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ABSTRACT

Biomolecular phase separation has recently attracted broad interest, due to its role in the spatiotemporal compartmentalization of living cells. It governs the formation, regulation, and dissociation of biomolecular condensates, which play multiple roles *in vivo,* from activating specific biochemical reactions to organizing chromatin. Interestingly, biomolecular phase separation seems to be a mainly passive process, which can be explained by relatively simple physical principles and reproduced *in vitro* with a minimal set of components. This Mini review focuses on our current understanding of the fundamental principles of biomolecular phase separation and the recent progress in the research on this topic.

1. INTRODUCTION

A living cell is an assembly of various organelles. Some of them are surrounded by a membrane, while others float inside the cell as spherical droplets made of proteins, nucleic acids, lipids, and other small molecules (1). The membrane-bound organelles (such as nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, and lysosomes) have been known for many decades and studied extensively, but our knowledge of the membrane-less organelles, often called *biomolecular condensates*, was limited until recently (2). Although biomolecular condensates do not enclose their components within a membrane, the components do not mix with their surroundings. This demixing had been a mystery, but recently it was found that it can occur spontaneously via liquid-liquid phase separation (LLPS), a phenomenon that has been known in physics and chemistry for more than a century (3).

Many cellular reactions can be triggered by a change in the concentrations of specific molecules, in which cases LLPS can be utilized for efficient cell functioning as it can dramatically increase the local concentration of the recruited molecules (4, 5). Moreover, due to their liquid-like properties, biomolecular condensates can quickly recruit specific molecules in response to perturbations such as temperature changes. Cells can use this mechanism to quickly respond to abrupt environmental changes. Hence, biomolecular condensates have a significant role in a wide range of cellular functions including cell signalling, ribosomal biogenesis (5), cytoskeletal regulation (6), stress response (7), cell polarization (8), and cytoplasmic branching (9). LLPS is also observed in many processes

such as miRISC assembly (10), innate immune signalling (11), stress granule assembly (12), autophagy (13), nucleolus formation (14), and transcription (15). In addition, LLPS even has implications for the pathogenesis of cancer and many neurodegenerative such as Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (16-18).

In this review, we highlight the recent findings on the underlying mechanisms of phase separation driven by proteins and the effects of nucleic acid on this process. We start with a brief introduction of the major principles of LLPS (Section 2), and move to the recent advancement in the field (Section 3): new biomolecular condensates and their functions in the cytosol (Section 3.1) and nuclei (Section 3.2), studies on molecular principles of biomolecular LLPS (Section 3.3), design and engineering of artificial condensates (Section 3.4), and implications of LLPS in diseases (Section 3.5). We collected recent publications (specifically published in two years) related to biomolecular LLPS, and found that the four topics mentioned above can summarize the field well.

2. PRINCIPLES OF LIQUID-LIQUID PHASE SEPARATION

A multi-component system like cellular cytoplasm can exist as a homogeneous, well-mixed mixture or a soup of distinct phases (phase separation), depending on the interactions of the constituent molecules including the solvent. The thermodynamics of a multi-component system can be explained by a tug of war between entropy and enthalpy; demixing occurs if the energy gain for demixing is greater than the entropic loss for demixing (19, 20). The miscibility is strongly dependent on the components' concentrations, temperature, pressure, pH, and crowding agents. If the separated phases maintain their liquidity, the demixing is called *liquid-liquid phase separation* (LLPS). We underline that phase separation of a liquid system does not necessarily imply a change in material properties (*e.g.*, a change from liquid to solid), although the latter can take place during the "maturation" of liquid-like condensates (21, 22). A phase diagram shows the region in which phase separation occurs in the parameter space of the concentration and the other factors such as temperature (Figure 1).

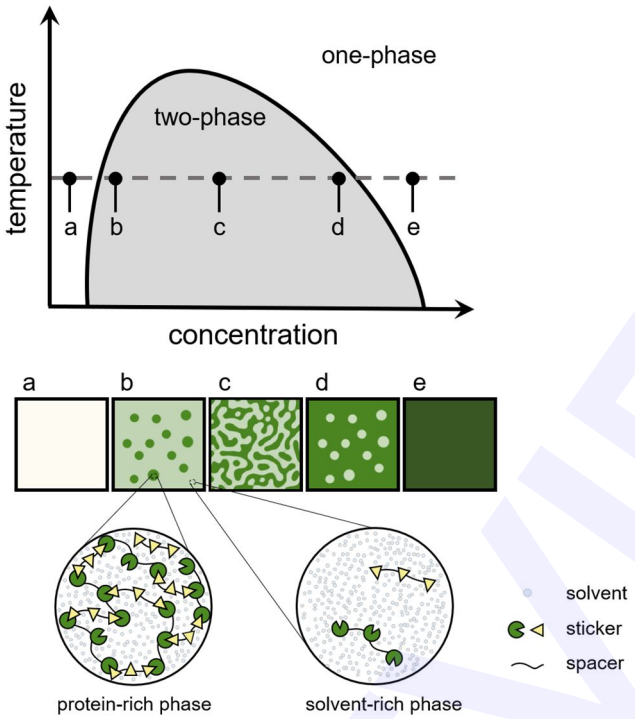

Figure 1. Schematic of a typical phase diagram. The coexistence line (black) denotes the boundary between the one-phase regime (a, e) and the two-phase regime (b, c, d). In the two-phase regime, two distinct phases can be observed: the protein-rich phase and solvent-rich phase. The molecular network formed by stickers is shown as a driving force for phase separation.

