrioles (Conduit et al., 2014b). In

fly embryos, the SPD-2 scaffold can recruit Polo Kinase and centrosomin, which results in the formation of a centrosomin scaffold that is independent of the SPD-2/Polo scaffold (Conduit et al., 2014b, 2014a).

Unlike mitotic spindles, meiotic spindles are usually acentrosomal and their spindle pole consist of a unique liquid-like spindle domain (LISD) (Helmke et al., 2013). LISDs contain an ensemble of phase separating centrosomal, centriolar, and MT-associated proteins and form dynamic spherical protrusions both into and beyond the acentrosomal spindle (So et al., 2019). LISD condensation is controlled by AuroraA kinase, its substrate TACC3, and the clathrin heavy chain CHC17 that binds to MTs along with TACC3. Inhibition or depletion of major LISD components results in smaller and less stable spindles and a decreased MT growth rate. Interestingly, in contrast to centromeric spindles, spindle pole LISD condensates do not enrich tubulin and lack BuGZ (So et al., 2019), yet AuroraA is needed for LISD formation. Considering the activation of AuroraA by BuGZ (Huang et al., 2018), one may conclude that acentromeric LISD formation may occur downstream of BuGZ dependent centromeric spindle matrix assembly.

Recent work established the importance of phase separation also for MT branching during spindle formation (King and Petry, 2020). The spindle MT regulator TPX2, which stabilizes and enhances the interactions between αβ-tubulin dimers within and in between MT protofilaments (Zhang et al., 2017a; Alfaro-Aco et al., 2017), binds MTs via its C-terminus while activating AuroraA kinase through its N-terminus; TPX2 binding to MTs is essential for nucleating new branching MTs (Zhang et al., 2017a; Alfaro-Aco et al., 2017; Anderson et al., 2007). TPX2 was shown to condensate in vitro and in *Xenopus* egg extracts, mediated by its positively charged N-terminus. Co-condensation of TPX2 with tubulin on existing MTs increases local αβ-tubulin concentration and thereby facilitates the γ-TuRC-dependent nucleation of branching MTs (Fig. 2B). Interestingly, the presence of importin-α/β abolishes TPX2 condensation and inhibits MT branching (Safari et al., 2021). It seems that weak dispersed interaction between the importin-α/β complex and TPX2 suppress the molecular interactions needed for TPX2 condensation and thereby also inhibit TPX2-mediated branch nucleation.

Another MT based organelle, the primary cilia, is regulated by the juvenility-associated protein Bex1, which affects the growth of cerebellar granule cells (Spassky et al., 2008), retinal epithelial and endothelial cells (Bujakowska et al., 2017), and kidney function (Guay-Woodford, 2006). Bex1 forms biomolecular condensates in the presence of molecular crowding agents that concentrate tubulin and nucleate GTP mediated tubulin polymerization (Hibino et al., 2022).

During bacterial cell division, PomX/PomY/PomZ complexes guide and promote cytokinetic ring formation by LLPS-mediated nucleation of FtsZ filaments (Ramm et al., 2022). Condensation of PomY on a PomX scaffold and the recruitment of FtsZ, a bacterial tubulin homolog, into PomY condensates results in the nucleation and GTP-dependent bundling of FtsZ filaments.

3.1.2. Nucleation and growth tuning of MTs by MAP condensates (independent of spindles)

MT dynamics are fine-tuned by plus end interacting proteins (+TIPs) that concentrate at the growing MT plus ends while exhibiting a weak affinity for the MT shaft. The group of +TIPs is comprised of the end-binding protein family (e.g., EBs), the CAP-gly family (cytoskeleton-associated protein glycine-rich, e.g., CLIP-170), and other family members (Akhmanova and Steinmetz, 2010). Earlier studies reported the formation of "patches" upon overexpression of EB3 and CLIP-170 (Pierre et al., 1994; Goodson et al., 2003), which later was shown to have liquid-like properties suggesting they are biomolecular condensates (Wu et al., 2021; Jijumon et al., 2022). EB3 and CLIP-170 co-condensates recruit tubulin dimers onto the plus end of existing MTs, which enhances MT growth speed and decreases growth pausing (Miesch et al., 2022). +TIP phase separation was also demonstrated for EBs in budding and fission yeast, although the LLPS potency of orthologues varied between organisms (Maan et al., 2021; Meier et al., 2021; Jijumon et al., 2022). Spatiotemporal control of MT dynamics via MAP condensates thus seems to be a unifying principle across species.

