

Polymer physics of intracellular phase transitions

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Intracellular organelles are either membrane-bound vesicles or membrane-less compartments that are made up of proteins and RNA. These organelles play key biological roles, by compartmentalizing the cell to enable spatiotemporal control of biological reactions. Recent studies suggest that membrane-less intracellular compartments are multicomponent viscous liquid droplets that form via phase separation. Proteins that have an intrinsic tendency for being conformationally heterogeneous seem to be the main drivers of liquid–liquid phase separation in the cell. These findings highlight the relevance of classical concepts from the physics of polymeric phase transitions for understanding the assembly of intracellular membrane-less compartments. However, applying these concepts is challenging, given the heteropolymeric nature of protein sequences, the complex intracellular environment, and non-equilibrium features intrinsic to cells. This provides new opportunities for adapting established theories and for the emergence of new physics.

An essential aspect of biological function is the compartmentalization of biomolecules. The most well known biological compartment is the cell, and cellularization was undoubtedly crucial for the evolution of living matter. Cells themselves are further compartmentalized to achieve physical separation of different processes and components from one another. These compartments, known as organelles, are either membrane-bound vesicles or membrane-less structures that are involved in a variety of different biological functions.

Membrane-less organelles often contain both RNA and protein molecules, and are referred to as RNP bodies or granules. Examples include stress granules and processing bodies in the cytoplasm, as well as nucleoli, Cajal bodies, and PML bodies in the nucleus (Fig. 1). Other examples of membrane-less structures composed primarily of protein molecules include so-called inflammasomes¹, signalling complexes², purinosomes³, centrosomes⁴, postsynaptic density⁵ (PSD), and dynamic inclusion bodies such as JUNQ that contain soluble misfolded proteins⁶. Membrane-less subcellular structures are thus ubiquitous in cells, and contribute to numerous important biological functions, including the storage and processing of RNA and other biomolecules. There is also a growing appreciation of the relevance of these structures to pathological protein aggregates^{7,8}. Recent studies have focused on identifying the components, physical properties and mechanisms that lead to the assembly of these structures.

Membrane-less compartments are condensed liquids

An increasing body of work suggests that membrane-less organelles are multicomponent, viscous liquid-like structures that form via spontaneous phase transitions. These transitions occur on molecular supersaturation, which is achieved through regulated changes in protein/RNA concentration (gene expression), charge state (post-translational modifications), and changes to salt/proton concentration and/or temperature (osmotic or pH shocks).

Key insights into the role of phase transitions have come from the study of *Caenorhabditis elegans* P granules—RNA/protein-rich bodies that are implicated in specifying and protecting the fate of the worm's germ cells. Like many other RNP bodies, P granules have roughly spherical shapes. They fuse into larger droplets when

they come into contact with one another and they flow in response to applied shear stresses, exhibiting classic wetting and dripping behaviours that are the hallmarks of liquid-like behaviour⁹. During the first asymmetric cell division, gradients in several key molecules are established across the anterior–posterior axis of the embryo. These gradients control the saturation concentration of P granule molecules, and lead to preferential droplet condensation in the posterior of the embryo^{9,10}. The assembly and material properties of P granules seem to be tunable by RNA, salt concentration and the presence of other molecules, which may be important for RNA processing functions^{11,12}.

The nucleus contains many different types of RNP bodies that share similarities with P granules. The most prominent nuclear body is the nucleolus, which assembles at specific nuclear loci defined by transcriptionally active repeats of ribosomal DNA (rDNA) genes. Work in *Xenopus laevis* oocytes has revealed that these structures are liquid-like droplets that are roughly four orders of magnitude more viscous than water¹³. Large oocytes contain hundreds of these and other RNP bodies, representing an emulsion of liquid-phase organelles, which are stabilized against gravitational sedimentation and coarsening by a filamentous actin scaffold¹⁴. The *C. elegans* embryo was recently exploited to manipulate the concentration of nucleolar components, revealing that nucleoli assemble only when the concentrations of certain protein components cross threshold values^{15,16}.