Biopolymers are biological heteropolymers and their behaviour can be explained by modifying the theories which were originally developed for the associative polymers (23). Among them, the *stickers-and-spacers* framework helps quantitative and qualitative explanation of LLPS of biopolymers (24-26); each polymer consists of several sticky regions called *stickers*, which are covalently connected by flexible regions called *spacers*. Stickers form reversible physical crosslinks with a combination of short- and long-range interactions with one another, which give rise to a connected network of biopolymers. Spacers provide scaffolds for stickers and modulate the chain properties (27). LLPS is driven by the interplay of sticker-sticker, sticker-spacer, and spacer-spacer, as well as sticker-solvent and spacer-solvent interactions. The valence of the stickers, *i.e.* the number of interacting domains or motifs, is one of the key determinants in the process (28, 29).

Two types of proteins are known to drive the LLPS: multi-domain proteins and intrinsically disordered proteins (IDPs). Multi-domain proteins are proteins with folded domains linked by disordered linkers. Several multi-domain protein systems have been reported to exhibit phase separation (13, 20, 27), but most of the known LLPS driver proteins are the IDPs. The IDPs lack stable 3-dimensional structures (30), and their flexibility allows access to various structures which may lead to the effective formation of sticker-sticker contacts. In the case of IDPs, specific types of amino acids or short linear motifs (SLiMs) can play the role of stickers (24). Cation-anion interactions, cation-π interactions, and π-π interactions are frequently utilized in driving LLPS (31-33). For example, in the FUS family of proteins, aromatic residues such as tyrosine and phenylalanine were found to be the primary stickers that use π–π stacking interactions, whereas arginine was shown to be a context-dependent auxiliary sticker involved in cation-π interactions to form reversible non-covalent crosslinks (33).

Biomolecular condensates usually contain numerous types of molecules (34, 35), among which only a few play an essential role in forming the condensates (4). These molecules are called *scaffolds*, and are generally defined as biomolecules capable of forming droplets when separated *in vitro* (3). Other components, called *clients*, are recruited to the scaffold in a relatively passive manner (36). For example, in the case of the P body, RNA-binding proteins play the role of scaffold to form a condensate, and RNA is recruited into the condensate as a client (34). Clients cannot form condensates by themselves but can adjust the condensate properties (37).

Most biomolecular condensates are enriched in RNA, and nuclear condensates are in constant contact with DNA. Consequently, the role of DNA and RNA in intracellular phase separation has attracted attention (3, 38, 39). DNA and RNA contain the phosphate backbones, which are decorated with negative charges, and can participate in electrostatic interactions that may drive LLPS. It has been shown that RNA can dramatically change the phase separation propensity of the RNA-binding protein (40). In addition, the RNA itself can undergo LLPS by using its nucleotides as stickers (39). The interactions between DNA/RNA and proteins and their subsequent role in inducing LLPS have been studied extensively (41-43).

3. RECENT TRENDS IN RESEARCH OF BIOMOLECULAR LLPS

Many biomolecular condensates are produced by LLPS of biopolymers such as intracellular proteins and nucleic acids (3, 28, 44). The list of new biomolecular condensates formed by LLPS is dramatically expanding, for both prokaryotes and eukaryotes (28, 45), and their roles have been revealed. We can control the function of a biomolecular condensate by regulating its LLPS behaviors by changing the experimental conditions and/or the protein sequence by mutagenesis (41, 46, 47). In addition, LLPS has been implicated in neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases (16, 18, 48, 49). It has been hypothesized that LLPS of the pathological proteins can accelerate their nucleation and consequent fibrillation (50). We can also design artificial condensates like DNA nanostructures by applying the principles governing LLPS. In the following section, we will sketch some of the recent works in the field of biomolecular LLPS (Figure 2), which may help the readers to grasp the overall landscape of the field, although our description is not complete or thorough.

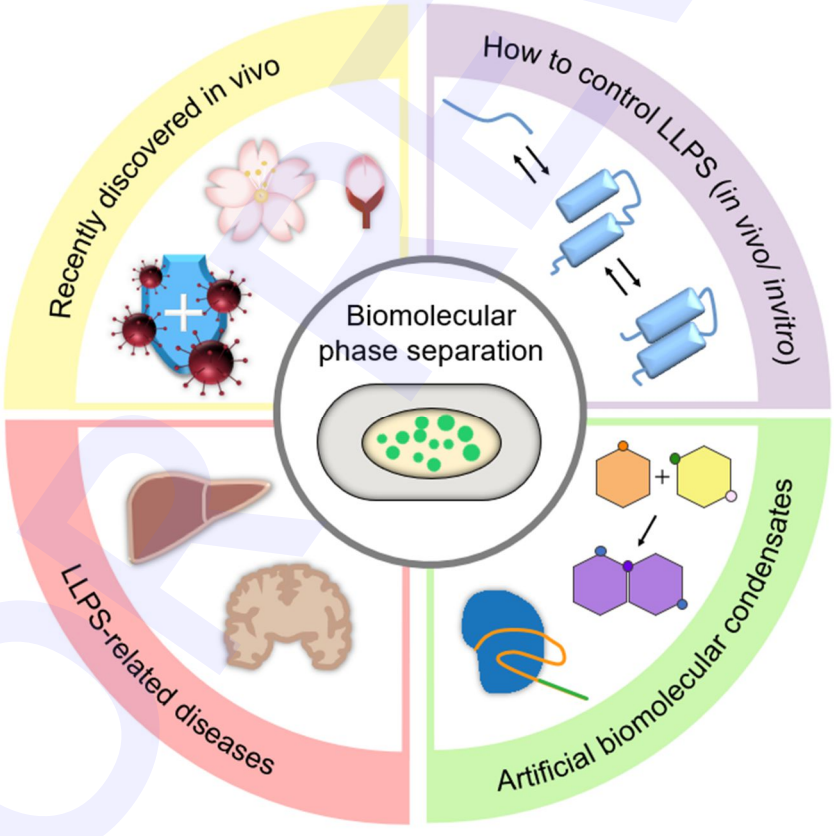

Figure 2. Recent trends in the research of biomolecular phase separation.