Another example for the nucleation of MTs from MAP condensates is provided by the neuronal Tau protein, which regulates MT stability and transport in axons of the central nervous system (Brandt and Lee, 1993; Stamer et al., 2002; Dixit et al., 2008; Kadavath et al., 2015). Tau is an intrinsically disordered protein that binds MTs through its C-terminal repeat domain (+short flanking regions; (Mukrasch et al., 2007; Kadavath et al., 2015)). Phosphorylation in these regions decreases Tau-MT binding and also seems to play a role in Tau aggregation observed in Alzheimer's and related neurodegenerative diseases (Fischer et al., 2009; Morris et al., 2015; Wegmann et al., 2021). Owing to its inhomogeneous charge distribution and intrinsic disorder, Tau can undergo LLPS in vitro by itself, in the presence of crowding agents, and through coacervation (=co-condensation of positively with negatively charged polymers) with polyanionic molecules like RNA and heparin (Ambadipudi et al., 2017; Hernández-Vega et al., 2017; Zhang et al., 2017b; Wegmann et al., 2018; Boyko et al., 2020). Condensation of Tau in these systems can be tuned by Tau PTMs, e.g. phosphorylation and acetylation (Wegmann et al., 2018; Hochmair et al., 2022; Ukmar-Godec et al., 2019), and disease mutations (Wegmann et al., 2018; Kanaan et al., 2020). In cells, light induced oligomerization of Tau drives Tau to MTs, suggesting that Tau condensation is tightly coupled to its MT binding (Zhang et al., 2020). *In vitro*, pre-formed Tau condensates recruit tubulin dimers and initiate the formation and bundling of MTs (Hernández-Vega et al., 2017; Hochmair et al., 2022). During MT outgrowth from Tau-tubulin condensates, the condensates deform and shrink, whereby Tau molecules spread along the growing MTs, reminiscent of liquid condensed Tau wetting onto the MT surface (Hernández-Vega et al., 2017). The wetting behavior of Tau on MTs appears to depend on the composition of the Tau condensates, for example, the presence of RNA induces de-wetting of MTs (for details see 3.1.3.).

Importantly, Tau molecules in liquid condensates formed in the presence of molecular crowding and/or RNA or heparin, and, with time, can structurally convert and adopt features of pathological brain Tau (Wegmann et al., 2018; Kanaan et al., 2020; Hochmair et al., 2022), for example, the formation of SDS-stable dimers and oligomers and the seeding of Tau aggregation in sensor cell models (Hochmair et al., 2022). During the structural conversion, the molecular diffusion of Tau molecules inside condensates, as measured by fluorescence recovery after photobleaching (FRAP), drastically decreases, which can be envisioned like a "polymerization" process. Interestingly, the rate of polymerization and the production of seeding-competent Tau species seem to depend on Tau condensate composition; compared to crowding-induced Tau condensation, co-condensation of Tau with RNA and heparin results in slow polymerization but efficient Tau oligomerization. It remains to be examined whether proteins that coacervate with Tau, like tubulin (Hochmair et al., 2022) or 14–3–3 (Han et al., 2022), also reduce the polymerization rate or even inhibit Tau oligomerization inside condensates. Negatively charged small molecules, like the curcumin fragment C1 (Pradhan et al., 2021), appear to modulate Tau LLPS and inhibit the pathological transition of Tau in condensates, which gives them some therapeutic potential to interfere with aberrant Tau condensation leading to aggregation. It remains unexplored how these approaches interfere with physiological Tau condensation.

3.1.3. Interaction of condensates with the microtubule surface

The interaction of liquid-like MAP condensates with the MT surface is characterized by their surface wetting behavior, first described for Tau (Hernández-Vega et al., 2017). Liquid Tau condensates formed in the presence of crowding agent PEG and added to preformed MTs, first attach and then spread onto the MT surface. Interestingly, the presence of polyanionic clients, i.e., RNA, in the condensed Tau phase drastically changes the wetting behavior of Tau by holding it back in a condensed phase and thereby promoting de-wetting of MT bundles (Hochmair

et al., 2022), whereby Tau-RNA condensates remained attached to MT bundles and showed partial wetting of their surface. How other clients of Tau condensates, such as 14–3–3 (Han et al., 2022) that was suggested to compete with MTs for the binding of phosphorylated Tau (Hashiguchi et al., 2000), change the wetting of Tau on MTs is unknown.