A variety of other intracellular bodies also appear to represent liquid-phase droplets, including other RNP bodies and signalling complexes^{17–21}. The liquid-like behaviour of these bodies can be recapitulated *in vitro* using purified protein constructs. These approaches have begun to help uncover the key features of the molecular components that drive intracellular phase separation^{11,19,22–24}.

Molecular drivers of intracellular phase transitions

Intracellular bodies are multicomponent entities, typically containing dozens of different types of proteins and RNA. However, *in vitro* it is possible to reconstitute droplets that are similar to native intracellular bodies, often using only one or two components. This suggests that for some intracellular bodies a

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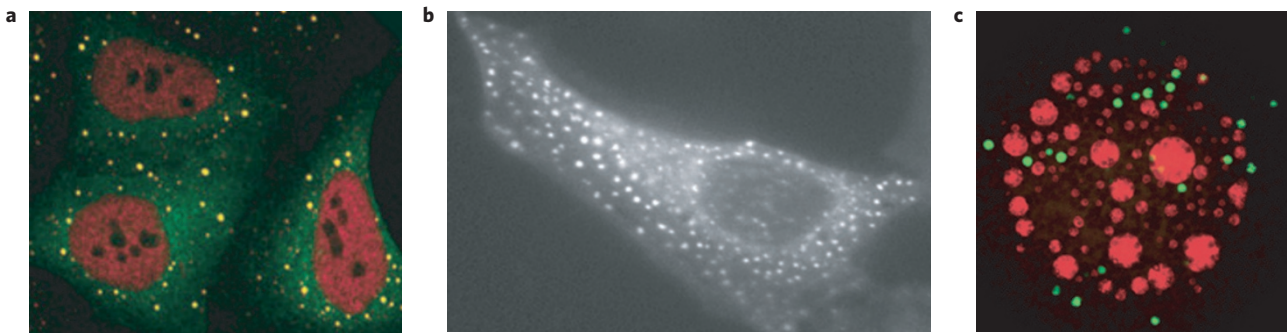


Figure 1 | Examples of membrane-less bodies in cells. a, P bodies (yellow) in tissue culture cells (adapted from ref. 63, NPG). **b,** Purinosomes (adapted from ref. 3, AAAS). **c,** Nucleoli (red) and histone locus bodies (green) in the nucleus of a large *X. laevis* oocyte (adapted from ref. 14, NPG).

single protein may be necessary and sufficient to drive assembly. Many of the components of these bodies contain repeats of weakly binding interaction domains, which may bind to either RNA or complementary binding partners on other proteins. Recent work on synthetic systems consisting of repeats of two interaction domains has shown that these biopolymers are sufficient to drive phase separation *in vitro*, and may also phase separate *in vivo*^{19,25}. Many signalling molecules within cells also exhibit such repetitive interaction domains, and it is likely that phase separation driven by these ‘multivalent’ motifs is important for organizing intracellular signalling networks.

Many of the proteins that drive intracellular phase transitions exhibit significant conformational heterogeneity and are referred to collectively as intrinsically disordered proteins/regions (IDPs/IDRs; refs 26,27). The biased amino acid sequences of IDPs encode an intrinsic preference for conformational disorder, and an inability to fold into singular well-defined three-dimensional structures under physiological conditions. As an example, numerous P granule proteins contain long stretches that are predicted to be disordered²⁸. These include MEG proteins that promote assembly of P granules depending on whether they are phosphorylated or unphosphorylated²⁹. LAF-1 is another P granule protein that contains an N-terminal disordered domain which is necessary and sufficient for droplet formation *in vitro*¹¹.

IDPs that drive phase transitions are typically low-complexity sequences, characterized by long stretches with low overall diversity of amino acids²⁸. The sequences are often repetitive and are enriched in polar sidechains glycine (G), glutamine (Q), asparagine (N) and serine (S), positively charged sidechains arginine (R) and lysine (K), negatively charged sidechains aspartic acid (D) and glutamic acid (E), or aromatic sidechains phenylalanine (F) and tyrosine (Y). The sequences of interest often encompass multiple short motifs such as YG/S-, FG-, RG-, GY-, KSPEA-, SY- and Q/N-rich regions, and blocks of alternating charges (Fig. 2). Ongoing studies are beginning to elucidate the physics behind how these motifs drive phase separation in cells.

