2149-Plat

Ebola viral factories are biomolecular condensates with specialized assembly dynamics for viral RNA synthesis

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A hallmark of Ebola virus (EBOV) infection is the formation of membrane-less organelles termed viral factories (VFs). Accumulation of viral proteins drives the formation of VFs, which offer a specialized compartment for viral replication. Multiple EBOV proteins are present inside VFs, but the involvement of individual viral components in VF assembly is unclear. Key to VF function is the recruitment of EBOV polymerase L, which, in complex with viral cofactor VP35, mediates replication and transcription of viral RNAs. How VFs spatially accommodate and control EBOV RNA synthesis remains elusive. Here, we detected both connected network-like and spherical Ebola VFs during infection, suggesting that spinodal decomposition and nucleation-and-growth both contribute to VF assembly. Using fluorescence recovery after photobleaching with live cell imaging, we measured highly dynamic molecular exchange within Ebola VFs reconstituted with mixtures of two viral proteins, NP and VP35. Co-expression of EBOV L partitions L into VFs but immobilizes a fraction of VP35, which likely results from L forming an arrested network pattern with interconnected foci inside VFs. We further introduced a surrogate viral genome into reconstituted VFs to allow L-directed viral RNA synthesis, from which we observed an intriguing link between the spacing of interconnected L-foci and a functional switch of viral RNA synthesis. We examined the same reconstituted VFs with electron microscopy and observed electron-dense materials in the cytoplasm, which resemble arrested L-network patterns observed with light microscopy. EBOV polymerase complex (L-VP35) localizes to select sites at the boundary of electron-dense materials, indicating viral RNA synthesis could initiate at multicomponent phase boundaries and benefit from the directional flux of viral proteins. In summary, our work provides an unprecedented view of EBOV propagation, which exploits fundamental rules of phase transition to regulate virus biogenesis.

2150-Plat

Nuclear phase separation directs HOX transcription in acute myeloid leukemia

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Cancer requires transcriptional misregulation to preserve a stem cell-like state, as in acute myeloid leukemia (AML) through sustained activation of *HOX* genes. Most drivers of *HOX* in AML have established roles in transcriptional regulation in the nucleus. In contrast, the most common driver, mutant Nucleophosmin1 (NPM1c) is a cytoplasmic oncoprotein resulting from the *de novo* addition of a nuclear export sequence to the essential nucleolar RNA binding protein NPM1. Our lab and others have shown that NPM1c is necessary for *HOX* activation; however, the mechanism underlying nuclear activity by this predominantly cytoplasmic protein remains perplexing. We used high-resolution microscopy to investigate NPM1c's localization and role in *HOX* transcription. Unexpectedly, we observed a fraction (<10%) of NPM1c resides in unreported nuclear puncta or condensates with >100-fold higher enrichment than in the cytoplasm. Biophysical characterization reveals that NPM1c condensates are phase-separated. These condensates require a critical concentration of NPM1c to form and exhibit a concentration-dependent increase in size and number across multiple cell lines. Modulation of NPM1c concentration and subsequent condensate number dictated *HOX* mRNA levels and cell growth. These results are consistent with a role for NPM1c condensates in *HOX* misregulation. Further evaluation of a dozen truncations of NPM1c allowed us to unequivocally establish a link between NPM1c condensates, *HOX* activation, and preservation of the stem cell-like state. Moreover, we observed NUP98 and MLL—proteins found in leukemic oncofusions—enrich in NPM1c condensates, leading us to characterize the ability of numerous fusion oncoproteins to drive nuclear phase separation and activate *HOX*. Our results suggest that nuclear phase separation is a ubiquitous mechanism for *HOX* activation, providing a common therapeutic vulnerability across AML subtypes and possibly other cancers.

2151-Plat

Effect of a membrane protein condensation phase transition on reaction-diffusion kinetics

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Reversible biomolecular phase transitions, such as liquid-liquid phase transition in cytosolic proteins, and phosphorylation-driven condensation in membrane-bound systems have been cited as underlying mechanisms for numerous important cellular processes such as signal transduction. For example, condensation of the T cell signaling protein LAT, and, separately, Epidermal Growth Factor Receptor has been shown to elongate the membrane dwell time of the Ras-GEF SOS, enabling it to overcome a series of slow, membrane dependent activation steps. However, evidence from in-vitro reconstitution experiments suggests that formation of a condensed phase may not solely be beneficial to membrane-associated enzyme kinetics. SOS is both a scaffolding component, and a downstream effector of signaling condensates upstream of the Ras/Erk pathway. In such systems, its substrate Ras needs to have access to SOS for nucleotide exchange to take place. Sequestration of SOS proteins within a networked condensate may inhibit the ability of Ras to encounter SOS by diffusion. To investigate, we reconstitute protein condensation phase transitions consisting of the phosphotyrosine tails of either LAT or EGFR on supported lipid bilayers, along with the crosslinking components Grb2 and the PR domain of SOS to induce condensation. Under these conditions, single molecule TIRF microscopy can be used to localize and track membrane diffusion and partitioning between the condensed domains and surrounding sparse phase.

2152-Plat

Interface resistance of biomolecular condensates

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A hallmark of biomolecular condensates formed via liquid-liquid phase separation is the dynamic exchange of condensate components with the surroundings. Such material exchange can be key to condensate function, as the rate of component exchange can impact biochemical reaction rates in the condensates, the speed of response of condensates to a changing environment, and their number and sizes. How is this exchange rate controlled? Intuitively, this rate can be limited by the flux of materials from the dilute phase or by the speed of mixing inside the dense phase. Surprisingly, recent experiments suggest that this rate can also be limited by the dynamics of molecules at the droplet interface, implying the existence of an ''interface resistance''. Here, we combined theory and simulation to show that an interface resistance can arise when incident molecules contact the interface without entering the dense phase. We find that such ''bouncing'' occurs for incident molecules that are capable of self-collapse into a non-sticking conformation. Our work highlights the underappreciated role of interface resistance in condensate exchange dynamics, with implications for natural and synthetic systems.

2153-Plat

Molecular determinants and modifiers of the toxicity, condensate dynamics, and droplet morphology of the ALS/FTD-associated protein Matrin-3

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Matrin-3 is a DNA- and RNA-binding protein (RBP) implicated in amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and a distal myopathy. Many RBPs with prion-like domains, including TDP-43 and FUS, have been implicated in ALS/FTD and Matrin-3 has key similarities and differences as compared to these proteins. To better understand the key features of Matrin-3, we have developed a new yeast model of Matrin-3 proteotoxicity