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Prospering phase separation

Phase separation is an important mechanism for biomolecule condensate assembly and is involved in multiple biological activities. Understanding its molecular mechanism provides a unique perspective for gaining insights into its role in cellular physiology and for developing new tools for the manipulation of cellular function.

Phase separation (PS) refers to the de-mixing process of a homogenous phase to form dense and dilute phases under certain conditions. It was proposed long ago that crowding in the cytoplasm may cause PS of biological macromolecules in cells. However, biologists did not pay close attention to PS until Clifford Brangwynne and Anthony Hyman discovered the liquid-like properties of P-granules formed via condensation in *Caenorhabditis elegans*. Subsequent studies have revealed that PS is a universal phenomenon across all kingdoms of life, acting as one of the fundamental mechanisms for the assembly of subcellular biomolecular condensates (also called membraneless organelles) and membrane-bound organelles. In this Focus issue, we present a collection of Reviews, Perspectives and Articles that aim to reveal the molecular and chemical principles underlying phase-separated condensate formation, promote the development of new tools for the study of PS biology, and broaden the application of these tools in chemical biology and biomedicine.

Although the phase-separating biomolecule condensates initially discovered comprised mainly proteins and nucleic acids (especially RNA), further studies showed that condensates can be formed by proteins alone, as well as by other macromolecules, such as lipids and polysaccharides. Among these components, multiple interactions drive the PS process and affect the mesoscopic properties of the resulting condensates, including their shape, size, material properties, etc. For example, changing the hydrophobic interactions within a condensate by changing the hydrophobic domain length of elastin alters its liquid-to-solid transition. A Review by Villegas, Heidenreich and Levy further details how the molecular features of phase-separating biomolecules influence their separation behavior, and how the sequence and structural characteristics of proteins interact with the complex cellular environments to shape the PS process. In addition, the dynamic nature of PS assembly endows an organism with the flexibility to respond to environmental changes. An example of this is SEUSS, a transcription regulator in *Arabidopsis* that undergoes condensation after high-salt stimulation to induce the expression of stress-related genes.

The mesoscopic properties of condensates are closely related to their functions, such that anomalies can lead to aberrant cell functions and diseases. For example, changing the liquidity of PopZ condensates in the bacterium *Caulobacter crescentus* affects their cellular localization and interferes with cell division. In eukaryotes, most proteins involved in neurodegenerative diseases, including TDP-43, FUS, tau and α-synuclein, are believed to undergo abnormal PS into solid or gel-like pathologic condensates. Loss of interaction between TDP-43 and the chaperone HSPB1 destroys the liquid property of TDP-43 condensates, which promotes the progression of pathological fibrils.

The critical roles of biomolecular condensates in physiology and disease make them attractive targets for drug development. For example, Xie et al. found that liquid–liquid PS of the androgen receptor induced by androgen receptor antagonists contributes to the resistance associated with these drugs in the treatment of prostate cancer, which can be overcome by a small molecule that disrupts the androgen receptor's PS. Moreover, the specific microenvironments inside condensates lead to the selective distribution of small molecules, providing a new modality for drug design with improved specificity and potency and reduced side effects. A Perspective by Kilgore and Young describes the chemical properties that govern the selective partitioning of small molecules in biomolecular condensates, and proposes how these properties inform the development of condensate-targeting drugs.

In addition to drug development, growing knowledge about PS mechanisms, combined with chemical biological technologies, has enabled the design of synthetic protein condensates with desired functions in response to artificial signals. For example, Li et al. developed a membrane-tethering liquid–liquid PS system for clustering cell membrane proteins and regulating downstream intracellular signaling events. A Review by Qian, Huang and Xia summarizes the currently available building blocks for synthetic condensates, and how these tools contribute to cellular and metabolic engineering by sequestering or enriching needed molecules and/or by excluding unwanted byproducts.

Collectively, the programmable and quantitative properties of these synthetic condensates make it possible to obtain new insights into PS itself. Using a chemically inducible condensate system for SUMOylation, Peeples and Rosen revealed that PS increases enzyme reaction activities, not only due to the enrichment effect of enzyme and substrates within the condensates but also dependent on the molecular organization in the condensates. This notion was further confirmed by a synthetic kinase condensate system for controlling phosphorylation.

As research output on these and other topics in the PS field continues to increase, a key outstanding issue is how to identify biologically important condensates and establish causative relationships between PS and its biological functions. In a Perspective from Gao, Li, Li and Lin, the authors propose a pipeline for PS-related biological studies, highlight the proper experimental designs with which to validate and characterize PS, introduce strategies for establishing causative connections to biological functions, and flag some caveats to avoid when designing experiments.

We are excited to see that PS has evolved from an underappreciated phenomenon to a widely accepted mechanism involved in various biological functions across different kingdoms of life. We believe the intrinsic connection between chemical biology and PS will afford new perspectives for understanding life processes and sophisticated tools for manipulating biological systems.

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