Towards a physics of intracellular phase separation

The polypeptide backbone, common to all sequences, is essentially a chain of dipoles. Aqueous solvents are also dipolar, and thus three types of dipolar interactions are possible: peptide–solvent, peptide–peptide and solvent–solvent. For the simplest homopolymers, the tendency to undergo phase separation into polymer-rich and solvent-rich phases is described by the Flory interaction parameter, χ , which quantifies the competition among these three types of interactions (see Box 1). The net attraction between peptide dipoles makes water a poor solvent ($\chi > 0$) for polypeptide backbones, giving rise to chain compaction in dilute solutions and bulk phase separation at higher concentrations³⁰. The ‘chain of dipoles’ picture can be extended to Q/N/S-rich sequences that are deficient in other

types of amino acids. These sequences also form compact globules in dilute solutions^{31–33} and they separate into soluble and insoluble phases at low micromolar concentrations³⁴.

The conceptual framework for chains of interacting dipoles can be captured in a mean-field model with suitable parameterization of χ . However, this basic framework needs to be generalized to explain the phase behaviour of sequences that are enriched in charged and/or aromatic sidechains. Sequences of several IDPs—including LAF-1 (ref. 11), the related germ granule protein DDX4 (ref. 22), the fungal septal pore protein SPA5 (ref. 35), and neurofilament sidearms³⁶—highlight the role of electrostatic interactions in driving phase separation. In these and other systems, complementary electrostatic interactions between blocks of oppositely charged residues seem to enhance the driving forces for phase separation through long-range interchain attractions. Developing a fundamental understanding of these driving forces requires building on theoretical approaches developed for describing the phase separation of polyelectrolytes, such as that of Overbeek and Voorn (Box 1). The adaptation of this theory and its generalization require careful consideration of the patterning of oppositely charged residues within linear sequences and the correlated fluctuations they encode.

Recent work has begun to probe the impact of charge patterning on the conformational properties of IDPs and the driving forces for phase separation^{22,37}. Nott *et al.* uncovered the contributions to phase separation that derive from short-range, directional interactions between positively charged arginine residues and aromatic residues within and between DDX4 molecules. Short-range directional interactions involving aromatic groups are also likely to be important in repetitive FG motifs within the cohesive proteins that form the permeability barrier of the nuclear pore complex³⁸. Overall, there seems to be a hierarchical interplay between the different types of interactions: electrostatic interactions between charged motifs promote long-range interactions, with additional specificity achieved through short-range, directional interactions of dipoles (G, Q, N, S) or positive charges (R) with aromatic groups (F and Y). Elucidating this interplay will lead to a molecular biophysical understanding of the sequences that drive the formation of intracellular liquid phases.

Metastable liquids and pathological solids

Intriguingly, many of the amino acids and sequence elements enriched in the IDPs that drive liquid–liquid demixing in cells are also common in proteins that are known to cause pathological protein aggregation. For example, Huntington’s disease is caused by genetic mutations that lead to aggregation of peptides with expanded polyQ tracts. Similarly, low-complexity Q/N-rich sequences are prevalent in prion aggregation³⁹ and amyotrophic lateral sclerosis (ALS; ref. 40). These pathologies are associated with solid-like inclusions comprised of semi-crystalline

Box 1 | Essential physics of polymer phase separation.

Consider homopolymers of length N in a low molecular weight solvent. The volume fraction of the polymer is denoted as ϕ_p and that of the solvent is ϕ_s . As $\phi_p + \phi_s = 1$, it follows that $\phi_s = 1 - \phi$ if we denote ϕ_p as ϕ . The solution is modelled as an infinite lattice with coordination number z . Each lattice can be occupied by either a solvent molecule or a polymer bead. The Flory–Huggins free energy of mixing per lattice site can then be derived using a mean-field assumption^{64,65}:

$$\frac{F}{k_B T} = \frac{\phi}{N} \ln \phi + (1 - \phi) \ln(1 - \phi) + \chi \phi(1 - \phi) \quad (1)$$

In equation (1), the first two terms represent the mean-field entropy of mixing per lattice site and the third term represents the energy of mixing per lattice site. The Flory χ parameter quantifies the balance between chain–chain and chain–solvent interactions, and is written as:

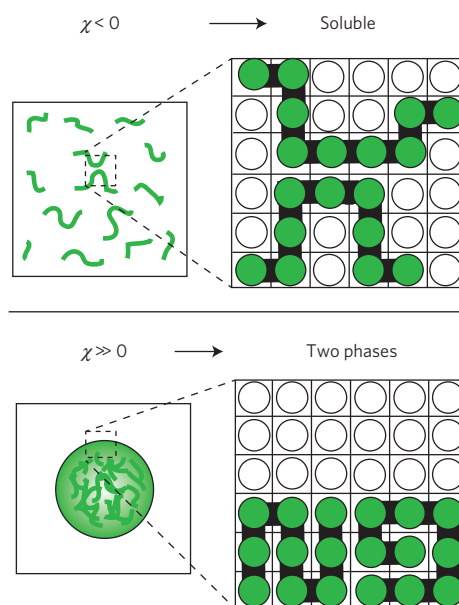
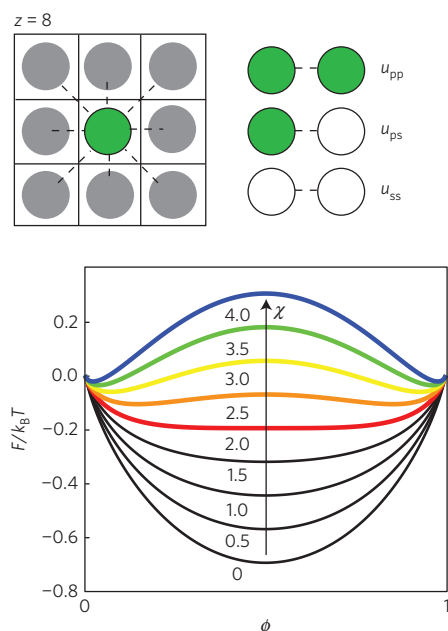
$$\chi = \frac{z}{k_B T} \left[u_{ps} - \frac{1}{2}(u_{pp} + u_{ss}) \right] \quad (2)$$

Here, u_{ps} , u_{pp} and u_{ss} are the mean-field energies per site that quantify the strengths of interactions between polymer and solvent units, pairs of polymer units, and pairs of solvent units, respectively (see above diagram). The magnitude of χ quantifies the energetic cost of having sites adjacent to the polymer site being occupied by solvent particles—the higher the magnitude of χ , the larger this cost. For $\chi > 0$, the polymer is in a ‘poor solvent’, favouring phase separation. Above a critical value of χ , the energetic term will overcome the mixing entropy, such that the free energy of mixing has a region of negative curvature (coloured curves in the above diagram). The resultant instability is resolved by phase separation: one phase deficient in polymers that coexists with a polymer-rich phase. It has been established that aqueous solutions are poor solvents ($\chi > 0$) for archetypal IDPs that are rich in polar amino acids such as G, Q, N and S (ref. 66). Despite its simplicity, this mean-field model has been widely utilized for

synthetic polymeric systems, and increasingly as a framework for understanding intracellular phase behaviour^{10,16,22}. Overbeek and Voorn extended the Flory–Huggins formalism to describe phase separation in the presence of electrostatic interactions⁶⁷. Consider a system comprised of polycations of length N and valence Z^+ and polyanions of length N and valence Z^- , such that $Z^+ = Z^-$. Both polyions have similar molecular volumes and are dissolved in a 1:1 aqueous electrolyte. The term involving χ is neglected on the grounds that the energy scales for long-range electrostatic interactions are at least an order of magnitude stronger than shorter-range interactions between neutral groups. The pseudo-ternary system may be treated as an effectively binary system of polymers of volume fraction $\phi = \phi_+ + \phi_-$ in a solvent. The mean-field free energy per lattice site is written as:

$$\frac{F}{k_B T} = \frac{\phi}{N} \ln \frac{\phi}{2} + (1 - \phi) \ln(1 - \phi) - \alpha(\sigma\phi)^{3/2} \quad (3)$$