Upon addition of soluble non-condensed Tau to pre-formed MTs at low (nM range) concentrations below c_{sat} of Tau in solution, Tau "islands" with liquid-like FRAP behavior form on individual MTs (Siahaan et al., 2019; Tan et al., 2019). With increasing Tau concentrations, individual islands extend to cover more MT surface and eventually merge with neighboring islands. This observation suggests a pre-wetting scenario (Nakanishi and Fisher, 1982), in which the cooperative monolayer binding of Tau molecules at distinct MT positions produces Tau "islands", independent of condensation (Mitchison, 2020; Wiegand and Hyman, 2020)(Fig. 1B). However, additional recruitment of soluble Tau into Tau islands could lead to multilayer adsorption and phase transition at the MT surface (Fig. 1 C). The positions at which condensed Tau islands form on MTs seem to be encoded by the MT lattice (Siahaan et al., 2019). Of note, in these in vitro TIRF imaging experiments, MAP2 showed island formation and preference for positions of disturbed MT lattice similar to Tau and could co-condensate with Tau on MTs. In contrast, MAP4 did not form islands but uniformly coated the MT surface. Functionally, condensed Tau islands on MTs have been implicated in the regulating motor protein processivity (Siahaan et al., 2019; Tan et al., 2019), whereby Dynein paused and Kinesin-1 could not pass and fell off the MT at the Tau condensate phase border. The super-processive molecular motor kinesin-8 could bind MTs regardless of the presence of Tau and even initiated the dissolution on Tau island. Furthermore, Tau islands protected MT against severing proteins Katenin and Spastin. These results suggest a complex role of Tau condensation in the regulation of local MT Tau concentration, MT stability, and molecular motor transport.

Like for Tau, MT-induced phase separation was also reported for components of the inner centromere, +TIP proteins, and TPX2 (Trivedi et al., 2019; Setru et al., 2021; Meier et al., 2021; Wu et al., 2021). *In vitro,* TPX2 condensates can uniformly coat MTs, whereby the liquid TPX2-coat later breaks up into regularly spaced "liquid islands", reminiscent of discontinuity of a liquid film produced by Plateu-Rayleigh instability (Haefner et al., 2015; Xuan and Biggins, 2017) (Fig. 1D). The resulting TPX-2 islands can cluster MT regulators thus facilitating nucleation of new branched MT (Setru et al., 2021; Thawani et al., 2019) (Fig. 2B).

Recently, it was shown that stress-induced protein degradation condensates, formed by BAG2, attach to MTs like beads on a string (Carrettiero et al., 2022). The BAG2 condensates, which contain proteasome components and the chaperone Hsp70, seem to be involved in the clearance of MT-bound Tau via the ubiquitin independent proteasome system. Whether BAG2 condensates locally change the MT wetting behaviour of MAPs remains speculative.

3.1.4. Influence of MTs on cytosolic condensates, and vice versa

In addition to direct interaction with condensates, the cellular MT network influences transport and formation of different biomolecular condensates indirectly. For example, the formation of stress granules is impacted via active transport of granule components along MTs (Chernov et al., 2009; Ivanov et al., 2003; Kolobova et al., 2009). It was recently suggested to exploit the targeted transport of condensates along the MT cytoskeleton as a platform for synthetic functional organelle design in mammalian cells (Reinkemeier et al., 2019; Reinkemeier and Lemke, 2022). Furthermore, it has been suggested that MTs regulate stress granule size by encouraging droplet fusion and non-specific adhesive interactions between them (Alfaro-Aco et al., 2017; Böddeker et al., 2022). Similarly, during cell division, mitotic chromatin forms condensed phases that resist perforation by growing MTs and, thus, can be pushed by polymerizing spindle aster MTs to enable chromatin movement (Schneider et al., 2022). This observation gave first insights into how forces between the cytoskeleton and non-interacting condensates can enable condensate movement in absence of active transport.

In contrast, cellular condensates that interact with cytoskeletal fibers can contribute to the organization of the cytoskeleton and membrane-bound organelles (Fig. 2 C). For example, simultaneous attachment of Tau-RNA coacervates or phospho-Tau condensates to more than one MT (bundle) produces distinct MT arrangements (Hochmair et al., 2022; Hernández-Vega et al., 2017). The high surface tension in this system is governed by cohesive forces inside condensates that enable the arrangement of MTs while maximizing the interaction surface between condensates and MTs. Attachment of condensates to parallelly oriented cytoskeletal fibers could promote their bundling. Capillary forces of the thin liquid-condensed layer between fiber surfaces would produce strong tensile forces that prohibit fiber displacement perpendicular to the fiber axis, but would still allow the gliding of fibers (Fig. 2D). Notably, these general considerations also apply to condensates wetting membrane surfaces and provide a frame work of how biomolecular condensates can connect the cytoskeleton with membrane-bound organelles and the plasma membrane.