3.1 Discovery of new biomolecular condensates and their functions

Biomolecular condensates perform a variety of functions *in vivo*. The list of new condensates and their biological roles is rapidly expanding. The molecular mechanisms of formation, regulation, and dissolution of the condensates are frequently explained by LLPS. In this subsection, we will introduce some recent works that report the discovery of new biomolecular condensates and their functions.

Some biomolecular condensates change their physical properties with changes in experimental variables, such as concentration, temperature, or pH. They utilize these changes to perform special functions within the cell. For example, tardigrade-unique IDP (TDP) has a propensity to assemble into fiber or fibrillar gel at high concentration and low temperature, which can encapsulate soluble biomolecules to protect them from cold. This is one of the molecular strategies for tardigrades to survive in extreme environments. The TDP assembly is mediated by the α1 segment, which makes up the central helical domain of proteins, and a coiled-coil propensity of the segment has been shown to play a great role in tardigrade anhydrobiosis (51).

LLPS is also associated with biological immunity. An intrinsically disordered short lysine-repeating sequence of NLRP6, an innate immune receptor, is responsible for multivalent interactions, phase separations, and inflammasome activations, and induces LLPS by interacting with lipoteichoic acid and dsRNA acting as a ligand *in vivo* (52).

Cells employ biological condensates for inter-cell interactions. The formation of biomolecular endocytic condensates is driven by the interactions of proteins with PLDs (prion-like domains) that have higher glutamine (Q) contents compared to asparagine (N). Hemispherical puncta, which originate from yeast, have the characteristics of these condensates. Cohesive interactions between the endocytic condensates and interfacial tension enable actin-independent endocytosis by membrane remodeling (53).

LLPS can explain how plants bloom during flowering time, as biomolecular condensates are involved in the biological clock mechanism. Vegetations use seasonal temperature signs for measuring the conversion to reproduction over time. The binding of two proteins, FRIGIDA and FLC, triggers flowering. However, low-temperature conditions immediately accelerate the formation of FRIGIDA condensates that do not co-localize with FLC, and FRIGIDA cannot bind to its binding partner. This process ensures that the flowering time coincides with the warm weather, *i.e.*, spring (54).

The mitotic spindle composed of microtubules is crucial for the assembly and separation of chromosomes during cell division of the eukaryotic cells, and the formation of the mitotic spindle is related to phase separation. It was shown that the NuMA protein regulates the organization, structural dynamics, and functions of the mitosis spindle through liquid-liquid phase separation (LLPS) controlled by Aurora-A phosphorylation (55).

Similar to the eukarotic cells, bacteria also utilize biomolecular condensates for cellular functions (45). One example is the ParABS system. Segregation of bacterial chromosomes and plasmid is driven by a tripartite system composed of two protein components (ParA and ParB) and a DNA-site (*parS*). Oligomerization of ParB is promoted by CTP *in vivo* and *in vitro*, and it results in LLPS forming ParB condensates. The condensate specifically binds to the *parS* to form a partition complex (56).

3.2 Biomolecular condensates and nucleic acids *in vivo*

Research in the past several years has made significant strides toward understanding the molecular mechanisms for the formation, regulation, and function of biomolecular condensates in the presence of nucleic acids. For example, in a cell nucleus, diverse RNA species, RNA-binding proteins, and DNA molecules are mixed and intertwined. The complex interactions among them can lead to the formation of specific condensates through LLPS, and cells employ the condensates to regulate multiple functions. Particularly, the role of phase separation in transcriptional regulation has been actively studied, as LLPS was shown to mediate the formation of *super-enhancers* (15, 57). Common transcription components, including the RNA polymerase II (pol II), can drive LLPS (58).

Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a prototypical transcriptional coactivator controlling context- and/or tissue-specific transcriptional networks. PGC-1α binding controls gene transcription by compartmentalizing multi-protein complex within a transcriptionally active liquid-like nuclear condensate (59).

Med15, one of the Mediator complex subunits that plays a major role in transcriptional activation, forms nuclear condensates that can promote enhancer-promoter interaction and gene activation within the mammalian cells. Both glutamine-rich intrinsically disordered regions and short downstream hydrophobic motifs are required to form Med15 nuclear

condensates, and both regions contribute to intracellular phase separation to assemble transcription coactivator condensates in the nucleus (60).

Heterogeneous nuclear ribonucleoproteins (hRNPs) are complexes of RNA and RNA-binding protein (RBP) in the nucleus, and they regulate major affairs of RNA biogenesis. hnRNPH1, one of the hRNP proteins, has two low-complexity (LC) domains, LC1, and LC2. The LC1 domain can form liquid droplets or amyloid-like fibrils through phase separation. This promotes the interaction between hnRNPH1 and other RBPs and plays a role in regulating splicing. In contrast, the LC2 domain did not induce phase separation but was associated with the transcription activation of hnRNPH1 when bound to the DNA binding domain (61).

The transcription apparatus assembles at the super-enhancers through LLPS to form transcriptional condensates. Super-enhancers, the high-density cluster of enhancers, regulate transcription at the promoters. The fate of transcriptional condensates depends on the amount of RNA produced at each stage of transcription. CTCF, CCCTC-binding factor, organizes mammalian genomes into 3D chromatin loop structures. These structures help the assembly of the transcription apparatus at specific sequences (YSPTSPS) to regulate the eukaryotes gene expression (62).