In equation (3), the electrostatic effects are described using a Debye–Hückel theory for the screened interactions between charges of the polyions. The parameter α is determined by the charge per site, thermal energy $k_B T$, and the partial molar volume of the solvent. The parameter σ denotes the linear charge density on each polyion, which is crucial for phase separation. To a reasonable approximation, the critical point in the Overbeek–Voorn mean-field theory satisfies the condition $\sigma^3 N \approx 0.5$. If $\sigma^3 N$ exceeds this threshold, then the mixture of polyions will undergo phase separation into a dilute phase and a dense droplet phase of complexed polyions; this type of phase separation is referred to as complex coacervation. Although the mean-field description of complex coacervation provides a useful starting framework, a generalization of these concepts is called for in light of the sequence features that presumably drive phase separations via correlated fluctuations and the patterning of charged residues within polyampholytic IDPs (see Fig. 2).



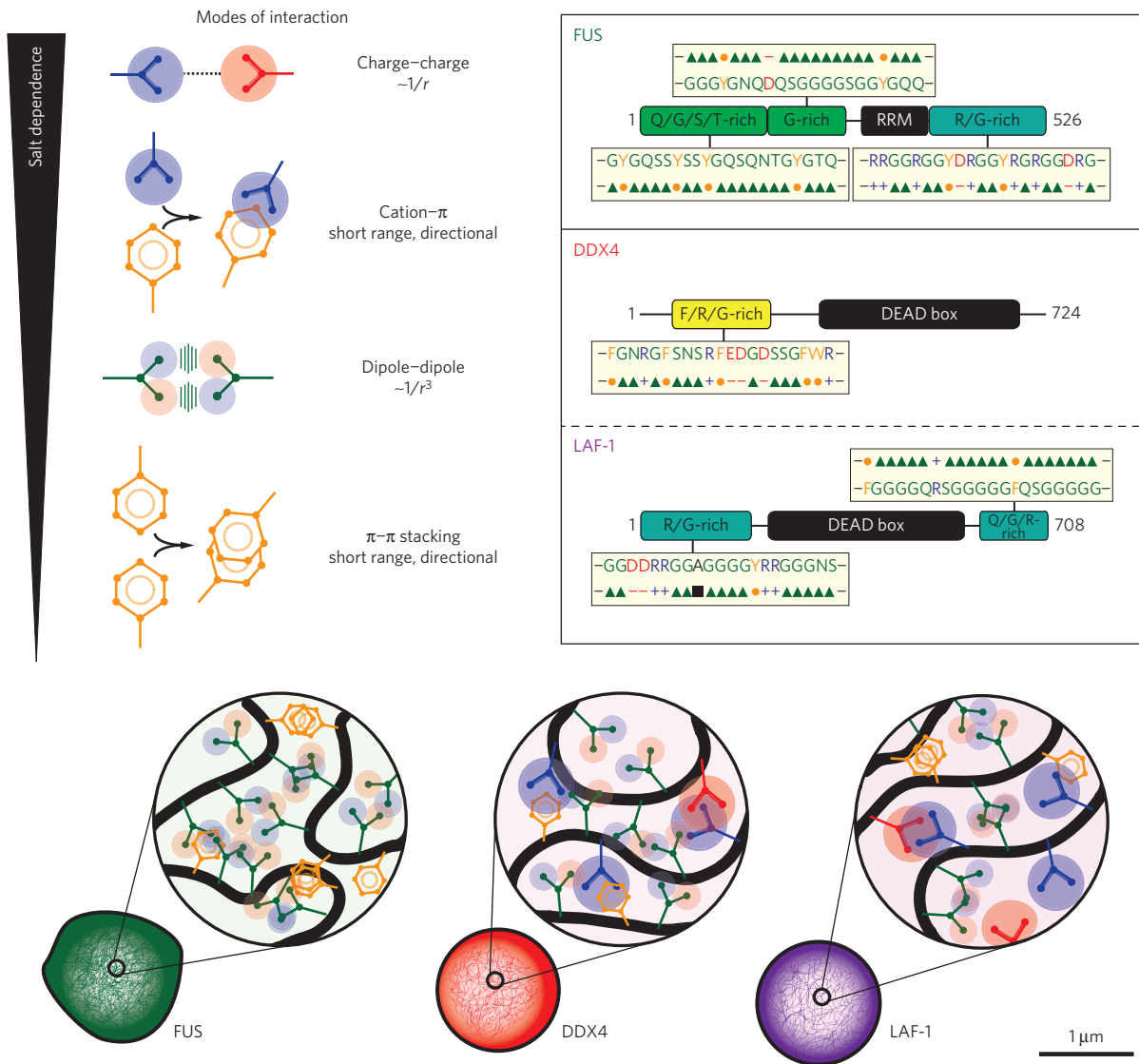


Figure 2 | Molecular interactions underlying intracellular phase transitions. The three panels at the top right show the domain architectures for FUS, DDX4 and LAF-1. In each panel, the numbers shown to the right of the domain architecture denote the lengths of the corresponding proteins. The amino acid biases within low-complexity regions of each protein are highlighted in square boxes that show portions of the actual amino acid sequence and annotation of the sequence stretch using symbols to denote dipoles (triangles), charges (+ or -), aromatic groups (circles) and aliphatic groups (squares). For the sequences of DDX4 and LAF-1, the sequence patterning ensures the linear segregation of oppositely charged residues. The panel to the top left shows schematics of the hierarchical modes of interactions that are likely to drive the phase separation of different sequences. This schematic is annotated to depict the types of interactions, their ranges, and their expected salt dependencies. The bottom panel shows schematic representations of micron-sized droplets formed by each of the three proteins. The insets for each droplet depict the dominant interactions that are expected to prevail among molecules that make up the dense liquid. The contours of individual polypeptide backbones are shown in black. In all of the droplets the interactions are likely to involve interplay among electrostatic, dipolar and short-range directional interactions involving aromatic groups.

amyloid fibres. Given the recent findings regarding the sequence determinants of intracellular liquid phases, the presence of similar compositional biases in proteins that form pathological solids is surprising.

