Biomolecular condensates: organizers of cellular biochemistry

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Abstract | Biomolecular condensates are micron-scale compartments in eukaryotic cells that lack surrounding membranes but function to concentrate proteins and nucleic acids. These condensates are involved in diverse processes, including RNA metabolism, ribosome biogenesis, the DNA damage response and signal transduction. Recent studies have shown that liquid–liquid phase separation driven by multivalent macromolecular interactions is an important organizing principle for biomolecular condensates. With this physical framework, it is now possible to explain how the assembly, composition, physical properties and biochemical and cellular functions of these important structures are regulated.

Cajal bodies
Biomolecular condensates in eukaryotic nuclei containing coilin and survival motor neuron protein (SMN) as well as many factors involved in mRNA splicing. Cajal bodies are thought to have a role in assembling spliceosomal small nuclear ribonucleoproteins.

PML nuclear bodies
Biomolecular condensates in eukaryotic nuclei containing promyelocytic leukaemia (PML), death domain-associated protein (DAXX) and Sp100. PML nuclear bodies are thought to have a role in apoptotic signalling, antiviral defence and transcriptional regulation.

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A fundamental problem in cell biology is how the densely packed cellular space is organized to enable control over complex biochemical reactions in space and time. One way to achieve spatiotemporal control is to regulate the localization of reaction components: concentrating components together can increase reaction kinetics, whereas segregating them can slow or inhibit reactions. These differences can alter flux through specific pathways and protect cells from damaging activities such as proteolysis, inappropriate covalent modifications and effects of low pH. Indeed, *in vivo* enzymatic reaction components are often packaged within distinct subcellular compartments.

Classic organelles, such as the endoplasmic reticulum or Golgi apparatus, are compartments defined by surrounding lipid bilayer membranes. These membranes are impermeable to most biological molecules. Thus, the interior and exterior of classic organelles are physically separated, and organelle compositions are regulated through specialized membrane transport machineries.

However, many cellular compartments are not bound by membranes (FIG. 1a). Examples include RNA–protein granules such as nucleoli, Cajal bodies and PML nuclear bodies in the nucleus[1], as well as stress granules and germ granules in the cytoplasm[2,3]. Clusters of signalling molecules at membranes can be viewed in a similar light. These micron-scale structures are all defined by their ability to concentrate proteins and nucleic acids at discrete cellular sites. As these cellular compartments lack a physical barrier to separate their internal components from the surrounding medium, it remained elusive for many years how they concentrate molecules, maintain and regulate their structures, control their compositions and modulate internal biochemical activities.

In this Review, we discuss cellular and biochemical observations that have led to a new physical model of membraneless compartments based on principles of polymer chemistry and soft matter physics. This model unites many of the observed behaviours of membraneless compartments, both membrane-associated molecular clusters and cytoplasmic or nuclear structures, under a common framework. We discuss how the model explains many aspects of the assembly and dissolution, composition and function of membraneless compartments. We suggest mechanisms by which these features can be regulated in cells. Finally, we conclude with a series of major open questions in this exciting area of biology.

Phase-separated liquid compartments

The first membraneless compartment was observed within the nucleus of neuronal cells in the 1830s and was later termed the nucleolus[4]. Since then, many such compartments have been discovered in the nucleus, cytoplasm and on membranes of essentially all eukaryotic cells. High-resolution microscopy imaging and descriptions of their molecular components have revealed similarities in their shape, dynamics and manner of assembly, despite differences in their composition, location and function. Each type of membraneless compartment contains many molecular components. These components can remain stably concentrated within the structures for hours to days; however, decades of photobleaching recovery experiments consistently showed that many of these compartments could exchange with the surrounding medium on time-scales of seconds to minutes[5–7]. These compartments also displayed unexpected behaviours, such as two of the same type fusing upon contact[8–14] (Supplementary information S1–S4 (movies)). Until recently, it remained unclear

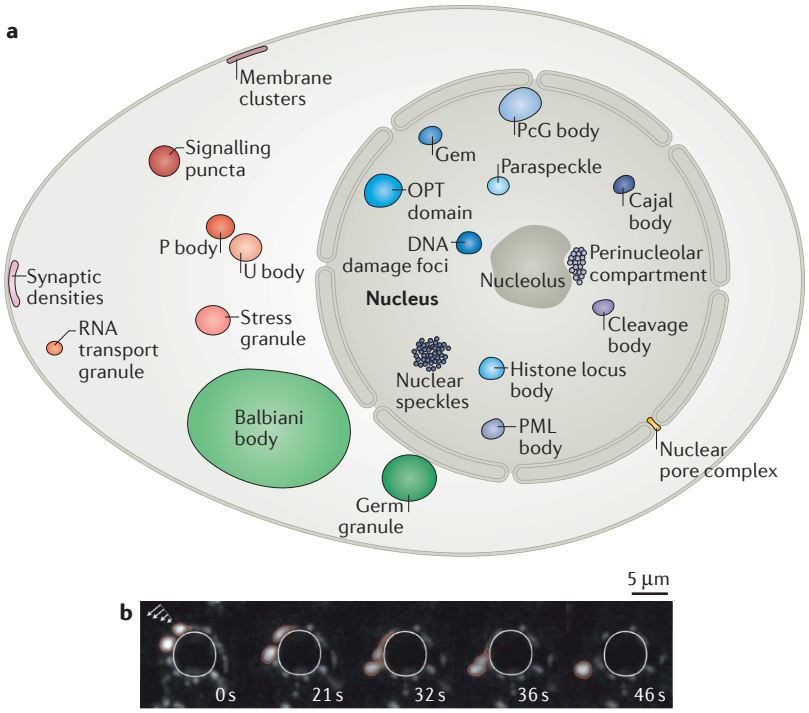


Figure 1 | **Biomolecular condensates in eukaryotic cells. a** | Schematic of the numerous condensates in the nucleus, cytoplasm and membranes of eukaryotic cells. Some compartments occur only in specific cell types, but are shown here for completeness. For example, Balbiani bodies and germ granules are specific to germ cells (green hues), and RNA transport granules and synaptic densities are specific to neuronal cell types (pink hues). See Supplementary information S6 (table) for more information on individual condensates. **b** | *Caenorhabditis elegans* germ granules, P granules, are perinuclear condensates that behave like liquids. A montage of live time-lapse imaging of P granules under shear force (arrows, top left). P granules deform, drip and fuse with one another around a nucleus (circular structure in the middle outlined in white; time points (in seconds): 0, 21, 32, 36 and 46. See also Supplementary information S1–S4 (movies). Gem, Gemini of Cajal body; OPT, OCT1/PTF/transcription; PcG, Polycomb group; PML, promyelocytic leukaemia. From REF. 14 (Brangwynne, C. P. *et al.* Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science **324**, 1729–1732 (2009). Modified with permission from the AAAS.

how these properties could be explained in physical and molecular terms.