3.2. Condensation processes involving Actin

Actin is a major constituent of the cytoskeleton and participates in more protein-protein interactions than any other known protein. The cytoskeletal fibrillar form of actin, F-actin, is polymerized through linear head-to-tail assembly of globular actin (G-actin) monomers. Monomeric and filamentous states of actin are interchangeable under the control of accessory proteins, nucleotide hydrolysis, and ions (Cingolani and Goda, 2008; Merino et al., 2020). Individual actin filaments are assembled into two general types of structures, actin bundles or actin networks, which play different roles in the cell (Janmeysonll et al., 1994; Kraikivski et al., 2008). Actin bundles consist of parallel or antiparallel cross-linked F-actin and provide protrusive or contractile strength. Actin networks are orthogonal arrays of loosely cross-linked F-actin that can form a 3-dimensional mesh with the properties of semisolid gels. The F-actin meshwork renders cells resistant to mechanical stress and enables the formation of stable cell-cell junctions for intercellular communication. More detailed descriptions of cellular actin fibres and networks can be found (Michelot and Drubin, 2011; Blanchoin et al., 2014).

3.2.1. Fluid properties of F-actin assemblies

In vitro studies have shown that actin cross-linking proteins can induce phase separation of F-actin networks, thus endow the filamentous network with new physical characteristics. For example, filamin, an important actin cross-linker, can aggregate F-actin into viscoelastic hydrogels (Wang and Singer, 1977) that can be resolved by gelsolin, a potent actin-severing protein, in a calcium-dependent manner (Yin and Stossel, 1979; Yin et al., 1981). These observations provided early insights into the mechanical properties and regulation of F-actin networks. More recently, short F-actin polymers, promoted by the actin crosslinker filamin, were shown to rapidly demix into spindle-shaped structures (Weirich et al., 2017). These 'tactoids' displayed liquid-like properties such as fusion and dynamic exchange of individual short F-actin polymers with the surrounding medium. Interfacial tension and viscosity of the tactoids depended on cross-link density and influenced the overall tactoid shape. Also Myosin phosphatase rho-interacting protein (MPRIP), which localizes to phosphatidylinositol-4,5-bisphosphate containing nuclear structures, acts on short F-actin units through a condensation process. Overexpression of MPRIP was shown to trigger the formation of nuclear liquid-like MPRIP condensates that reversibly bind nuclear F-actin and thereby initiate F-actin fibres (Balaban et al., 2021).

3.2.2. F-actin nucleation from condensates

Phase separated condensates can concentrate cytoskeletal proteins inside the dense phase to values above their polymerization

concentration (Fig. 2 A). In addition, co-condensation of polymerization modulators can regulate the dynamics of polymerization (Li et al., 2012; Case et al., 2019). In the case of actin, the nucleation promoting factor N-WASP (Neural Wiskott–Aldrich-syndrome protein), its adaptor protein Nck, and the actin nucleator Arp2/3 (actin related protein 2/3) complex control local F-actin assembly (Goley and Welch, 2006). A role of condensation in this process is evident from recent observations in podocyte cells, in which F-actin assembly and branching at the plasma membrane seems to be initiated by LLPS of the nephrin/NcK/N-WASP signalling cascade. Phosphorylation of the transmembrane protein nephrin in its intracellular cytoplasmic tail triggers the interaction with Nck that in turn binds to the N-WASP/Arp2/3 complex via Nck-SH3 domain and N-WASP-proline rich motifs (PRMs) (Jones et al., 2006). Synthetic tandem repeats of N-WASP PRMs co-phase separated with the Nck-SH3 domain in vitro (Banjade et al., 2015). Furthermore, co-condensation of nephrin/NcK/N-WASP depends on the phosphorylation state of nephrin (Kim et al., 2019) and correlates with increased Arp2/3-induced actin polymerization in vitro and in cells (Li et al., 2012). Signalling molecules and actin regulatory components (e.g., ARP 2/3) recruited to Nephrin/Nck/N-WASP condensates appeared to have an increased activity, and their stoichiometry influenced the dwell time of Nephrin/Nck/N-WASP condensates on supported lipid membrane (Case et al., 2019; Su et al., 2016). In T-cells at the immunological synapse, F-actin polymerization can be nucleated from membrane-associated LAT (linker for activation of T cells) microclusters upon T-cell activation. LAT phosphorylation regulates its clustering together with actin adaptor proteins SLP-76 and Nck, which in turn initiate actin polymerization by increasing the activity of N-WASP in Nck/N-WASP/Arp2/3 complexes(Su et al., 2016). These observations suggest that actin polymerization in response to receptor phosphorylation can be further regulated on the levels of Nck/N-WASP/Arp2/3 condensation, condensate stoichiometry, and component activity inside condensates (Su et al., 2016; Case et al., 2019).