Although the DNA organization of the nuclear genomes has been studied extensively and the role of phase separation therein is being revealed, how the mitochondrial genome assemblies and functions remain unclear. One of the recent works compiled mitochondrial nucleic acids through *in vitro* reconstitution and revealed that mitochondrial transcription factor A (TFAM) goes through phase separation with mitochondrial DNA (mtDNA) to induce nucleic acid self-assembly. Furthermore, nucleoid droplet production promotes the recruitment of transcription machinery through co-phase separation that facilitates mtDNA transcription (63).

3.3 Molecular aspects of the biomolecular condensates

For a fuller understanding of biomolecular LLPS, we need to know how biomolecular LLPS works at the molecular level. Physical and chemical analysis for a controlled experiment *in silico* and *in vitro* is useful to dissect molecular principles. In this section, we list a few recent works that systematically study the molecular aspects of the biomolecular LLPS.

TDP-43 can be condensed by homomeric interactions with the helices at the C-terminal domain (CTD). Recently, phase separation of TDP-43 has been investigated *in vitro*, using CTD sequence variants. The mutants showed weaker binding affinity to another binding domain, while maintaining the binding and regulation of other RNAs. It was suggested that the homomeric interaction of the proteins selectively promotes condensation on the molecular scale (64).

α-synuclein undergoes LLPS in the presence of high protein concentrations (≥ 200 μM) and salts. The formation of amyloid fibrils through LLPS and subsequent liquid-solid phase transition was studied *in vitro* by controlling various experimental parameters, such as salt, protein concentrations, pH, surface, and polyvalent cations. N-terminal acetylation was shown to increase the critical concentration of phase separation and modulate the kinetics (65).

FUS proteins, one of the pathological proteins of amyotrophic lateral sclerosis (ALS), can specifically recognize and bind to G-quadruplex (G4)-DNA/RNAs. The interaction between FUS and G4-RNA can induce LLPS and subsequent liquid-to-solid transition of FUS. Ishiguro *et al*. found that ALS-linked FUS mutations lose the binding affinity to G4-RNA and show different fibrillation behaviors (66).

Equilibrium thermodynamics predicts that all similar biomolecular condensates will eventually merge into one single large droplet. However, we cannot observe this in our laboratories. This could be attributed to a longer timescale of merging than the experimental timescale, but there have been other proposals (67). Recently, it has been shown that cells use Pickering emulsions to maintain the average size of P granules. In the Pickering emulsions, solid particles can be adsorbed on the interface of water droplets, and this can prevent roughening of the emulsifier. Similarly, the dynamics of the P granules are regulated by nano-sized protein clusters adsorbed on the surface of the condensate (68).

The dense networks of chromatin around nuclear biomolecular condensates can suppress the growth of the condensates. In a recent *in silico* study, the dynamic process of phase separation promoting nucleolus formation in the existence of chromatin networks was modelled. The interactions between nucleoli and chromatin promote the coarsening of droplets due to coupled movements of the droplets and chromatin networks but hinder their coarsening (69).

3.4 Artificial biomolecular condensates

The biggest challenges in studying *in vivo* phase separation are the complexity and dynamic nature of the intracellular environment. One way to address these challenges is to use the artificial biomolecular condensate model and try to predict molecular interaction with the help of the polymer physics theories about LLPS. Most of these models have more specific, stable, and simple configurations than their *in vivo* counterparts, and they can be designed to reproduce phase behaviors in a more predictable way, which will help us dissect the effects of different factors systematically.

In a recent study, a new type of short peptide derivative that induces phase separation has been developed. The peptide consists of phenylalanine dipeptides connected by hydrophilic spacers. This spacer's disulphide binding allows dynamic control of the assembly through redox chemistry. In addition, they could functionalize the coacervates as a catalyst for aldol and hydrazone formation reaction (70).

Another study showed that side-chain amino acid-functionalized α-helical homo polypeptides can form droplet phases in aqueous media through a high degree of side-chain conformational disorder and hydration around the ordered backbone (71).

A DNA nano-star, the multivalent DNA assembly, was designed to study the relationship between intermolecular interactions and phase behaviour in biomolecular LLPS. The structural flexibility of the nano-stars affected attractive interactions between the sticky ends. It led to condensation and as a result, the phase diagram was highly modulated (72).

Sato and Takinoue used DNA nanostructures to induce lateral LLPS. If the DNA nanostructures are introduced to the oil-water system, the nanostructures localize at the oil-water interface. Depending on the DNA sequences, they could induce different patterns of two-dimensional phase separation. The phase separation of DNA nanostructures led to the formation of hydrogels (73).

3.5 LLPS and diseases

LLPS plays a significant role in many body processes. One of its implications is that the malfunction of LLPS can lead to a lethal disease. Particularly, the interplay between LLPS and fibrillation of pathological proteins for neurodegenerative diseases has attracted a lot of interest (16). Our understanding of the interplay may provide an insight into a new cure for neurodegenerative diseases, which are considered almost incurable for now.

In one of the recent studies, it was shown that disease-associated stop codon mutation of the prion protein (PrP) at tyrosine 145 (Y145Stop) produces a high disordered N-terminal IDR, and the high positive charge of this IDR sequence promotes intermolecular interactions, resulting in LPS. Promoted LLPS is predicted to cause disease by promoting protein fibrosis (74).

Cancer cells can utilize LLPS for its efficient function. They increase glycogen storage and cell turnover by lowering the regulation of glucose-6-phosphatase (G6PCs), which catalyzes glycogenolysis, to enhance survival. Amassed glycogen experiences a phase separation process to inhibit Hippo signaling, and glycogen phase separation triggers the assembly of the Laforin-Mst1/2 complex in the liquid droplets. By inactivating Mst1/2, Yap is activated for cell survival and growth. This blocks Hippo signaling and accelerates the development of liver tumors (75).

