Title: Condensates regulate the actin cytoskeleton

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Abstract:

The formation of actin filament networks with specific architectures is essential for diverse cellular processes. In this issue of *Developmental Cell*, Walker et al. demonstrate that biomolecular condensates containing F-actin binding proteins have an inherent capacity to assemble and bundle actin filaments, despite the proteins lacking polymerase or bundling activities.

The dynamic assembly of actin cytoskeleton networks is controlled by numerous actin binding proteins, and the formation of actin filament networks with specific architectures is essential for diverse cellular processes including cell motility, cytokinesis, and endocytosis. Biomolecular condensates are membraneless cellular compartments that form through phase separation of their constituent molecules. Several actin binding proteins, including N-WASP and VASP, have been found to undergo phase separation and localize within condensates[1,2]. Recently, several studies have shown that condensates containing proteins that promote actin polymerization can regulate actin assembly[1–4]. In this issue of *Developmental Cell*, Walker et al. demonstrate that actin filament assembly and bundling are general, emergent properties of condensates containing F-actin binding proteins[5]. Thus, condensates can promote actin filament assembly without canonical actin polymerases or nucleators.

Lamellipodin is an F-actin binding protein found in lamellipodia and filopodia. The authors purified a minimal Lamellipodin (mini-Lpd) containing a dimerization domain and the c-terminal intrinsically disordered region[6,7]. They found that mini-Lpd can undergo phase separation on its own or with its binding partner VASP, a processive actin polymerase. Mini-Lpd formed liquid-like biomolecular condensates that readily fuse and exchange proteins with the dilute phase. Surprisingly, mini-Lpd condensates promote actin filament assembly and bundling, similar to VASP condensates, despite Lpd lacking intrinsic polymerase activity. Using an agent-based mathematical model, they predicted that any condensate-forming protein that binds F-actin could bundle actin filaments through dynamic, multivalent crosslinking. Indeed, experiments showed that monomeric Lpd could form condensates that bundle actin filaments, suggesting a stable dimer is not required for this phenomenon. Moreover, adding a single F-actin binding domain to the condensate forming protein Eps15 is sufficient to drive F-actin assembly and bundling within Eps15 condensates. Together, these data suggest that any F-actin binding protein that undergoes phase separation may be capable of promoting F-actin assembly and bundling.

These findings suggest a general principle of actin network organization through multivalent interactions within condensates. Inside condensates, F-actin binding domains are highly concentrated and can facilitate dynamic multivalent interactions with actin filaments. As actin filaments grow within condensates, they partition to the surface of condensates to reduce the filament curvature energy. Actin filaments initially assemble as a shell on the condensate surface but rearrange to form ring-like bundles. Eventually, the actin bundle rigidity overcomes the condensate surface tension, allowing the filaments to straighten and the condensates to deform into rod-like structures. The end result is a condensate containing a bundle of parallel actin filaments (Figure 1). The ability of actin binding domains to promote filament assembly and bundling within condensates is specific to F-actin binding domains. G-actin binding domains are not capable of promoting filament assembly and bundling within condensates, and strong G-actin

partitioning into condensates is not required for filament assembly. However, the mechanism of F-actin assembly in these condensates remains unclear. F-actin assembly can be increased by increasing G-actin concentration, increasing the rate of filament nucleation, or increasing the rate of filament elongation. How these different parameters are impacted within these condensates is not known.

In addition to concentrating macromolecules, condensates also have emergent physical properties such as surface tension and viscosity. Different condensates have distinct physical properties. For example, condensate viscosities have been measured between 0.1 – 1,000 Pa•s[8]. The modeling in this study suggests that condensate physical properties might influence how F-actin networks assemble within condensates. The authors tested a range of condensate viscosities (0.001 to 1,000 Pa•s) in their model. They found that at viscosities above 10 Pa•s, the transition from actin shell to actin ring was significantly slower. Furthermore, condensates with higher surface tensions might be more difficult to deform[1]. Thus, the emergent physical properties of condensates could directly influence the assembly of F-actin networks. Additionally, condensate composition can also change the type of F-actin network that forms within condensates. VASP condensates contain F-actin binding domains and can assemble and bundle actin filaments[1]. However, adding the branched actin nucleator Arp2/3 complex to VASP condensates changes the F-actin network architecture. At lower actin to VASP ratios, Arp2/3 complex inhibits VASP mediated bundling. At higher actin to VASP ratios, Arp2/3 complex promotes the formation of asters, where bundled actin emerges from a central branched actin core[3]. Thus, the emergent actin network architecture within condensates likely depends on condensate composition (i.e. the relative stoichiometry of F-actin binding domains, F-actin, and Arp2/3 complex), as well as the condensate physical properties (i.e. surface tension and viscosity) (Figure 1).