A recent study in *C. elegans* oocytes demonstrated non-equilibrium orchestration of N-WASP and F-actin co-condensates in the early stage of actin meshwork formation (Yan et al., 2022). Recruitment of G-actin into N-WASP condensates triggers F-actin polymerization and actin network assembly. However, as F-actin accumulates, N-WASP is removed and the condensates dissolve. This dynamic assembly/disassembly instability of actin/N-WASP condensates is reminiscent of the dynamic instability of MTs (Mitchison and Kirschner, 1984) and allows for autocatalytic control of F-actin nucleation; it also suppresses Ostwald ripening of existing condensates (Zwicker et al., 2015; Yan et al., 2022). This mechanism was suggested to prevent runaway growth and resultant clumping of F-actin, which is important for successful activation of cytoskeletal actin meshworks (Yan et al., 2022). Of note, general differences in LLPS behaviour of G-actin and F-actin have also been suggested from in vitro experiments in a simple biphasic polymer system of molecular crowders, PEG and DEX. Whereas F-actin seemed to enrich in DEX-rich microdroplets surrounded by PEG-rich solution, monomeric G-actin remained uniformly distributed in both phases (Waizumi et al., 2021). Depolymerization of F-actin into G-actin by fragmin removed its association with the DEX-phase, suggesting that G-actin and F-actin have different preferences for the DEX-rich phase.

Other proteins that have been implicated in the regulation of actin polymerization through condensation include vasodilator-stimulated phosphoprotein (VASP), actin binding LIM Protein 1 (abLIM1), and synaptopodin (Falahati et al., 2021; Graham et al., 2022; Harker et al., 2019). VASP associates with growing barbed ends of F-actin and catalyses processive elongation of F-actin in filapodial tips (Harker et al., 2019). Around ~60% of the VASP protein are intrinsically disordered, and VASP homo-tetramers can act as a scaffold for dynamic protein assemblies. In the presence of molecular crowding agent, VASP forms liquid-like condensates, into which G-actin can co-partition and F-actin polymerization is initiated, evident from the deformation (elongation) of VASP condensate shape (Graham et al., 2022). abLIM1 is an actin-bundling protein that ensures stable interaction of the cortical actin meshwork with the plasma membrane. abLIM1 condensates act as actin-nucleators and organize actin-asters into webs of actin bundles in vitro. In response to chemical depolymerisation of the actin network, abLIM1 condensates re-emerge (Yang et al., 2022). Synaptopodin regulates actin-bundling in the dendritic spine apparatus and in renal podocyte foot processes (Asanuma et al., 2005). When expressed in cells lacking endogenous synaptopodin (Falahati et al., 2021), synaptopodin clusters can recruit alpha-catenin, a binding partner involved in actin bundling. The presence of Pdlim7, another protein involved in synaptopodin-mediated actin bundling, decreases the molecular diffusion (by FRAP) inside synaptopodin clusters, indicating a stabilization of molecular interactions inside the condensed phase. Interestingly, low expression of synaptopodin targeted to the ER led to dot-like actin structures, whereas high expression resulted in large linear F-actin structures, suggesting a concentration dependent actin recruitment, nucleation, and bundling activity of synaptopodin clusters at the ER surface. Via this mechanism, condensation of synaptopodin in dendritic spines could couple the F-actin cytoskeleton to spine ER and thereby form and stabilize the spine apparatus.

3.2.3. *Influence of actin cytoskeleton on cellular condensates*

Recent studies have shown that cytoskeletal dynamics can directly impact cytosolic condensates. For example, Arp2/3 derived branched actin networks seem to be required for the formation of functional p62 bodies, condensates that targeted ubiquitinated proteins for lysosomal degradation (Feng et al., 2022a). The interplay between the motor protein Myosin1D and ATP-dependent p62 recruitment along F-actin filaments locally increases p62 concentration resulting in the formation of small p62 clusters. Asymmetrically distributed actin structures increase the proximity of these p62 clusters and thereby contribute to their fusion into larger p62 bodies. Similarly, in immunological synapses, F-actin networks can influence the movement of LAT condensates depending on the density of Nck and WASP proteins in the network (Ditlev et al., 2012). In the nucleus, a role of F-actin in the structural organisation of the nucleoplasm has been suggested. The disruption of F-actin using small molecule inhibitors (e.g., Latrunculin-A) induces fusion of nucleoli in *X. laevis* oocytes, suggesting a role of F-actin in constraining dynamics of nuclear condensates (Feric and Brangwynne, 2013; Feric et al., 2016). Nucleoli are multi-layered phase-separated condensates, in which rRNA transcription and ribosome assembly are initiated (Lafontaine et al., 2020). However, the presence of nuclear F-actin is unclear and seems to be coupled to cellular stress response (Sanger et al., 1980; Hofmann and De Lanerolle, 2006; Belin et al., 2013).