CONCLUSIONS

This review illustrates our current knowledge on how biomolecular LLPS works *in vitro* and *in vivo*. We highlight the importance and relevance of the LLPS concept in the formation of biomolecular condensates, and their role in various biological processes. We also take a glimpse of how we can design artificial LLPS systems. The recent adaptation of the concept of LLPS in biology has revealed many important cellular mechanisms. We are beginning to understand that this phenomenon plays an essential role in physiology and disease.

Although the experimental and computational data on specific LLPS systems have been rapidly accumulated, our understanding is yet far from complete. For example, it is still challenging to predict the *in vitro* phase behavior of a specific IDP purely based on its sequence. The role of LLPS in gelation, glass transition, and fibrillation is widely unexplored. The cellular environment is filled with numerous molecules of various types, whose actions

and interactions are far from equilibrium. How do crowding, heterogeneity and out-of-equilibrium thermodynamics change biomolecular phase behaviors in a cell? This question may address the similarities and differences between *in vitro* and *in cellulo* environments. There also remain uncharted domains of our knowledge on the biological and pathological consequences of LLPS or other phase behaviors of biomolecules. We anticipate that the next decade will witness a giant leap in the field of biomolecular LLPS, which will lead to our fuller understanding on living systems and its wider application to biomedical treatments.

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CONFLICTS OF INTEREST

The authors declare no competing conflicts of interest.

References

1. Harmon TS, Holehouse AS, Rosen MK and Pappu RV (2017) Intrinsically disordered linkers determine the interplay between phase separation and gelation in multivalent proteins. eLife 6, e30294
2. Shin Y and Brangwynne CP (2017) Liquid phase condensation in cell physiology and disease. Science 357, eaaf4382
3. Ryu J-K, Hwang D-E and Choi J-M (2021) Current Understanding of Molecular Phase Separation in Chromosomes. International Journal of Molecular Sciences 22, 10736
4. Banani SF, Rice AM, Peeples WB et al (2016) Compositional Control of Phase-Separated Cellular Bodies. Cell 166, 651-663
5. Feric M, Vaidya N, Harmon TS et al (2016) Coexisting Liquid Phases Underlie Nucleolar Subcompartments. Cell 165, 1686-1697
6. Banjade S and Rosen MK (2014) Phase transitions of multivalent proteins can promote clustering of membrane receptors. eLife 3, e04123
7. Kroschwald S and Alberti S (2017) Gel or Die: Phase Separation as a Survival Strategy. Cell 168, 947-948
8. Riback JA, Katanski CD, Kear-Scott JL et al (2017) Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response. Cell 168, 1028-1040.e1019

9. Molliex A, Temirov J, Lee J et al (2015) Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization. Cell 163, 123-133
10. Sheu-Gruttadauria J and MacRae IJ (2018) Phase Transitions in the Assembly and Function of Human miRISC. Cell 173, 946-957.e916
11. Xiao Q, McAtee CK and Su X (2022) Phase separation in immune signalling. Nature Reviews Immunology 22, 188-199
12. Wippich F, Bodenmiller B, Trajkovska Maria G, Wanka S, Aebersold R and Pelkmans L (2013) Dual Specificity Kinase DYRK3 Couples Stress Granule Condensation/Dissolution to mTORC1 Signaling. Cell 152, 791-805
13. Fujioka Y, Alam JM, Noshiro D et al (2020) Phase separation organizes the site of autophagosome formation. Nature 578, 301-305
14. Nicolas E, Parisot P, Pinto-Monteiro C, de Walque R, De Vleeschouwer C and Lafontaine DLJ (2016) Involvement of human ribosomal proteins in nucleolar structure and p53-dependent nucleolar stress. Nature Communications 7, 11390
15. Cho W-K, Spille J-H, Hecht M et al (2018) Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science 361, 412-415
16. Boyko S and Surewicz WK (2022) Tau liquid–liquid phase separation in neurodegenerative diseases. Trends in Cell Biology
17. Wang B, Zhang L, Dai T et al (2021) Liquid–liquid phase separation in human health and diseases. Signal Transduction and Targeted Therapy 6, 290
18. Alberti S and Dormann D (2019) Liquid–Liquid Phase Separation in Disease. Annual Review of Genetics 53, 171-194
19. Brangwynne Clifford P, Tompa P and Pappu Rohit V (2015) Polymer physics of intracellular phase transitions. Nature Physics 11, 899-904
20. Flory PJ (1942) Thermodynamics of High Polymer Solutions. The Journal of Chemical Physics 10, 51-61
21. Hyman AA, Weber CA and Jülicher F (2014) Liquid-Liquid Phase Separation in Biology. Annual Review of Cell and Developmental Biology 30, 39-58
22. Dolgin E (2018) What lava lamps and vinaigrette can teach us about cell biology. Nature 555, 300-303
23. Martin EW, Holehouse AS, Peran I et al (2020) Valence and patterning of aromatic residues determine the phase behavior of prion-like domains. Science 367, 694-699
24. Wang J, Choi J-M, Holehouse AS et al (2018) A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins. Cell 174, 688-699.e616
25. Rubinstein M and Semenov AN (1998) Thermoreversible Gelation in Solutions of Associating Polymers. 2. Linear Dynamics. Macromolecules 31, 1386-1397
26. Choi J-M, Hyman AA and Pappu RV (2020) Generalized models for bond percolation transitions of associative polymers. Physical Review E 102, 042403
27. Huggins ML (1942) Theory of Solutions of High Polymers1. Journal of the American Chemical Society 64, 1712-1719
28. Banani SF, Lee HO, Hyman AA and Rosen MK (2017) Biomolecular condensates: organizers of cellular biochemistry. Nature reviews. Molecular cell biology 18, 285-298
29. Choi J-M, Holehouse AS and Pappu RV (2020) Physical Principles Underlying the Complex Biology of Intracellular Phase Transitions. Annual Review of Biophysics 49, 107-133