An important clue towards understanding the physical processes that drive the formation of membraneless compartments came from the discovery that P granules in germ cells of *Caenorhabditis elegans* are liquid-like. P granules are perinuclear membraneless compartments composed of many proteins and RNAs (FIG. 1b). The relatively large size of P granules (2–4 µm diameter) compared with most other cellular bodies (200–1,000 nm diameter) enabled quantitative analysis of their formation. P granules fuse with one another[14] and subsequently relax back into a spherical shape (FIG. 1b; Supplementary information S1 (movie)). Photobleaching experiments demonstrated that proteins are highly mobile within P granules and exchange rapidly with the surrounding cytoplasm[14]. Crucially, under shear force, P granules can freely flow and deform around surfaces of other structures as well as undergo fission[14]. Together, these observations suggested that P granules are liquids that form through liquid–liquid demixing (phase separation) from the surrounding cytoplasm (see next section). The concept of phase separation suggested how P granules could segregate asymmetrically during the first cell division in *C. elegans* embryos[15]. We note that such phase-separated structures are distinct in physical properties and functions from canonical macromolecular assemblies (such as ribosomes; for details see Supplementary information S5 (box)). Since this study of P granules, other compartments, such as nucleoli[13], DNA damage repair sites[16,17] and stress granules[16], were also shown to exhibit liquid-like properties, highlighting the possibility that phase separation is a common mechanism by which membraneless compartments form[18]. As detailed below, principles of phase separation can indeed explain the formation of such structures with diverse material properties as well as the complex organization of such structures (organization into subcompartments, for example). The presence of a phase boundary explains how molecules can be concentrated in one place in a cell without a surrounding membrane but still provide an environment suitable for cellular biochemistry that depends on rapid diffusion. Phase separation also provides a unifying principle that explains the formation of membraneless compartments from diverse types of molecule.

Non-membrane-bound compartments are highly diverse in their physical properties, dimensionality (membrane-associated or soluble), molecular composition, subcellular location and functions. Throughout the years, these compartments have been referred to by various names, including cellular bodies, nuclear bodies, membraneless organelles, granules, speckles, aggregates, assemblages and membrane puncta. Here, we propose a new name — biomolecular condensates — which emphasizes the two features common to all of the structures: their ability to concentrate molecules and that they comprise biological molecules, independent of all other characteristics. We apply this name to both membrane-associated structures and various non-membrane-bound organelles and granules because we believe that these structures are formed through similar mechanisms. The term also provides a link to concepts in condensed matter physics[19], which, as we discuss below, are important in understanding the formation of these structures.

Multivalency-driven phase separation

Molecules will be miscible in solution until they reach their solubility limit, the threshold concentration at which they phase separate. As detailed in BOX 1, this behaviour can be understood using classic thermodynamics. In the cell, the existence of separate phases enables the maintenance of a chemical equilibrium between compartments of different chemical properties (for example, concentration) through the rapid movement of molecules between them.

Biomolecular condensates are often enriched with multivalent molecules — that is, molecules that harbour multiple elements that govern intra- or inter-molecular interactions[20–24]. As detailed in BOX 2, this multivalency is important because classic concepts in polymer science indicate that multivalent molecules naturally assemble into large oligomers or polymers when mixed.

Entropy
A measure of disorder in a given system. Specifically, the number of microstates possible for a given state. Systems tend to approach states that maximize their entropy.

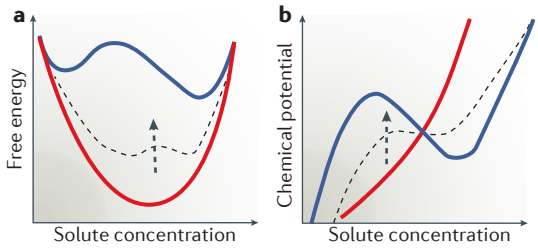
Free energy
The energy available in a thermodynamic system to work. Systems tend to approach states that minimize their free energy.

Stereospecificity
A property of binding reactions whereby the specificity is largely dictated by the complementary geometries of the ligand and receptor molecules.

This assembly will inherently decrease the solubility of the molecules due to entropy-driven effects[25], thus promoting their phase separation. The coupled assembly and phase separation of multivalent macromolecules has emerged as an important organizing principle for biomolecular condensates. This idea can be applied broadly to understand the phase separation behaviour of diverse multivalent molecules. Such molecules include proteins composed of multiple modular interaction domains and proteins containing disordered regions that provide multiple weakly adhesive sequence elements. RNA and DNA molecules, which can harbour multiple regions that bind to other nucleic acid molecules and proteins, can also undergo phase separation. Furthermore, as discussed below, this mechanism naturally leads to a biological means of regulating phase separation, as well as the composition, physical properties and biochemical functions of biomolecular condensates (FIG. 2).

Phase separation of proteins with modular domains. There are now many examples of phase separation of natural proteins composed of modular interaction domains. The first example studied in detail was the actin-regulatory signalling pathway consisting of the multivalent proteins nephrin, Nck and neural Wiskott–Aldrich syndrome protein (N-WASP). These proteins assemble into high-order oligomers through interactions between phospho-tyrosine (pTyr) residues in nephrin and Src homology 2 (SH2) domains in Nck and between SH3 domains in Nck and proline-rich motifs (PRMs) in N-WASP (FIG. 2a, left). This assembly produces both phase-separated liquid droplets suspended in solution[20] and phase-separated clusters on lipid bilayers when nephrin is attached to membranes *in vitro*[26] or in cells (M.K.R., unpublished observations). An analogous system is one controlling actin organization in T cells. This system comprises the proteins linker for activation of T cells (LAT), growth factor receptor-bound protein 2 (GRB2), GRB2-related adaptor protein 2 (GADS), son of sevenless (SOS) and SH2 domain-containing leukocyte protein of 76 kDa (SLP76; also known as LCP2) and forms membrane puncta *in vitro* and in cells in response to stimulation of the T cell receptor[27]. In addition to these signalling systems, the P body components mRNA-decapping enzyme subunit 2 (DCP2) and enhancer of mRNA-decapping protein 3 (EDC3) (FIG. 2b), the nucleolar protein nucleophosmin (NPM1) (FIG. 2c) and the postsynaptic density proteins SynGAP and postsynaptic density protein 95 (PSD95; also known as DLG4) were shown to phase separate through multivalent interactions of folded domains. In these cases, the interactions are with ligands harbouring disordered regions[24,28,29].

Box 1 | **Thermodynamics of phase separation**



To understand phase separation, we first consider free energy of the solution (see the figure, panel **a**) and the chemical potential (see the figure, panel **b**), which is its first derivative (with respect to molecular composition). These properties are dictated by the energy possessed by each molecular species within its chemical bonds, its location and its concentration in the system. For a simple system of non-interacting solute molecules in a solvent, free energy as a function of solute concentration is unimodal and the chemical potential is monotonic (see the figure, unbroken red curves). A given value of chemical potential corresponds to a unique solution composition. Under these conditions, the solute molecules are, on average, distributed homogeneously to maximize the entropy of the system. Fluctuations that produce transient inhomogeneities in concentration (and in chemical potential) are dissipated by diffusive flux, which equalizes the differences in chemical potential across the system and minimizes free energy (for further discussion, see REF. 19).

However, when solute molecules interact, the free energy curve becomes multimodal and the chemical potential curve becomes non-monotonic (see the figure, broken curves and arrow). Then, some values of chemical potential correspond to two different solute concentrations, and the free energy of the system can be minimized by separating the solute molecules into two compartments of different concentrations but equal chemical potentials (see the figure, unbroken blue curves)[25,116].

In molecular terms, all macromolecules exhibit varying degrees of weak, nonspecific interactions with each other and with solvent (water in biology). These interactions tend to be very low in affinity, short-lived, lacking stereospecificity and distributed throughout the surface of the molecule. Essentially, the solubility of macromolecules — the concentration at which they phase separate — is governed by the balance between the weak interactions between macromolecules and those between the macromolecule and water. When interactions between macromolecules are weaker than those between macromolecules and water (so-called good solvent conditions), the macromolecules remain miscible in solution at all concentrations. However, when the macromolecule–macromolecule interactions are sufficiently stronger than macromolecule–water interactions (poor solvent conditions), the macromolecule has limited solubility and gains the propensity to phase separate[117]. In such systems, phase separation occurs at the concentration at which the favourable energetics of macromolecule–macromolecule interactions begin to overcome the entropic tendency of the solution to remain homogeneously mixed. At this solubility limit, the molecular mixture separates into two phases: a large volume, low concentration dilute phase, and a small volume, high concentration condensed phase. The phase-separated state in such systems has the minimum free energy (equilibrium). The chemical potential in both phases is equal, eliminating net diffusive flux between the phases while allowing individual molecules to move between them. Thus, the concentrated compartment is maintained persistently. At equilibrium, phase-separated liquid systems allow a cell to maintain concentration differences without constant input of energy. By contrast, gradients of soluble molecules in non-phase-separating systems, as seen for instance in cell polarity systems[118], require a constant input of energy.

