Membranes regulate biomolecular condensates

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Membranes regulate biomolecular condensates

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Abstract

Biomolecular condensation has emerged as a fundamental mechanism for cellular organization, but less is known about the regulation of condensate subcellular location and size. A new study reports that membrane tethering of protein and RNA directly influences the assembly, size, and material properties of ribonucleic condensates.

Article

Biomolecular condensates are cellular compartments that concentrate a specific group of molecules without a surrounding membrane. Condensate formation has emerged as a

fundamental mechanism for cellular organization and compartmentalization. Many condensates form through liquid-liquid phase separation driven by multivalent molecular interactions. Phase separation is a thermodynamic process; above a threshold concentration the solution demixes into a dense and dilute phase. In liquid-liquid phase separation, the coexisting phases have liquid-like material properties, resulting in the formation of condensates that behave as liquid droplets. Over the last decade, biochemical, cellular, and theoretical studies have provided a detailed understanding of how condensates form. However, less is known about how cells control the subcellular location of condensates or limit the size of condensates. In this issue of *Nature Cell Biology*, Snead et al. find that membranes can directly influence condensate assembly and size[1].

Whi3, a glutamine rich RNA-binding protein, forms punctate ribonucleic condensates that regulate cell cycle and cell polarity in the multinucleate fungus *Ashbya gossypii*[2]. Endogenous Whi3 condensates often colocalize with the endoplasmic reticulum *in vivo*, suggesting a role for membrane localization in Whi3 regulation[1]. In this study, the authors combined purified protein and RNA on supported lipid bilayers and identified several ways in which recruitment of Whi3 or RNA to membrane surfaces impacts these ribonucleic condensates (Fig. 1)[1]. First, membranes can reduce the threshold concentration required for condensate formation. Recruitment of molecules to membranes locally increases their concentration to favor condensation. When Whi3 is tethered to the membrane, condensates form at physiological conditions (50 nM Whi3, 150 mM KCl). In contrast, previous studies found that in the absence of membranes, Whi3 undergoes liquid-liquid

phase separation in solution only at non-physiological conditions (28 μM Whi3, 75 mM KCl)[2]. Second, condensates formed on membranes can have different material properties than condensates nucleated in solution. Whi3 droplets formed in solution exhibit liquid-like material properties such as rapid fusion, while Whi3 condensates nucleated from membranes are less liquid-like and do not fuse. Finally, condensates formed on membranes can have reduced size due to altered growth. Initially, RNA-Whi3 condensates nucleated on membranes grow by coalescence (i.e. two condensates fuse to form a larger condensate), but this growth stops within 5 minutes. As condensates grow their mass increases, which decreases their diffusion and the likelihood for two condensates to encounter and coalesce.

In this study, the authors also observed that membrane localization can have different effects on condensates depending on which species of molecule is directly tethered to the membrane surface. Tethering either Whi3 or RNA to a fluid membrane was sufficient to promote condensate nucleation at physiological conditions. However, tethered protein and tethered RNA resulted in condensates with distinct properties. When Whi3 protein was tethered to the membrane, RNA molecules did not partition into the center of Whi3 condensates, but rather initially interacted with the condensate surface. Furthermore, Whi3-tethered condensates did not undergo fusion or rounding. When RNA was tethered to the membrane, Whi3 strongly partitioned into condensates and colocalized with RNA. RNA-tethered condensates also displayed liquid-like material properties and underwent fusion and rounding. Thus, simply changing which molecule is tethered to the membrane resulted in condensates with different chemical and material properties.

This study provides evidence that membranes can play an important role in regulating condensate size. Condensates reconstituted *in vitro* from purified protein or RNA are usually much larger than their cellular counterparts. Phase separation is a thermodynamic process, and *in vitro* condensates grow over time until a single large droplet remains. However, condensates in cells more commonly exist as a collection of much smaller puncta that do not increase beyond a certain size (often only 100 nm – 300 nm in diameter)[2]. The discrepancy in size between cellular condensates and reconstituted condensates could be explained by active cellular process, such as the controlled translation and degradation of proteins, that may limit condensate size *in vivo*[3]. Reconstitution of ribonucleic condensates on membranes demonstrates that tethering molecules to a two-dimensional membrane surface is another mechanism that can restrict condensate size and reduce growth[1]. Cellular membranes could be important regulators of condensate size, and the localization of molecules to membrane surfaces may provide a passive mechanism to limit condensate growth. How the size of a condensate relates to its emergent properties and function remains unclear[4]. If small condensates have distinct properties from larger condensates, the control of condensate size by cellular membranes could have important implications for regulating condensate function.