30. Jia P, Li X, Wang X et al (2021) ZMYND8 mediated liquid condensates spatiotemporally decommission the latent super-enhancers during macrophage polarization. Nature Communications 12, 6535
31. Zhang H, Ji X, Li P et al (2020) Liquid-liquid phase separation in biology: mechanisms, physiological functions and human diseases. Science China Life Sciences 63, 953-985
32. Paloni M, Bailly R, Ciandrini L and Barducci A (2020) Unraveling Molecular Interactions in Liquid–Liquid Phase Separation of Disordered Proteins by Atomistic Simulations. The Journal of Physical Chemistry B 124, 9009-9016
33. Bremer A, Farag M, Borcherds WM et al (2022) Deciphering how naturally occurring sequence features impact the phase behaviours of disordered prion-like domains. Nature Chemistry 14, 196-207
34. Buchan JR and Parker R (2009) Eukaryotic stress granules: the ins and outs of translation. Molecular cell 36, 932-941
35. Fong K-w, Li Y, Wang W et al (2013) Whole-genome screening identifies proteins localized to distinct nuclear bodies. Journal of Cell Biology 203, 149-164
36. Song D, Jo Y, Choi J-M and Jung Y (2020) Client proximity enhancement inside cellular membrane-less compartments governed by client-compartment interactions. Nature communications 11, 1-13
37. Ruff KM, Dar F and Pappu RV (2021) Ligand effects on phase separation of multivalent macromolecules. Proceedings of the National Academy of Sciences 118
38. Rhine K, Vidaurre V and Myong S (2020) RNA Droplets. Annual Review of Biophysics 49, 247-265
39. Roden C and Gladfelter AS (2021) RNA contributions to the form and function of biomolecular condensates. Nature reviews. Molecular cell biology 22, 183-195
40. Wei M-T, Elbaum-Garfinkle S, Holehouse AS et al (2017) Phase behaviour of disordered proteins underlying low density and high permeability of liquid organelles. Nature chemistry 9, 1118-1125
41. Le Vay K, Song EY, Ghosh B, Tang T-YD and Mutschler H (2021) Enhanced Ribozyme-Catalyzed Recombination and Oligonucleotide Assembly in Peptide-RNA Condensates. Angewandte Chemie International Edition 60, 26096-26104
42. Grese ZR, Bastos AC, Mamede LD, French RL, Miller TM and Ayala YM (2021) Specific RNA interactions promote TDP-43 multivalent phase separation and maintain liquid properties. EMBO reports 22, e53632
43. Shao W, Bi X, Pan Y et al (2022) Phase separation of RNA-binding protein promotes polymerase binding and transcription. Nature Chemical Biology 18, 70-80
44. Li P, Banjade S, Cheng H-C et al (2012) Phase transitions in the assembly of multivalent signalling proteins. Nature 483, 336-340
45. Azaldegui CA, Vecchiarelli AG and Biteen JS (2021) The emergence of phase separation as an organizing principle in bacteria. Biophysical journal 120, 1123-1138
46. Fritsch AW, Diaz-Delgadillo AF, Adame-Arana O et al (2021) Local thermodynamics govern formation and dissolution of *Caenorhabditis elegans* P granule condensates. Proceedings of the National Academy of Sciences 118, e2102772118
47. Hong K, Song D and Jung Y (2020) Behavior control of membrane-less protein liquid condensates with metal ion-induced phase separation. Nature Communications 11, 5554
48. Zbinden A, Pérez-Berlanga M, De Rossi P and Polymenidou M (2020) Phase separation and neurodegenerative diseases: a disturbance in the force. Developmental cell 55, 45-68