Box 2 | **Multivalency promotes phase separation**

In addition to the very low affinity interactions dominating solubility (BOX 1), biological macromolecules form complexes through relatively long-lived interactions that occur with high(er) affinity and high stereospecificity; for example, the binding of modular signalling domains in proteins to their cognate ligands. When such interactions occur between multivalent molecules, the molecules assembly into large oligomers or polymers, resulting in the formation of complexes with varying stoichiometries[119]. Increasing the affinity between the interacting modules or the number of these modules (referred to as the valency of the molecule) promotes the formation of larger complexes[25,120].

Importantly, interactions governing solubility and those governing the formation of polymeric complexes are thermodynamically coupled, so that in poor solvent conditions (which appear to apply to many macromolecules in water) the solubility of a complex decreases as its size increases[117,121]. This phenomenon arises because the entropic cost of confining a complex into the condensed phase is lower than the cost of confining its components individually.

This phenomenon may also be viewed as increased avidity of the weak, solubility-determining interactions as the size of the assembly grows. Thus, oligomerization and phase separation are linked for non-covalently associating multivalent molecules. By increasing the average size of complexes, oligomerization can enhance the weak, nonspecific interactions between molecules, thereby decreasing solubility and promoting phase separation. Because phase separation concentrates molecules into a condensed phase, it further increases the degree of binding in that phase, thereby promoting the formation of larger complexes.

We note that, to our knowledge, previous conceptions of the assembly of multidomain macromolecules have focused largely on the networks created by strong, specific interactions, without consideration of the extremely weak, nonspecific interactions that govern solubility, and how they would be affected by the assembly process. We argue that considering the coupling between the strong and weak interactions, and therefore the ability of multivalency to promote phase separation, is essential to understanding the behaviour of multivalent biological molecules[122].

Finally, in some systems, such as disordered proteins, interactions may occupy intermediate regimes on the spectrum of strong, stereospecific contacts and weak, nonspecific contacts. In such cases, the distinction between interactions that govern assembly and those that govern solubility is blurred. Such systems may be considered either through the lens of simple phase separation or multivalency-driven phase separation. Nevertheless, as disordered polymers become less soluble as they grow longer or become more adhesive, in either view, the presence of multiple points of contact between molecules provides an important driving force for phase separation.

Phase separation has also been explored in various engineered proteins composed of repeated folded domains connected by flexible linkers. These simplified model systems enable a more precise understanding of the influence of individual physical parameters than would be possible in more complex, naturally occurring proteins. Examples include polySH3 proteins binding to polyPRM ligands and proteins with multiple RNA-binding domains binding to repeated RNA oligonucleotides[20]. Proteins comprising multiple repeats of small ubiquitin-related modifier (SUMO) domains (polySUMO proteins) binding to polySUMO-interaction-motif (polySIM) ligands have also been explored[30] (FIG. 2a, right). Experiments with these molecules have identified valency (that is, the number of interaction modules) and affinity between the interacting modules as key parameters controlling phase separation. Higher valency and affinity both promote assembly into larger structures, enabling phase separation at lower concentrations (FIG. 2j), and decrease the dynamic rearrangements of molecules within phase-separated droplets[20,26].

We note that when molecules are highly soluble (that is, are characterized by a high solubility limit), assembly does not necessarily lead to phase separation. For example, engineered proteins consisting of tandem repeated WW domains readily polymerize when mixed with multivalent PRM-containing partners. However, this assembly remains a single, macroscopically homogeneous phase[31,32]. These observations illustrate the idea that molecular assembly and phase separation of multivalent systems are distinct phenomena, even if often coupled.

Phase separation of proteins with intrinsically disordered regions. Proteins containing large intrinsically disordered regions (IDRs) represent a second, abundant class of macromolecules that can phase separate under physiological conditions[33]. IDRs lack a defined 3D structure but often contain repeated sequence elements that provide the basis for multivalent weakly adhesive intermolecular interactions.

IDR-containing proteins are enriched in many biomolecular condensates, particularly those that also concentrate RNA, such as P bodies, stress granules, germ granules and many nuclear structures. Many such proteins can phase separate on their own *in vitro* under various solution conditions[23,34–37]. The IDRs of these proteins have low sequence complexity and are enriched in a limited number of amino acid types — primarily glycine, serine, glutamine, asparagine, phenylalanine and tyrosine. Some IDRs are also enriched in charged residues — lysine, arginine, glutamate and aspartate. The lack of sequence diversity generates multiple Gly/Ser-Phe/Tyr-Gly/Ser sequences and/or poly-Gln and poly-Asn tracts in these molecules, as well as blocks of positive or negative charge[21,23,38–41]. These repetitive motifs are important for their targeting to RNA granules[38–41] or the mitotic spindle[42] and for phase separation *in vitro* and in cells[23,35,36,42,43].

Several recent studies have pointed to a particularly important role of aromatic residues in the interactions that enable IDRs to phase separate (FIG. 2e). The IDR in DEAD-box helicase 4 (DDX4), for example, contains numerous Phe-Gly repeats whose aromatic rings appear to promote phase separation by engaging in cation–pi interactions with Arg residues intramolecularly and intermolecularly[23] and probably in pi-stacking interactions. Similarly, mutations in aromatic residues in BuGZ (also known as ZNF207) and in the nephrin intracellular domain (NICD) decreases the ability of these proteins to phase separate[42,44]. Sequences enriched in Gln, Asn or Ser residues also contribute to the driving force for phase separation through dipolar interactions of their side chains[45,46]. Finally, the phase separation of IDR-containing proteins can also be promoted by interactions between blocks of oppositely charged residues — either between two different molecular types (FIG. 2f) or as alternating blocks in the same molecular type[16,17,23,34,44] (FIG. 2g). In these systems, the patterning of charged residues is important: for the same net molecular charge, when the charge is uniformly distributed phase separation is disfavoured, whereas when charged residues are clustered phase separation is promoted[23,33,44,47].

WW domains
Small (~5 kDa) modular signalling domains found in numerous proteins that contain two conserved tryptophan residues. WW domains bind to proline-containing peptide motifs.

Cation–pi interactions
Noncovalent interactions between positively charged residues (for example, lysine) and pi electrons in aromatic residues (for example, phenylalanine).

Pi-stacking interactions
Attractive interactions between aromatic rings, such as those found in phenylalanine, tyrosine and tryptophan residues.

Dipolar interactions
Interactions between two molecules that are electrically polarized, wherein the partial positive charge on one interacts with the partial negative charge on the other.

Notably, all of these interaction types — aromatic, polar and charge–charge — are short lived and provide little structural order to the peptide chain, consistent with the dynamic nature of phase-separated liquids.