These examples show that the polymerization of G-actin into F-actin, as well as the assembly of F-actin into fibres, may be facilitated by F-actin binding protein condensates. Furthermore, the cellular actin cytoskeleton can influence condensate biology, directly through physical interactions or indirectly by restricting their diffusion.

3.3. *Condensation during intermediate filament assembly*

Intermediate filaments (IFs) are a group of cytoskeletal filaments that promote the mechanical stability of cells or organelles. Different IFs are built from different proteins - Vimentin, Keratin, Lamin, Neurofilaments, or glial fibrillary acidic protein (GFAP) - yet are formed through a similar molecular assembly process. All IF proteins have in common unstructured N-terminal head and C-terminal tail domains that are connected by 4 alpha-helical coil domains, which are spaced by short linker domains. Assembling of IFs is thought to progress through head-to-head oriented dimers, that assemble in a head-to-tail orientation into tetramers, which then connect with their head-tail domains to form protofilaments that bundle into mature IFs (for a review on IF structure and assembly see (Herrmann and Aebi, 2016)).

First evidence for a link between IFs and biomolecular condensates

came from a study on the toxicity and interactions of C9orf72 dipeptide repeat proteins with cytoskeletal fibers by the McKnight Lab (Lin et al., 2016). Recombinant condensates of PR_{20} peptides or FUS LCD attached to recombinant vimentin IFs like pearls on a chain, as shown by electron microscopy, reminiscent of the Raleigh instability of condensates wetting a rigid fiber rod that has been described for TPX2 on MTs (Setru et al., 2021). The attachment of RBP condensates to IFs could, for example, offer a way to localize RNA granules for local translation. It appears that the mostly unstructured head domains of various IF proteins - including vimentin, peripherin, internexin, vimentin, desmin, lamin, and light, medium, and heavy neurofilament (NFL) protein – self-assemble into hydrogels (Lin et al., 2016; Zhou et al., 2021). Interestingly, from whole mouse brain lysates, several proteins related to the cytoskeleton partitioned into NFL head domain and lamin LCD hydrogels, including tubulin, spectrin, MAPs, and motor proteins (Zhou et al., 2021), which indicates that these proteins may interact with the condensed state of NFL and lamin. The condensation of NFL and desmin head domains, as well as their in vitro IF assembly, was impeded by phosphorylation with PKA and point mutations in the head domain, exchanging Pro residues in the NFL head domain (causative for Charcot–Marie–Tooth disease) or Ser residues in the desmin head domain (occurring in cardiomyopathy). This suggests a role of NFL and desmin condensation, and its regulation by phosphorylation, for proper IF assembly. To which extent this mechanism is translatable to other IFs needs to be investigated. In primary astrocytes, phosphorylation in the head domain during cytokinesis disassembles GFAP IFs and initiates the formation of spherical GFAP assemblies without FRAP, reminiscent of gel-like condensates (Li et al., 2006). Notably, the phosphorylation dependent binding of GFAP to 14–3–3γ seems to trigger this process. This indicates that condensation also plays a role in the conditional (dis) assembling of GFAP.

3.4. Role of condensation in postsynaptic density formation, regulation, and actin coupling

The post-synaptic density (PSD) is an electron dense region in the glutamatergic synapse, that is comprised of scaffold proteins, transmembrane receptors, regulators, and cytoskeletal proteins. The major PSD scaffold proteins - PSD95, GKAP, SHANK and HOMER - assemble other effector/regulator proteins via multidomain interactions and stabilize the PSD assisted by the actin cytoskeleton. The most abundant scaffold protein, PSD95, is associated with the post-synaptic membrane through palmitoylation of its 3rd and 5th cysteine residues (Craven et al., 1999; El-Husseini et al., 2002), and it contains (from N- to C-terminus) 3 PDZ domains that can bind a multitude of post-synaptic transmembrane receptors, including NMDAR, AMPAR, nNOS, neuroligins and K^+-channels, a SH3 interaction domain, and a GK domain (guanylate kinase domain without nucleotide binding activity). PDZ domains are protein-protein interaction modules characteristic for signaling proteins and often bind to the C-terminal sequences of their target proteins. PSD95 interacts through its PDZ domains with ion channels and receptors on the post-synaptic membrane, which initiates downstream signaling cascades in the PSD. It is, thus, one of the key regulators of synaptic maintenance and plasticity.