49. Wang B, Zhang L, Dai T et al (2021) Liquid-liquid phase separation in human health and diseases. Signal transduction and targeted therapy 6, 290-290
50. Lednev IK (2014) Amyloid fibrils: The eighth wonder of the world in protein folding and aggregation. Biophysical journal 106, 1433-1435
51. Malki A, Teulon J-M, Camacho-Zarco AR et al (2022) Intrinsically Disordered Tardigrade Proteins Self-Assemble into Fibrous Gels in Response to Environmental Stress. Angewandte Chemie International Edition 61, e202109961
52. Shen C, Li R, Negro R et al (2021) Phase separation drives RNA virus-induced activation of the NLRP6 inflammasome. Cell 184, 5759-5774.e5720
53. Bergeron-Sandoval L-P, Kumar S, Heris HK et al (2021) Endocytic proteins with prion-like domains form viscoelastic condensates that enable membrane remodeling. Proceedings of the National Academy of Sciences 118, e2113789118
54. Zhu P, Lister C and Dean C (2021) Cold-induced Arabidopsis FRIGIDA nuclear condensates for FLC repression. Nature 599, 657-661
55. Sun M, Jia M, Ren H et al (2021) NuMA regulates mitotic spindle assembly, structural dynamics and function via phase separation. Nature Communications 12, 7157
56. Babl L, Giacomelli G, Ramm B, Gelmroth A-K, Bramkamp M and Schwille P (2022) CTP-controlled liquid–liquid phase separation of ParB. Journal of Molecular Biology 434, 167401
57. Hnisz D, Shrinivas K, Young RA, Chakraborty AK and Sharp PA (2017) A Phase Separation Model for Transcriptional Control. Cell 169, 13-23
58. Palacio M and Taatjes DJ (2022) Merging Established Mechanisms with New Insights: Condensates, Hubs, and the Regulation of RNA Polymerase II Transcription. Journal of Molecular Biology 434, 167216
59. Pérez-Schindler J, Kohl B, Schneider-Heieck K et al (2021) RNA-bound PGC-1α controls gene expression in liquid-like nuclear condensates. Proceedings of the National Academy of Sciences 118, e2105951118
60. Shi Y, Chen J, Zeng W-J et al (2021) Formation of nuclear condensates by the Mediator complex subunit Med15 in mammalian cells. BMC Biology 19, 245
61. Kim GH and Kwon I (2021) Distinct roles of hnRNPH1 low-complexity domains in splicing and transcription. Proceedings of the National Academy of Sciences 118, e2109668118
62. Lee R, Kang M-K, Kim Y-J et al (2022) CTCF-mediated chromatin looping provides a topological framework for the formation of phase-separated transcriptional condensates. Nucleic acids research 50, 207-226
63. Long Q, Zhou Y, Wu H et al (2021) Phase separation drives the self-assembly of mitochondrial nucleoids for transcriptional modulation. Nature Structural & Molecular Biology 28, 900-908
64. Hallegger M, Chakrabarti AM, Lee FC et al (2021) TDP-43 condensation properties specify its RNA-binding and regulatory repertoire. Cell 184, 4680-4696. e4622
65. Sawner AS, Ray S, Yadav P et al (2021) Modulating α-Synuclein Liquid–Liquid Phase Separation. Biochemistry 60, 3676-3696
66. Ishiguro A, Lu J, Ozawa D, Nagai Y and Ishihama A (2021) ALS-linked FUS mutations dysregulate G-quadruplex-dependent liquid–liquid phase separation and liquid-to-solid transition. Journal of Biological Chemistry 297, 101284
67. Wurtz JD and Lee CF (2018) Chemical-Reaction-Controlled Phase Separated Drops: Formation, Size Selection, and Coarsening. Physical Review Letters 120, 078102

68. Folkmann AW, Putnam A, Lee CF and Seydoux G (2021) Regulation of biomolecular condensates by interfacial protein clusters. Science 373, 1218-1224
69. Qi Y and Zhang B (2021) Chromatin network retards nucleoli coalescence. Nature Communications 12, 6824
70. Abbas M, Lipiński WP, Nakashima KK, Huck WTS and Spruijt E (2021) A short peptide synthon for liquid–liquid phase separation. Nature Chemistry 13, 1046-1054
71. Scott WA, Gharakhanian EG, Bell AG et al (2021) Active Controlled and Tunable Coacervation Using Side-Chain Functional α-Helical Homopolypeptides. Journal of the American Chemical Society 143, 18196-18203
72. Lee T, Do S, Lee JG, Kim D-N and Shin Y (2021) The flexibility-based modulation of DNA nanostar phase separation. Nanoscale 13, 17638-17647
73. Sato Y and Takinoue M (2022) Capsule-like DNA Hydrogels with Patterns Formed by Lateral Phase Separation of DNA Nanostructures. JACS Au 2, 159-168
74. Agarwal A, Rai SK, Avni A and Mukhopadhyay S (2021) An intrinsically disordered pathological prion variant Y145Stop converts into self-seeding amyloids via liquid-liquid phase separation. Proceedings of the National Academy of Sciences 118, e2100968118
75. Liu Q, Li J, Zhang W et al (2021) Glycogen accumulation and phase separation drives liver tumor initiation. Cell 184, 5559-5576.e5519