In addition to these amino acid side-chain interactions, interactions involving the polypeptide backbone are likely to have an important role in the phase separation of IDR-containing proteins. The IDRs from the RNA-binding proteins FUS, TATA-box binding protein associated factor 15 (TAF15), heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2), Ewing sarcoma (EWS) and cold-inducible RNA-binding protein (CIRBP) form solid-like hydrogels when concentrated *in vitro*[22,41,48]. However, this mechanism only occurs after initial liquid–liquid phase separation for some of these proteins (see below for a discussion of this temporal progression). Based on a combination of X-ray diffraction, electron microscopy and chemical footprinting data, these hydrogels contain long filaments that appear to be generated from interactions between stretches of

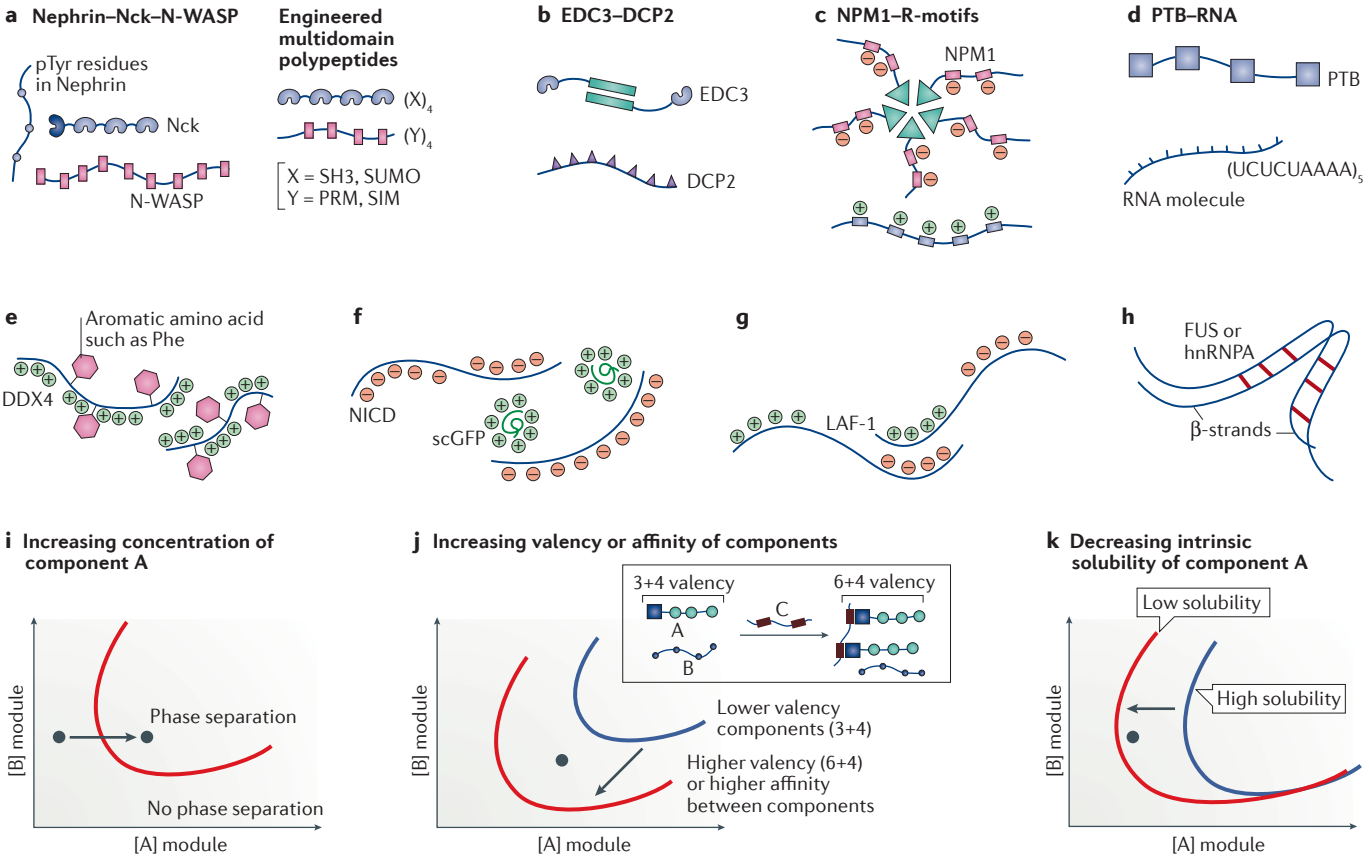


Figure 2 | **Different modes of multivalent interactions in synthetic and natural systems undergoing liquid–liquid phase separation. a** | (Left) Nephrin contains three phosphotyrosine (pTyr) motifs (light blue circles), which interact with the SRC homology 2 (SH2) domain (dark blue) on Nck. Nck also contains three SH3 domains (blue), which bind to the numerous proline-rich motifs (PRMs) (pink) in neural Wiskott–Aldrich syndrome protein (N-WASP). (Right) Engineered multivalent model systems, consisting of multiple SH3 or small ubiquitin-related modifier (SUMO) domains (blue), paired with multivalent ligands that contain multiple PRMs or SUMO-interaction motifs (SIMs) (pink). See REF. 20 for details. **b** | Enhancer of mRNA-decapping protein 3 (EDC3) dimerizes via its YJeF amino-terminal domain (green rectangles) and binds to the helical leucine-rich motifs (purple triangles) in mRNA-decapping enzyme subunit 2 (DCP2) via its LSm domain (blue). See REF. 28 for details. **c** | Nucleophosmin (NPM1) assembles into pentamers via its oligomerizing domain (green triangles) and binds to proteins that contain positively charged arginine-rich linear motifs (R-motifs) (blue rectangles) via its negatively charged acidic, tracts (pink rectangles). NPM1 can also bind to potentially multivalent nucleic acids via its nucleotide-binding domain (not shown). See REF. 24 for details. **d** | The RNA-binding protein polypyrimidine tract binding protein (PTB) interacts with UCUCU tracts in RNA (connected by AAAA linkers) via its RNA recognition motifs (blue squares). See REF. 20 for details. **e** | Association of intrinsically disordered regions (IDRs) via cation-pi interactions between aromatic and basic residues, as in DEAD-box helicase 4 (DDX4)[23]. **f** | Patterned intermolecular electrostatic interactions between acidic and basic tracts, as in the interactions between the nephrin intracellular domain (NICD) and positively charged partners, such as supercharged GFP (scGFP)[44]. **g** | Patterned electrostatic interactions between acidic and basic tracts in a single molecular species, as in the P granule protein LAF-1 (REF. 34). **h** | Polypeptide backbone interactions between β-strands in the polypeptide, as in FUS and heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) and hnRNPA2 (REFS 16,35,43,49). **i** | Phase diagram as a function of the concentrations of modules present in polymerizing multivalent components that are essential for condensate formation. Phase separation will be promoted by increasing the cellular concentration of component A (black dot, arrow). **j** | Regulation of condensate formation by increasing the critical concentration by increasing the valency of A and/or B or the affinity between A and B (arrow), whereas their cellular concentrations (black dot) remain fixed. Effective valency may be increased by the presence of a third interacting component as shown in the inset. **k** | Regulation of condensate formation by decreasing the intrinsic solubility of component A (arrow), whereas its cellular concentration (black dot) remains fixed. As molecule A becomes less soluble, phase separation can occur at lower concentrations of A.

β-strands (FIG. 2h), similar to those observed in amyloid fibres[22,41,48,49]. This result suggests that the interactions between β-strands that drive fibre and hydrogel formation when occurring thousands at a time may provide the weak multivalent adhesions that drive liquid–liquid phase separation when occurring only a few at a time[49]. Similarly, recent studies have demonstrated that short, evolutionarily conserved α-helical structures are important for phase separation of another RNA-binding protein, TAR DNA-binding protein 43 (TDP43)[50–52]. In any given IDR, the degree to which side chain and backbone interactions contribute to phase separation will depend on the amino acid composition and the overall sequence patterns of the protein. Predictive rules relating protein sequence to phase separation propensity are slowly emerging and remain an important area of future research[23,44,47,53].