Hints regarding the physics that links physiological liquids with pathological solids come from recent *in vitro* studies on polyQ peptides, which show that, as the total concentration of polymers increases beyond a threshold value, spherical liquid-like assemblies of 10–50 nm in diameter condense from solution³⁴. However, as the total concentration increases beyond a second threshold, a new two-phase equilibrium is established between soluble species and insoluble, amyloid fibrils. This concentration-dependent phase behaviour of Q-rich molecules might shed light on why the ALS proteins FUS, hnRNPA1 and hnRNPA2, under high-concentration

and low-temperature conditions, can form hydrogels comprised of amyloid-like fibres^{41–43}. Indeed, several recent studies have revealed that both FUS and hnRNPA1 form liquid droplets at moderate concentrations, but these droplets can nucleate amyloid-like fibres and begin to gel over time, a process accelerated when the protein exhibits disease-associated mutations^{44–46}. A similar time-dependent droplet gelation was recently described for the Q-rich protein Whi3, whose assembly into droplet-like structures may be important for regulating asynchronous cell divisions and branching in fungi⁴⁷. These observations are reminiscent of the metastable precursors which precede the formation of haemoglobin fibres that cause pathological sickle-shaped red blood cells⁴⁸, and are seen in supersaturated solutions of crystallizing proteins⁴⁹. These liquid-like mesophases are consistent with the two-stage nucleation model

of ten Wolde and Frenkel⁵⁰, according to which condensed liquid droplets serve as a metastable crucible to lower free energy barriers for crystal nucleation.

Non-equilibrium tuning

Given the commonly observed metastability of the liquid state, it is possible that cells utilize energy sources to actively prevent pathological fibril formation and gelation. Living cells are out of equilibrium, and equilibrium for biological systems may be synonymous with death. As the molecular ‘battery’ of the cell, ATP plays a central role in keeping cells out of equilibrium, and could potentially be used to control the fluidity of intracellular liquids. Indeed, experiments have suggested that ATP-dependent processes can modulate the properties of nucleoli¹³, P granules¹² and stress granules⁵¹, suggesting that these droplets may represent ‘active liquids’. Consistent with this, a large number of ATP-dependent protein chaperones, disaggregases, and RNA helicases are known to actively control the composition and three-dimensional architecture of assemblies of RNA and protein^{12,51,52}. Moreover, the activity of such ATPases is known to be important for combating the protein aggregation pathologies associated with ALS, Alzheimer’s and other neurodegenerative diseases⁵³.