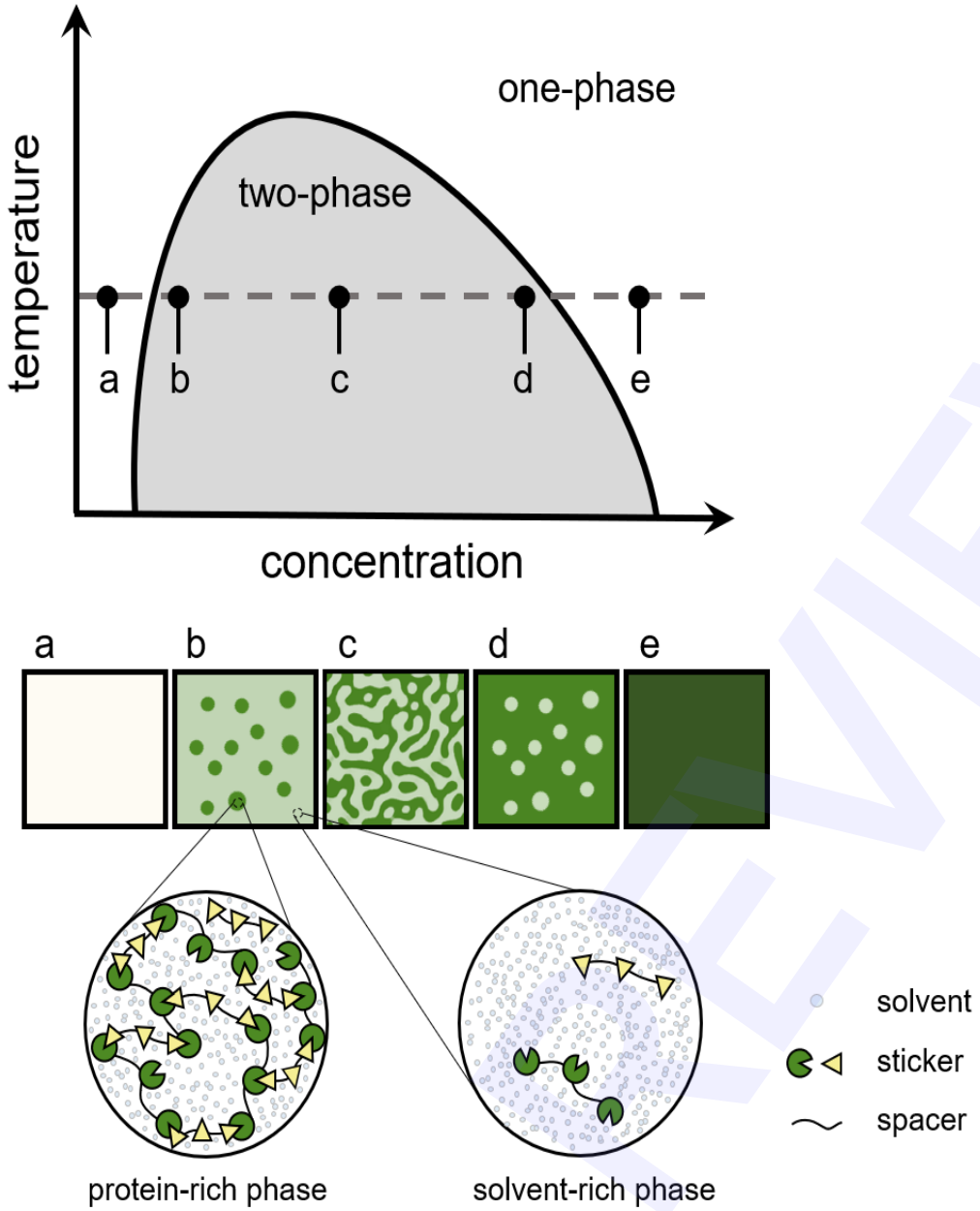

Fig. 1.

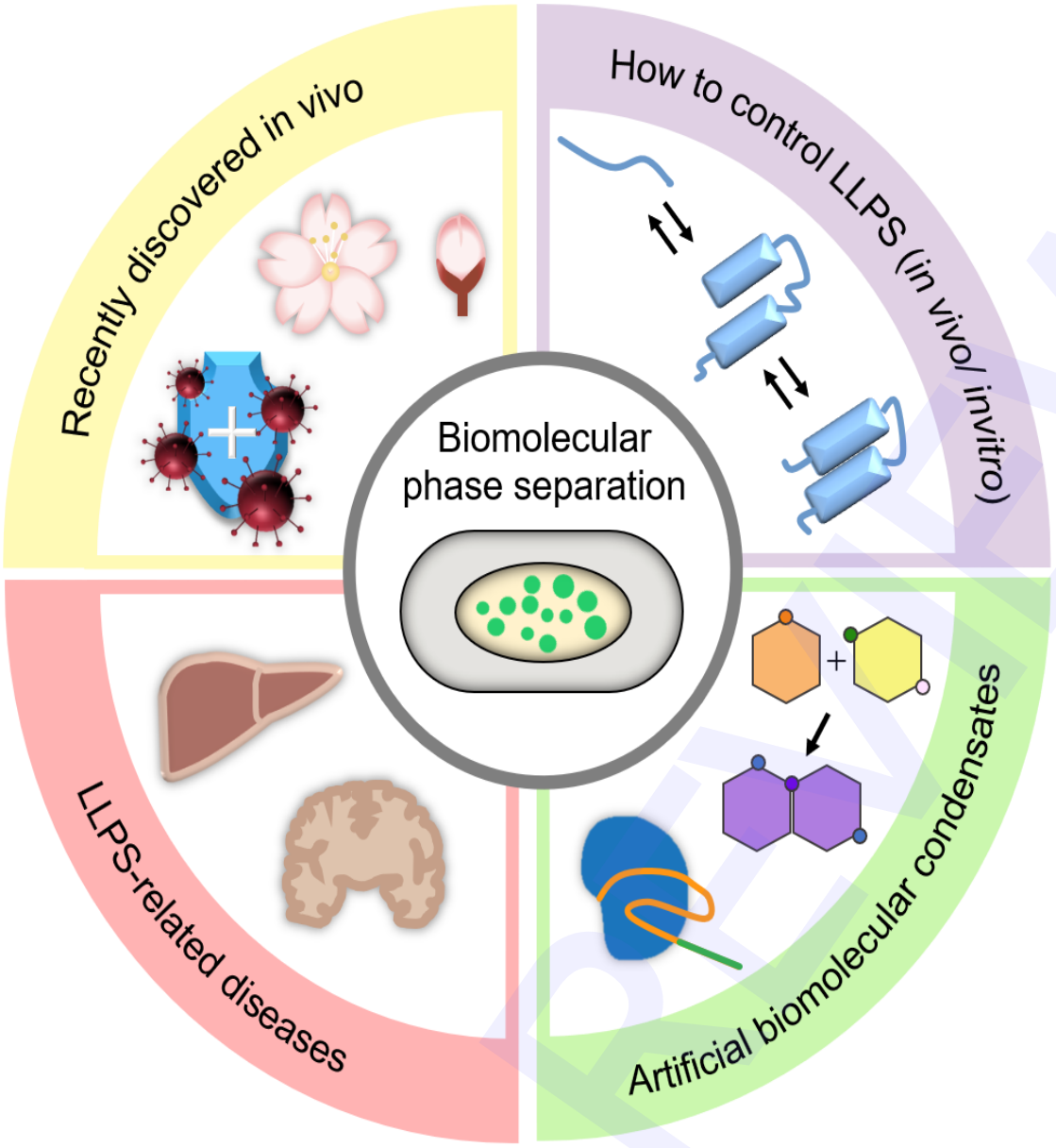

Fig. 2.