IDRs can therefore undergo various types of homotypic and heterotypic interactions. Although the individual interacting motifs are less well defined in IDR-containing proteins than in multidomain proteins, multivalency appears to play a central part in promoting phase separation of both types of molecule.

Regulation of assembly

The physical mechanisms promoting phase separation as outlined above (for more details see BOXES 1,2) suggest that there are means to controlling key features of biomolecular condensates, including their total volume, assembly and disassembly. Specifically, because molecules will phase separate until the values of the chemical potential of both species are matched in the two phases, this control can be achieved by altering the cellular concentration of biomolecular condensate components and/or their propensity to phase separate[19].

Control of cellular concentration. Because condensates form by phase separation, their appearance in cells is sharp when their core components reach their solubility limit (FIG. 2i). For example, nucleoli in *C. elegans* embryos form only when the nucleolar component FIB-1 (and perhaps other key molecules) is above a threshold concentration[54]. Under thermodynamic control, the total volume of the condensed phase (that is, the phase-separated entity) will then be determined by the extent to which the concentrations of its components exceed their solubility limits. This phenomenon has, indeed, been observed quantitatively with engineered DDX-4 and NICD as well as qualitatively with natural condensates, such as PML bodies, nucleoli, P bodies, stress granules and centrosomes[23,44], whose sizes scale with the expression levels of key components. Similarly, the formation of Cajal bodies, PML bodies, histone locus bodies, nuclear speckles (artificial) and nucleoli (natural) can be induced by experimentally concentrating their key components at a particular cellular site[55–59]. Furthermore, expanding the volume of isolated nuclei by placing them in a hypotonic solution leads to a reversible dissolution of PML bodies and nucleoli[60]. Moreover, by decreasing the concentration of nucleolar components in *C. elegans* embryos by increasing the cell volume, the nucleolar size decreases[54]. Essentially, any mechanism that alters the local concentration of key components, including changes in protein expression, degradation and localization, will influence the formation and total volume of the condensed phase.

Control of phase separation threshold. Condensate formation can also be controlled by modulating the phase separation threshold by changing the degree of molecular assembly (FIG. 2j) and/or intrinsic solubility (FIG. 2k) of key species. Post-translational modifications appear to be an important mechanism of achieving such control, as such modifications can change both the valency and intrinsic solubility of core condensate components. In the nephrin and LAT signalling assemblies (see above), for example, higher numbers of pTyr residues promote phase separation, enabling the control of phase separation by modulating the activity of kinases and phosphatases[20,26,27]. Similarly, phase separation of the nuage protein DDX4 is hindered by Arg methylation, which probably decreases the number of cation–pi interactions[23]. Moreover, the number and structure of PML nuclear bodies are influenced by the degree of sumoylation of the PML protein, which can alter self-assembly through its SIM[61]. Similarly, binding to interaction partners such as RNA can modulate protein solubility. For example, the solubility of a P granule component, PGL-3, decreases *in vitro* in the presence of RNA; in other words, PGL-3 phase separates at lower concentrations. Proteins that compete for RNA binding with PGL-3, such as MEX-5, can then increase the solubility limit of PGL-3 in the presence of RNA[15,23,26,62]. All these examples represent events that occur on rapid cellular time-scales (in a matter of minutes). However, processes occurring on much slower time-scales, for example alternative splicing or evolutionary processes, could also alter phase separation propensity by modulating the interaction valency.

Regulation of composition

Individual biomolecular condensates have a specific composition, typically concentrating from 10 to several hundred different proteins, and often also RNA molecules. Their composition is dynamically controlled; some components are constitutive, but many are recruited only transiently, for example, during particular stages of the cell cycle or in response to stimuli[61,63–66]. How can we understand this complexity? Little work has been conducted so far to understand the general compositional control of condensates. One recent attempt to develop a general framework to explain condensate composition was based on dividing condensate components into two qualitative classes[30]. The first are scaffolds, which are resident molecules essential for formation of the structure. Genetic studies have indicated that these scaffolds are often only a small subset of condensate components. For example, PML is the only protein known to be essential to form PML nuclear bodies[67]. Similarly, the following key components are necessary for forming particular biomolecular condensates: spindle-defective protein 5 (SPD-5) for centrosomes in *C. elegans*[68]; TIA1 for mammalian stress granules[69]; the NEAT1 non-coding

Chemical footprinting
Use of a small reactive chemical to modify solvent-exposed sites in a macromolecule, providing information on the structure of that macromolecule.

Chemical potential
The partial molar free energy within a system. Mathematically, the first derivative of free energy with respect to composition. Systems tend to approach states that dissipate gradients in chemical potential.

Histone locus bodies
Biomolecular condensates in eukaryotic nuclei containing nuclear protein, ataxia-telangiectasia locus (NPAT) and FLICE-associated huge protein (FLASH), and thought to be involved in the processing of histone mRNAs.

Nuage
Biomolecular condensates in metazoan germ cells thought to have a role in maintaining germ cell genomic integrity. This class of compartments includes P granules, polar granules and mammalian nuages.

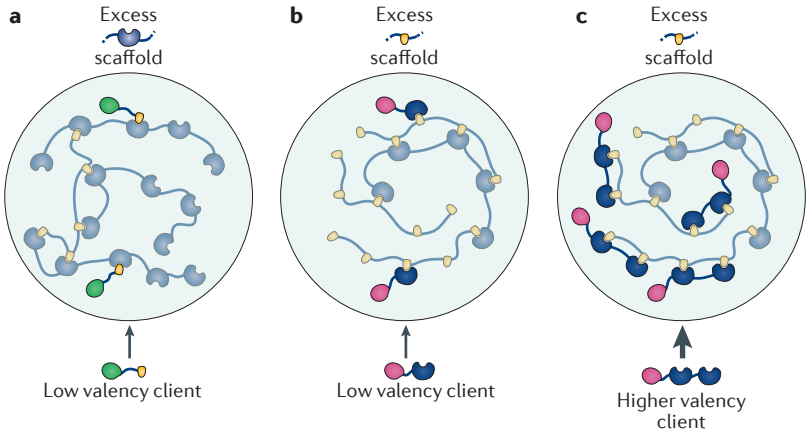


Figure 3 | **A model for compositional control of biomolecular condensates.** Multivalent molecules that constitute the scaffold of the condensate contain complementary modules (blue and yellow, for example, small ubiquitin-related modifier (SUMO) domains and SUMO-interaction motifs, respectively)), which enable the assembly of the scaffold to form the phase-separated structure (large circles). Client molecules in this example harbour interaction modules complementary to the scaffold components but at a lower valency, and are recruited to the structure by binding to free cognate sites in the scaffold (owing to stoichiometric excess of one of the modules). **a** | Stoichiometric excess of the scaffold component-containing blue modules yields free blue scaffold sites. Clients containing yellow modules can be recruited to the body by binding to the blue scaffold sites that are unoccupied by scaffold–scaffold interactions. **b** | Stoichiometric excess of the scaffold component-containing yellow modules yields free yellow scaffold sites. Clients containing blue modules can be recruited to the body by binding to the yellow scaffold sites that are unoccupied by scaffold–scaffold interactions. **c** | Higher valency of the blue client promotes stronger recruitment of this client when the yellow scaffold module is in stoichiometric excess (but not when the blue scaffold is in excess (not shown)). Figure modified with permission from REF. 30, Cell Press.

RNA for paraspeckles; and mRNAs for P bodies[2]. The second class of components, termed clients, consists of molecules that are dispensable for biomolecular condensate assembly. Clients constitute the majority of components and often localize to condensates in a regulated fashion by directly binding to scaffolds[61,63,66].