These findings are interesting in light of studies connecting metabolic processes to cytoplasmic states in bacteria^{54,55}, and the role of force fluctuations as enhancers of protein diffusion and fluidity in the cytoplasm of mammalian cells^{56,57}. Theoretical approaches from soft glassy rheology⁵⁸ or molecular glasses⁵⁹ may shed light on how active processes regulate the properties of condensed RNA/protein liquids. Of particular interest is the concept of biasing ‘suppressor fields’ (s-fields) that tune molecular motions in glasses. A positive s-field suppresses molecular motions, whereas a negative s-field enhances these motions⁶⁰. This formalism has been useful for understanding the dynamics of protein folding, with implications for protein aggregation⁶¹. Active processes could potentially play the role of negative biasing s-fields, to enhance the motions of specific macromolecular components within membrane-less bodies.

Another aspect of the out-of-equilibrium nature of living cells is their ability to dynamically tune the strength of intermolecular interactions. These interactions can be regulated through post-translational modifications, including phosphorylation of S, T, or Y residues, citrullination of R residues, and methylation of R or K residues. These modifications can either directly generate binding-competent recognition motifs¹⁹, or increase or decrease the net charge of the IDP above or below the critical threshold²²—see Box 1. Of relevance for nuclear bodies is the role of transcription, which generates RNA polymers at particular nuclear loci. Increasing the concentration of RNA at a transcriptionally active gene can shift the phase boundary for nucleolar assembly, causing localization by enhanced ripening at the expense of other droplets¹⁶. This is consistent with the dependence of nuclear FUS puncta on transcriptional activity⁴⁴.

In some cases, a local equilibrium may exist, as seems to be the case in P granule segregation. Here, an active process establishes a concentration gradient across the entire embryo, but locally the system exhibits an apparent equilibrium two-phase coexistence between the condensed liquid phase and the dispersed phase^{10,62}. However, some intracellular bodies may reflect non-equilibrium steady states, as has been suggested for centrosomes⁴. These ideas are particularly interesting, because coupling the assembly of RNA/protein bodies with reaction–diffusion mechanisms bridges emerging concepts of intracellular phase separation with the formalism of Turing-type biological patterning.

Future challenges

One of the key challenges for the future is to understand how and why only a subset of protein sequences promotes phase

transitions? How does the interplay between sequence complexity, conformational heterogeneity, and charge patterning enable phase separation within cells? Given a multicomponent body, how do we discern which components are necessary and sufficient for generating the phase transition and which components partition into the bodies in a signal/stress-dependent manner? Answers to these questions will require diverse structural and functional *in vitro/in vivo* studies, combined with advancements in molecular-level theory/simulation and systematic coarse graining approaches.

A second and related theme for future work concerns the mesoscopic properties—size, shape, viscoelasticity—of condensed phases in physiology and disease. The size and number of membrane-less organelles will strongly impact the residence time of component reactants, but how droplet nucleation and growth are controlled remains largely unknown. Moreover, the sequence features, substructures, conformational and mesoscale dynamics that determine the viscoelasticity of intracellular bodies also remain unknown. The dynamics and fluidity of condensed phases presumably contributes to biological function, but how mesoscale viscoelasticity is linked to reactivity and storage functions remains to be solved. And, finally, is there a unifying framework that links changes to the mesoscopic state of condensed phases—for example, amyloid fibre formation and gelation within metastable liquid phases—with the onset of protein aggregation pathologies? Answering these key questions is likely to illuminate both the function and dysfunction of intracellular bodies.

In addressing these issues, it is also important to recognize the complexity of the intracellular environment, which is densely crowded and intrinsically out of equilibrium. The assembly and properties of intracellular bodies seems to be strongly influenced by non-equilibrium activity. How is such activity controlled to tune assembly and mesoscale properties? Given the role intracellular bodies play in a wide variety of biological process, addressing these questions will open up an important new frontier for soft-matter physics in the cell.

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Additional information

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Competing financial interests

The authors declare no competing financial interests.