While condensates can regulate F-actin assembly, the actin cytoskeleton can also influence condensates. In C. elegans oocytes, N-WASP forms condensates on the actin cortex[9]. These condensates concentrate and polymerize actin filaments. However, actin filaments in turn negatively regulate N-WASP phase separation. Thus, in this system there is a delicate balance between N-WASP driven condensate formation and actin filament driven condensate dissolution. We are only beginning to understand the complex ways in which condensates interact with and regulate the actin cytoskeleton. The work of Walker et al. demonstrates that condensates containing F-actin binding proteins have an inherent capacity to assemble and bundle actin filaments, greatly expanding the set of actin binding proteins that can potentially regulate F-actin assembly[5]. Whether and where these condensates regulate F-actin networks within cells remains an open question. This biochemical study opens exciting new questions about how phase

separation functions in tangent with canonical actin nucleators, polymerases, and crosslinkers to regulate F-actin network assembly in cells.

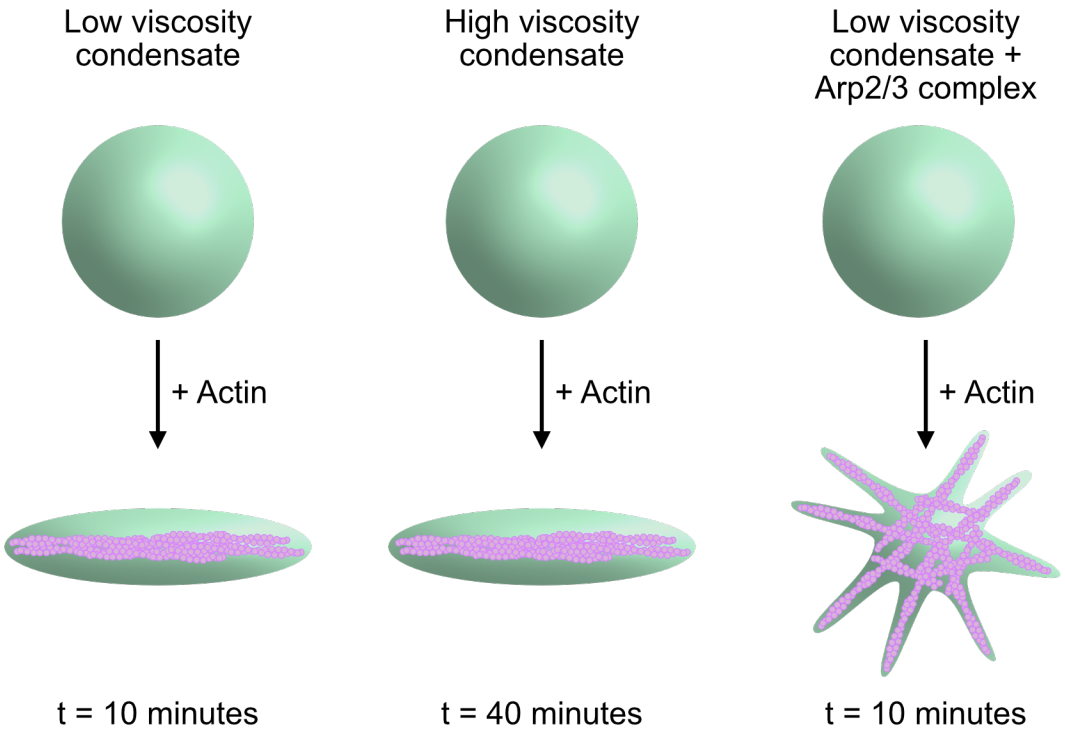

Figure 1. Biomolecular condensates that contain F-actin binding domains regulate actin assembly. Actin filament growth deforms low viscosity condensates within 10 minutes, while high viscosity condensates deform more slowly (40 minutes or longer). Addition of Arp2/3 complex can result in condensates with an aster shape.

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