To elucidate principles of compositional control of biomolecular condensates, simple model systems composed of multivalent scaffolds and their cognate low valency clients were used[30]. Both *in vitro* and in cells, phase-separated droplets formed by polySUMO–polySIM scaffolds differentially recruited low valency clients (for example, GFP-SUMO or GFP-SIM) depending on the relative stoichiometries of the scaffold components (FIG. 3a,b). Changes in composition could be induced rapidly by altering the SUMO:SIM ratio in the scaffolds. In particular, when the stoichiometry is nearly equal, even very small changes in relative concentrations of polySUMO and polySIM could drive large changes in client recruitment. In addition, clients with higher valency (for example, containing more than one SUMO domain) were recruited more strongly (FIG. 3c). Analogous behaviours were observed in mammalian PML nuclear bodies and yeast P bodies. In both cases, perturbing scaffold stoichiometries (by mutating sumoylation sites or modulating cellular mRNA levels, respectively), resulted in changes in client recruitment. Thus, despite the complexity of biomolecular condensates, their compositions may be partially explained by simple principles.

Paraspeckles
Biomolecular condensates in the mammalian nucleus that contain the long non-coding RNA nuclear paraspeckle assembly transcript 1 (NEAT1) and a variety of RNA-binding and other proteins. The functions of paraspeckles are not well understood, but include storage of certain RNAs.

Balbiani bodies
A transient collection of proteins, RNA and membrane-bound organelles (endoplasmic reticulum, Golgi and mitochondria) found in primary oocytes of all animals observed to date (flies, frogs, mice and humans).

In addition to specific binding interactions, general electrostatic properties can also influence client recruitment to condensates. This mechanism was shown recently in reconstituted LAT signalling clusters, which selectively exclude negatively charged proteins and concentrate positively charged proteins, probably because the scaffold components of this condensate are highly negatively charged[27]. The relative importance of specific binding and electrostatic interactions is likely to vary between systems.

IDR-based phase separating systems also show selective recruitment of clients. In some cases, this effect can be understood analogously to domain-based systems. For example, decreasing the number of Gly/Ser-Phe/Tyr-Gly/Ser motifs in hnRNPA2 and FUS decreases the efficiency of their recruitment into IDR-based droplets *in vitro*[49] and into stress granules in cells[41], respectively, by reducing the valency of these clients. In other systems, however, the molecular mechanisms governing selectivity of client recruitment are not yet understood[23,43].

Control of physical properties

Many biomolecular condensates possess liquid-like properties. However, some appear to behave more like solids[70] or have solid-like elements[52]. Moreover, physical properties and organization of phase-separated droplets can change over time. As these properties are likely to influence condensate functions (see below), they are probably regulated *in vivo*.

Maturation of IDR-based phases. Phase-separated droplets of FUS and hnRNPA1 rapidly exchange molecules with the soluble phase (as assessed by fluorescence recovery after photobleaching), contain largely disordered protein (shown for FUS by nuclear magnetic resonance) and behave macroscopically as liquids[16,35–37,43,49]. However, many droplets formed by IDR-containing proteins (for example, FUS, PUB1, LSM4, eIF4GII, TIA1, hnRNPA1, WHI3 and FIB1) that are initially fluid become more viscoelastic over the course of several hours, eventually behaving as solids and ceasing to exchange molecules with the surroundings. This process is referred to as maturation or hardening[16,35,37,43,49,71]. The material properties of these hardened states observed *in vitro* are currently unclear, but could be gels, glasses or two-phase solids. Maturation is also likely to occur *in vivo*, as some condensates behave as solids (for example, Balbiani bodies[72] and yeast stress granules[70]) or contain solid-like substructures (see below). Balbiani bodies are particularly interesting in this regard. They are large membrane-less structures that are present in immature oocytes and are thought to protect organelles during the many decades of oocyte dormancy. In *Xenopus* oocytes, Balbiani bodies are formed from a prion-domain-containing protein called Xvelo, which, when expressed from baculovirus, forms solid-like structures. Other proteins containing prion-like domains, such as FUS, form liquids *in vitro*[16]. Indeed, expression of the prion-like domain of FUS forms liquids, whereas that of Xvelo forms solids[72]. Therefore, it seems that the material properties are encoded in part by the prion-like domains. However, whether Xvelo forms a solid-like Balbiani body *de novo* or matures through a more liquid-like state is

not known. Note that maturation is not observed with phase-separated liquids formed by proteins composed of modular domains; in these systems dynamic behaviours are constant and determined by the affinity and kinetics of the modular domain–ligand interactions[24].

Several potential mechanisms could account for maturation (FIG. 4). Unfolded proteins have a propensity to form amyloid fibres through β-strand interactions[73]. This behaviour should be enhanced for IDRs within phase-separated droplets because of the high protein concentrations[43,44] and the tendency of polymers to adopt extended conformations in the condensed phase, which predispose the polypeptide chain to make β-strand-like contacts[25,74,75]. Thus, phase separation could promote increased rates of nucleation and/or growth of amyloid fibres, which could further crosslink through lateral contacts. Indeed, droplet maturation occurs *in vitro* concomitant with the macroscopic formation of filamentous structures[16,35–37,43,49], and fully matured droplets contain amyloid-like filaments observable by electron microscopy[22,43,72]. Balbiani bodies are rich in β-sheets as assessed by thioflavin T staining. Chemical footprinting studies suggest that fibre formation in condensates also occurs in cells[49]. As an alternative to fibre formation, some systems may be kinetically trapped or 'vitrified' in an amorphous, crosslinked state if their β-strand (or side chain) interactions form rapidly and dissociate slowly, preventing progression to regularized amyloid structures[75] (FIG. 4).

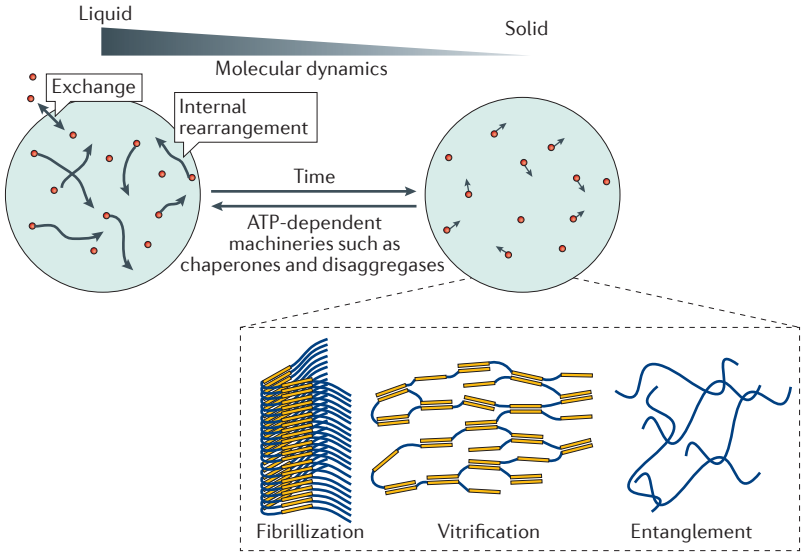


Figure 4 | **Changing material properties of biomolecular condensates.** Condensates composed of intrinsically disordered regions (IDRs) have the propensity to mature, changing their properties from liquid-like to solid-like. Initially, the components in the condensed phase exhibit only transient interactions and lack appreciable order. Thus, the molecules freely rearrange (and exchange with the surrounding solution) and the molecular dynamics can be described as that of a liquid. Over time, the liquid becomes more solid-like. Several potential mechanisms for this 'hardening' and the concomitant decrease in molecular dynamics have been proposed, as described in the main text. Briefly, these mechanisms could include nucleation and elongation of amyloid-like fibres, kinetic trapping into amorphous glasses (vitrification) or entanglement of the disordered polypeptides. ATP-dependent machineries such as chaperones and disaggregases are anticipated to act against these processes (other mechanisms that do not depend on ATP may act similarly).