In this study of reconstituted Whi3 condensates, Nickel-chelating lipids recruit his-tagged proteins and biotinylated lipids recruit neutravidin-coupled RNA molecules[1]. Although this system is synthetic, it demonstrates that the specific localization of molecules to fluid membrane surfaces is sufficient to reduce the molecular concentration and salt barrier

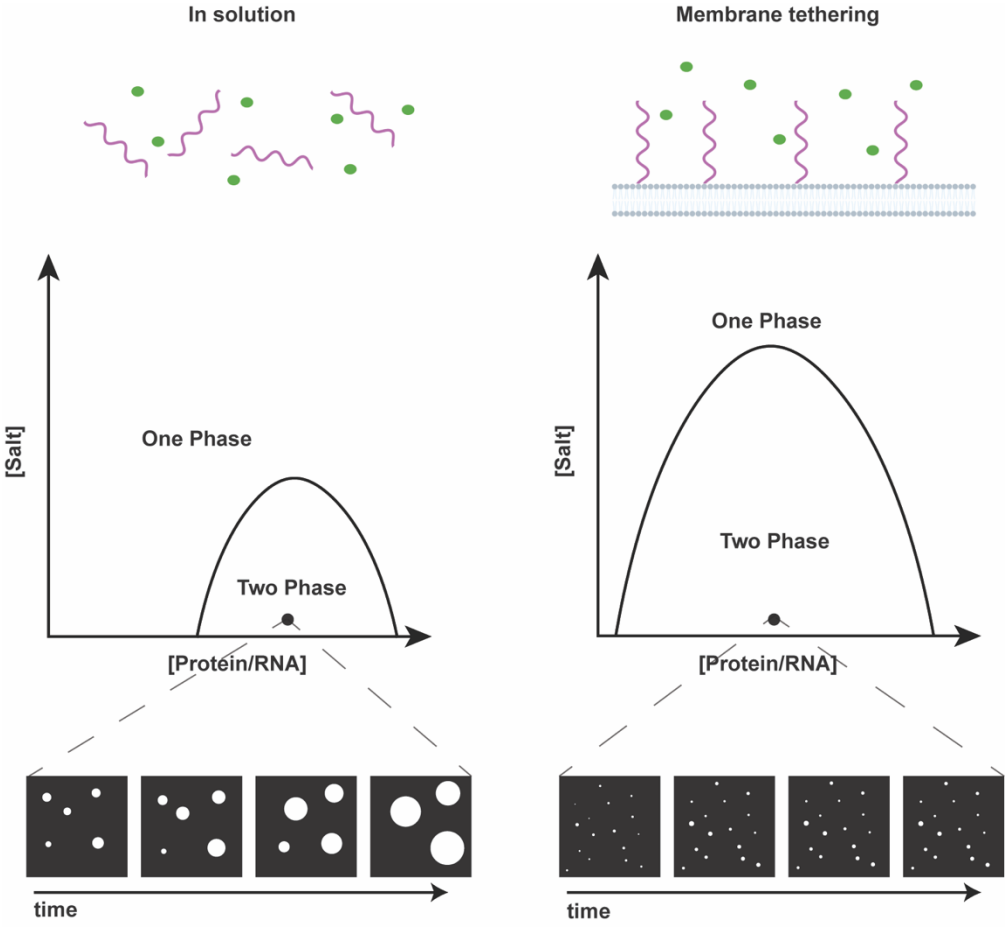
required for condensate formation (Fig. 1). In cells, specific localization of molecules to membranes can be controlled by a variety of factors. Recruitment of cytoplasmic molecules to membrane surfaces can be mediated by direct interactions with transmembrane proteins[5]; posttranslational modifications such as prenylation, myristoylation, and palmitoylation[6]; interactions with specific lipid species[7]; and sensing of membrane curvature[8]. Postranslational modification of proteins to promote specific protein-protein or protein-lipid interactions can be used to spatiotemporally regulate their membrane localization. Additionally, modification of lipid bilayers to alter lipid composition or membrane curvature can also spatiotemporally regulate recruitment of cytosolic molecules to membrane surfaces. Thus, modification of both the lipid bilayer and cytosolic molecules could trigger local concentration of molecules on membrane surfaces to promote condensate nucleation. Furthermore, the composition and organization of lipids within the bilayer can dramatically change diffusion of transmembrane proteins[9], and reduced diffusion can limit condensate size[1]. There are growing examples of diverse intracellular condensates that interact with and may be regulated by cellular membranes including the plasma membrane[10], the endoplasmic reticulum,[11,12] autophagosomes[13], lysosomes[14], and endosomes[15]. Membranes could be broadly used within cells to control condensate formation, location, and size. Condensates, in turn, could potentially alter cellular membranes. For example, phase separation of autophagy-related proteins at the yeast vacuole regulates subsequent autophagasome assembly[13]. More work is needed to understand the potential cross-talk between membrane-bound organelles and phase separated condensates.

In addition to membranes, condensates have been observed to associated with other intracellular surfaces including cytoskeletal filaments[10] and DNA[4]. Like membranes, binding to these biological polymers may increase local concentration and alter molecular diffusion. The work of Snead et al. demonstrates that assembly of macromolecules on biological surfaces could be important for the regulation of condensates in cells. Many biomolecular condensates interact with membranes or other intracellular surfaces, and these interactions may regulate condensates in complex ways. Uncovering these interactions and understanding how they influence condensate properties will be important in the ongoing efforts to understand the cellular function and regulation of condensates.

Competing interests

The author declares no competing interests.

Figure 1. Membrane localization controls the nucleation and size of condensates.
LEFT: protein (green dots) and RNA (pink curves) combined in solution. RIGHT: RNA is tethered to a membrane and protein is in solution. Membrane tethering shifts the phase diagram, promoting nucleation under lower protein and RNA concentrations and higher salt concentrations. Membrane tethering also leads to the arrest of condensate growth, resulting in fewer, smaller condensates. Figure created with Biorender.com.



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