In 2016, it was shown that PSD95, in the presence of its binding partner SynGAP, can undergo LLPS and form liquid-like co-condensates in vitro and in Hela cells (Zeng et al., 2016). When added at physiologically relevant ratios, other scaffold proteins, like GKAP, HOMER and SHANK, co-condensed with PSD95 and SynGAP as well. Furthermore, the C-terminus of NMDARs was recruited to PSD95 condensates, whereas the inhibitory post-synaptic protein Gephyrin was excluded from the condensed phase (Zeng et al., 2018). The co-partitioning of transmembrane client proteins into PSD95 condensates depended on their interaction with the PSD95 PDZ domains (Christensen et al., 2022; Hosokawa and Liu, 2021; Lv et al., 2022; Ukmar-Godec et al., 2019; Vistrup-Parry et al., 2021; Wu et al., 2020; Zeng et al., 2016, 2018), and PSD95 condensates could induce clustering of relevant receptors in the post-synaptic signaling pathway (Hosokawa et al., 2021; Hosokawa and Liu, 2021). Phase separation of PSD95 anchored to a supported lipid bilayer (Feng et al., 2022b; Zeng et al., 2016, 2018), led to spinodal de-mixing, whereby the threshold concentration for PSD95 LLPS on the lipid bilayer was significantly lower than that in solution(Erlendsson et al., 2019). These data indicated that the PSD could be formed via LLPS of PSD95 and its binding partners on the synaptic lipid membrane, and that the PSD phase composition is linked to molecular signaling processes and receptor clustering in the excitatory post-synapse.

A key component for the maintenance, stability, and compositional balance of the PSD is its connection to the actin cytoskeleton, which forms a supportive meshwork between the dendritic cytoplasm and the PSD. The interaction of F-actin with the PSD is facilitated via interactions of HOMER and SHANK with the Arp2/3 complex (Han et al., 2013) and cortactin (Naisbitt et al., 1999), which enables the dynamic regulation of the actin network in dendritic spines. PSD condensates that contain HOMER and SHANK on lipid membranes can recruit G-actin, Arp2/3 complex, and cortactin, and initiated F-actin bundles emerging from the condensates (Zeng et al., 2018). Another postsynaptic scaffold protein, the insulin receptor substrate protein 53 (IRSp53) that is involved in coupling membrane modulation with actin dynamics (Lowenthal et al., 2015), has also been shown to co-condensate with PSD95 or SHANK both in vitro and upon overexpression in neurons (Feng et al., 2022b). These condensates were also able to recruit G-actin and nucleate F-actin polymerization in solution and on supported lipid membranes. In summary, these observations suggest that the regulation of F-actin dynamics in response to synaptic processes, as well as the physical coupling of the spine actin cytoskeleton to the PSD, may be regulated by the composition and the liquid-like properties of the PSD.

Notably, in the pre-synapse, LLPS of key proteins such as synapsin-1, RIMs, RIM-BPs was suggested to play a role in the coordination of synaptic vesicle (SV) pools and active zones (Milovanovic et al., 2018; Wu et al., 2019). Two models have been proposed for the organization of SVs: The scaffold model suggests that clustering of SVs depend on their anchoring to the actin cytoskeleton by synapsin-1, which is supported by the observation that phosphorylation of synapsin-1 by CaMKII causes its dissociation from SVs and actin (Greengard et al., 1993; Pechstein et al., 2020; Menegon et al., 2006; Wang, 2008). The liquid phase model proposes that SVs are clustered by a reversible liquid phase of synapsin-1 that captures SVs and can be disrupted by CaMKII phosphorylation of synapsin-1 (Milovanovic et al., 2018; Pechstein et al., 2020). The pre-synaptic active zone scaffold proteins RIM and RIM-BP undergo phase separation in vitro at physiological concentrations and can recruit other partner proteins such as voltage-gated Ca2 + channels (Wu et al., 2019). Active zone proteins in *C. elegans*, like SYD-2 (homolog of mammalian liprin-α) and ELKS-1 (homolog of mammalian ELKS/ERC/CAST), undergo LLPS at developing active zones in vivo and can incorporate other components in vitro (McDonald et al., 2020). Although studies on LLPS mediated assembly of pre-synaptic structures is growing, a link to the actin or tubulin cytoskeleton has yet to be established.