Finally, increased entanglement of polymer chains (whereby the chains wrap around each other and cannot cross) could also change condensate properties in a manner akin to maturation[76]. These latter mechanisms may account for observations demonstrating that yeast stress granules behave as solids but do not appear to contain fibres[70]. Although detailed experimental studies are currently lacking, the slowed molecular dynamics and increasing hardness of droplets as maturation proceeds in these scenarios may result from increases in three attributes: fibre length, numbers, crosslinking density and strength; the density and strength of β-strand or side chain interactions; or in the degree of entanglement.

Regulation of physical properties by energy-consuming processes. Cells probably have mechanisms to limit the tendency of IDRs to mature in order to tune the dynamics and liquid-like properties of condensates into functionally appropriate regimes (see below). One likely mechanism involves the use of energy-dependent processes or machines to control the degree of fibre and/or crosslink formation within condensates. Thus, the formation of these structures is limited when condensates need to be dynamic but promoted when condensates need to be static (FIG. 4). This need for the dynamic regulation of condensate material properties may explain why chaperones, ATP-dependent disaggregases and molecular motors are present in many RNA granules[52,70]. Indeed, depletion of ATP increases the viscosity of stress granules and nucleoli[13,52]. Moreover, several ATPases can regulate the dynamics of stress granules, increasing or decreasing their persistence[50,52].

Recently, it has been suggested that imbalances between the thermodynamic drive of IDRs to form fibres and the opposing disaggregase machineries could lead to disease. In fact, a large body of data links dysregulation of RNA-containing condensates with neurodegenerative diseases. We direct readers to numerous recent reviews focused on this important topic[21,77–82].

In addition to controlling maturation, it is likely that energy-utilizing systems modulate many additional features of condensates. For example, the transcription of rRNA influences the nucleation and spatial distribution of condensing nucleoli in *C. elegans* embryos[59,83]. Furthermore, the size distribution of nucleoli and histone locus bodies is influenced by the actin cytoskeleton, the dynamics of which are controlled by ATP hydrolysis in actin filaments, motors and nucleation factors[84]. The actin cytoskeleton can also affect the localization of condensates. For example, phase-separated LAT clusters are moved radially at the T cell–antigen presenting cell interface by dynamic movements of the actin cytoskeleton[85,86]. Because energy consumption influences virtually all biological processes, these initial observations are probably exemplary of a more general phenomenon in the regulation of condensates. The study of energy-consuming, non-equilibrium materials — 'active matter' — is an area of great current interest in physics and materials science[87–90]. The application of physical theories should provide insight into the influence of cellular energy on the equilibrium processes of phase separation[13,91].

Multiphase biomolecular condensates

The studies we have discussed so far involve a single condensed phase and a more dilute surrounding phase. However, some biomolecular condensates are composed of distinct subcompartments; that is, they contain secondary condensed phases within the primary condensed phase[52,71,92,93]. A recent study examined this process in detail for nucleoli and demonstrated that the subcompartments have distinct viscosities, surface tensions and compositions[71]. The encapsulation of one subcompartment by another is enabled by the distinct surface tensions of the phases, which arises from distinct multivalent interactions based on IDRs or folded domains of the components. How the composition of such multiphase condensates is regulated and how the assembly of such structures is initiated in cells remain open questions.

The different subcompartments may have different propensities for maturation based on their compositions. The (inner) dense fibrillar subcompartment of the nucleolus, for instance, is more prone to maturation than the (outer) granular component and may exhibit a spectrum of viscoelastic behaviours in cells, which are likely to be subject to regulation[13,71]. As indicated by their names, electron microscopy images of these subcompartments show distinct textures[94]. Similarly, Cajal bodies sometimes exhibit coiled structures that can be visualized by electron microscopy[23,43,95] and at other times appear more isotropic. These coiled elements may represent fibrous structures embedded within a larger liquid phase. Recent biochemical and high-resolution imaging studies have revealed that other condensates also contain substructures that behave as solids[13,52,92,93]. Although the function of these subcompartments remains to be determined, it seems likely that cells regulate the relative amounts of solid versus liquid material to yield a functional effect, such as regulating reaction kinetics or stabilizing the structure against mechanical forces (see below).

Implications for function

We have discussed the physicochemical and molecular mechanisms that drive the formation of biomolecular condensates and how their assembly, composition and material properties can be regulated. These characteristics of condensates present unique opportunities, distinct from those provided by macromolecular complexes (Supplementary information S5 (box)), for controlling the biochemical environment of the cell[96]. In this section, we describe how the properties of condensates reflect their biological functions.

Effects of biomolecular condensates on reaction kinetics. Condensates substantially increase the local concentration of resident chemical species. In the simplest case, the increase in concentration should accelerate reactions inside the structure (FIG. 5a). Note, however, that the overall reaction rate will increase only if both enzyme and substrate of a reaction are concentrated in a condensed phase, but not if either is concentrated alone. This process has been observed for some cellular systems. For example, the rate of histone mRNA processing is significantly reduced when key components of this process fail to concentrate within the histone locus body[97]. Similar effects have been shown for components of Cajal bodies in zebrafish[98]; this finding is consistent with a computational model of small nuclear ribonucleoprotein assembly in these structures[99]. Acceleration of reactions by phase separation has also been observed biochemically. For example, the total solution activity of the hammerhead ribozyme can increase up to ~70-fold when it is concentrated along with its substrate RNA strand into phase-separated droplets *in vitro*[100]. Actin polymerization rates can also be substantially accelerated by concentrating the actin-related protein 2 (ARP2)–ARP3 complex (ARP2/3 complex) and N-WASP into nephrin–Nck–N-WASP-based droplets or clusters on model membranes[20,26].

However, concentration into a condensed phase does not always result in an acceleration of the overall reaction rate. For example, essential factors for small nuclear RNA modification, called guide RNAs, are normally concentrated inside Cajal bodies. However, disrupting Cajal body formation and thereby dispersing guide RNAs in the nucleoplasm does not seem to affect the efficiency of small nuclear RNA modification[23,101]. Similarly, the activity of enzymes in the purine biosynthetic pathway was not significantly enhanced when they were concentrated with their substrate into phase-separated droplets *in vitro*[102]. This lack of enhancement was due to the lower specific activities of the enzymes towards their substrates within the droplets compared with their activity in solution. In unpublished work, we have observed that the highly concentrated scaffolds and enzymes within phase-separated droplets frequently interfere with each other, with scaffold components inhibiting enzyme activities and enzymes dispersing droplets by covalently modifying scaffolds. In cells, it is likely that mechanisms exist to prevent or take advantage of such interference.

Many physical features of condensates could affect reactions that occur within them. Molecular crowding — the decrease in accessible volume owing to high macromolecule concentration — can affect allosteric regulation and binding affinity and in turn alter enzymatic activities[103]. Furthermore, condensates are porous structures (see below) and this porosity will also have complex effects on the movement of molecules within them. A solution containing high concentrations of a small molecule (for example, glycerol) will slow the movement of all molecules within it and therefore decrease reaction rates. But a solution containing a concentrated polymer matrix will behave differently. In this case, the free volume between the condensed scaffold components will behave as pores, through which small proteins will move as though the polymer was absent; only large molecules unable to permeate these pores or molecules that bind the polymer will move slowly[104]. The impact of such effects on biomolecular condensates — particularly those containing RNA — is likely to be significant because these condensates are composed of combinations of large RNA molecules, proteins of

Small nuclear ribonucleoprotein
A RNA–protein complex that is the primary constituent of spliceosomes, the eukaryotic splicing machinery.