4. Conclusion

The ability of condensates to locally increase client concentrations – e.g., G-actin and tubulin dimers - enables them to catalyze reactions like the polymerization of cytoskeletal fibers. Additionally, the physical fluid properties of biomolecular condensate give them the power to attach to the cytoskeleton through wetting, and to act and react according to fluid mechanics, governed by their rheology (viscoelasticity). The fluid mechanical aspects of condensates interacting with the cytoskeleton are of special interest since many of the functions attributed to cytoskeletal fibers – F-actin, MTs, and IFs – are actually of mechanical nature as well; cell motility, adhesion, and stability are all based on the buffering and exertion of mechanical forces by the cytoskeleton. In this context,

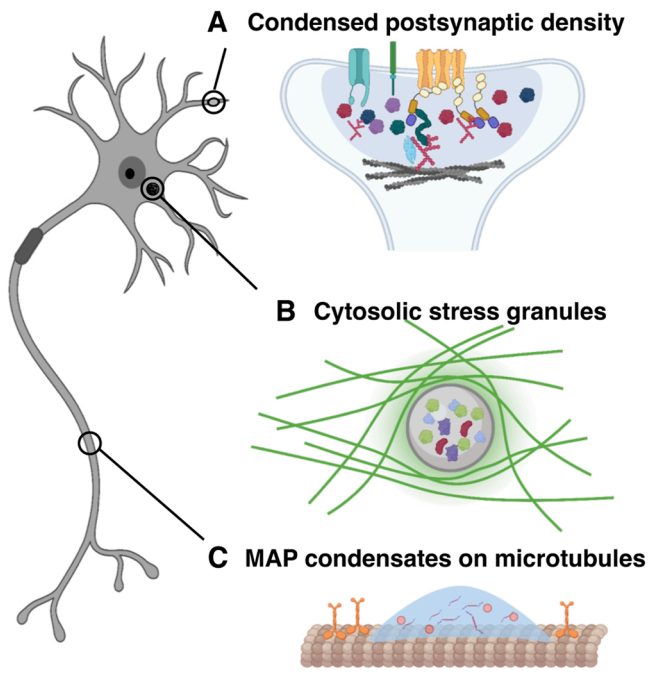

Fig. 3. Suggested phase separation of cytoskeleton associated proteins in neurons. In vitro experiments suggest that the postsynaptic density (PSD) is formed by condensation (Zeng et al., 2016) of PSD95 with its associated proteins. The recruitment of actin monomers into the PSD condensate could promote F-actin formation in the spine, and the wetting of the synaptic membrane and the spine actin cytoskeleton could stabilize the postsynaptic density, i.e., during long-term potentiation. Stress granules and the MT network repulse each other, which amplifies when microtubules polymerize to networks, resulting in high MT density around granules and restriction of stress granule growth (Böddeker et al., 2022). Tau and other MAPs condense and form condensate "patches" on the MT surface, which regulate motor protein processivity (Siahaan et al., 2019; Tan et al., 2019).

condensates could stabilize the cytoskeleton as mechanical adaptors between fibers, for example, enable MT bundling or connecting the tubulin with the actin cytoskeleton, or as adaptors between fibers and membranes. In contrast to rigid protein adaptor complexes, the fluid properties of adaptor condensates maintain a certain flexibility of the system and allow cytoskeleton deformation without breaking, which for cells is an important necessity.

Thin fluid sheets between surfaces provide large resistance against displacement perpendicular to the surfaces but allow gliding of surfaces upon comparable low shear stress exertion (Fig. 2D). This aspect of fluid mechanics could be used by liquid condensates of cytoskeletal associated proteins, like MAPs, to provide the firm attachment of individual fibers into bundles, yet allow the gliding of individual fibers against each other to allow bending of the bundles. Indeed, it was suggested that the *C. elegans* orthologue of Tau, PTL-1, together with spectrin, provides a "lubricant" function to MT bundles in neurons that are under constant bending stress during worm motility (Krieg et al., 2017). Whether PTL-1 and spectrin enable the gliding of MTs as liquid condensates is not known.

For neurons, that have long, thin and rather fragile protrusions (i.e., axons and dendrites) and heavily rely on the compartmentalization of reactions in their highly specialized structures (i.e., in pre-/postsynapses and dendritic shaft), the contribution of biomolecular condensates to stabilize the cytoskeleton and coordinate processes seems of high importance (Fig. 3). For example, biomolecular condensates of MAPs appear to be involved in MT stability, growth, branching, and transport. Additional functions may be provided by a conditional interplay of different MAP condensates on MTs, or in the interconnection of MTs with F-actin. In the postsynapse, condensates of PSD95, its clients and receptor C-termini appear to be involved in PSD function. Whether the liquid condensed PSD also connects the postsynaptic membrane with spine F-actin, e.g. to stabilize spines and enable efficient actin-dependent delivery of necessary substrates or removal of waste, is not known.

Data availability

No data was used for the research described in the article.

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