Hammerhead ribozyme
A catalytic RNA molecule involved in RNA cleavage found in organisms ranging from bacteria to mammals.

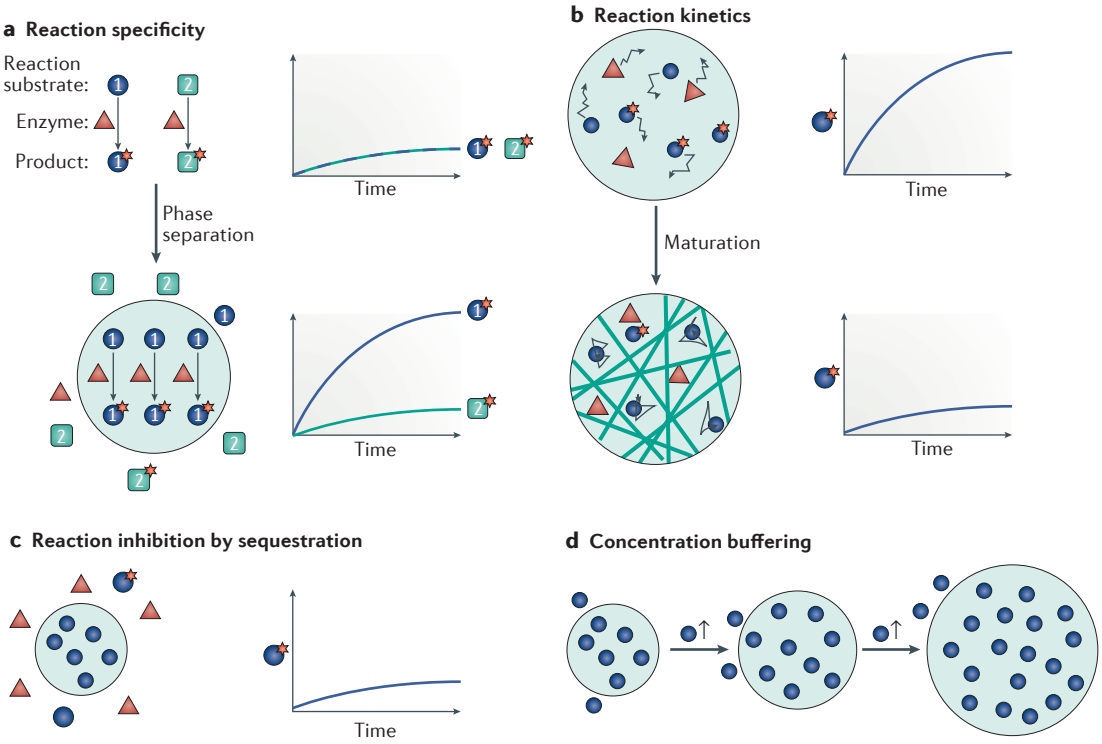


Figure 5 | **Functional consequences of forming biomolecular condensates. a** | Concentrating reactants inside condensates can increase reaction kinetics and specificities. An enzyme with two alternative substrates is shown. Colocalizing the enzyme with one of its substrates within the condensed phase (black circle) accelerates rates of reaction with that substrate. Additionally, excluding the substrate of an alternative pathway can direct a specific reaction to occur inside condensates. **b** | Changes in the physical properties of cellular bodies can affect the kinetics of reactions. For example, increased viscosity of cellular bodies by fibre formation (or other mechanisms of maturation, see main text and FIG. 4), may slow the diffusion of molecules, decreasing reaction kinetics. **c** | Sequestering molecules inside condensates can prevent reactions involving partners present in the bulk phase. This sequestration could control substrate flux through various pathways. **d** | The concentration of essential condensate components in the bulk phase is maintained at the phase separation threshold (defined by the solubility limit of the molecule). Thus, the concentration of these components in the bulk phase can be maintained despite fluctuations in expression or degradation.

various sizes and small organic compounds. In addition, the various viscoelastic properties of condensates, controlled, for example, by the degree of IDR maturation, interaction kinetics of multidomain scaffolds, RNA composition[37,105] or active (energy consuming) processes, are likely to influence the dynamics of molecules within them (FIG. 5b) and at the phase boundary. Changes in viscoelasticity could also affect condensate composition. Understanding of these behaviours will greatly benefit from additional experimental and theoretical work describing the chemistry within complex, heterogeneous media.

Regulating the specificity of biochemical reactions. Phase-separated compartments could concentrate a protein with a subset of its potential interaction partners while excluding others, imparting specificity to biochemical processes. For example, a condensate could concentrate an enzyme with a particular subset of its possible substrates, conferring specificity to a potentially promiscuous reaction (FIG. 5a). Similarly, a condensate could concentrate (and thus accelerate the chemistry of) molecules that act in one particular biological pathway while excluding components of alternative pathways, controlling biochemical flux. In this way, condensates could act analogously to classical scaffolding molecules in signalling pathways, which bind multiple, selected pathway components simultaneously to provide spatial proximity and structural organization, thus enhancing flux and selectivity[106]. Consistent with this idea, clustering the metabolic branch point enzyme carB with one downstream enzyme, pyrB, but not another, argI, was shown to direct the metabolic flux of carbamoyl phosphate to favour pyrimidine and disfavour arginine in *Escherichia coli*[107]. Many metabolic enzymes localize to condensate-like puncta in response to nutrient starvation, suggesting that such effects may be generally important in metabolic control[108,109]. In a related example from mammalian cells, reconstituted T cell receptor signalling clusters concentrate kinases but exclude phosphatases, stabilizing the phosphorylation-dependent clusters[27].

Sequestration of molecules. Condensates could also in principle sequester molecules, thus effectively inhibiting their activity outside the structure (FIG. 5c), as has been suggested for sequestration of the transcription factor death domain-associated protein (DAXX) in PML

bodies[110]. Stress and RNA transport granules have been ascribed similar storage functions[2]. An important consideration for such models is that for sequestration to be effective, most copies of the desired species must be captured in the condensate. As one type of condensate typically constitutes only 1–2% of the cellular volume (A.A.H. and M.K.R., unpublished observations), strong inhibition (high depletion of the molecule from the surrounding nucleoplasm or cytoplasm) would require very high partition coefficients for the sequestered component. Further quantitative analyses of condensates will be necessary to test these sequestration models.

Buffering cellular concentration of molecules. Once a phase-separated structure has formed, the volume of the condensed phase will grow as more scaffold components are added to the system; however, the scaffold component concentration in the surrounding solution will be maintained at the solubility threshold value (FIG. 5d). This phenomenon could be used to buffer against biological fluctuations (for example, in gene expression), making certain pathways more robust to noise or repressing pathways that require noise for their proper function[111].

Controlling function through dynamic regulation of phase separation. One important advantage of phase-separated structures is that all these potential functions can be switched on and off extremely rapidly by controlling the formation and dissolution of a condensed phase. At the solubility limit of a molecule, even minute changes in a physical parameter (such as concentration or temperature) can induce sharp phase transitions. For example, changes of 1 °C can cause condensation or dissolution of BuGZ, DDX4, hnRNPA1 or FUS droplets[23,35,42]. Modest changes in salt concentration can have similar effects[23,43]. In addition, as described above, condensate composition can also be regulated in a switch-like fashion with small changes in relative stoichiometries of scaffold components.

In conclusion, the biochemical environment within biomolecular condensates may be fundamentally different from that in the surrounding cytoplasm or nucleoplasm, and this difference may endow the cells with unique strategies for regulating cellular reactions.

Conclusions and perspectives

Research in the past several years has made significant strides towards understanding the molecular mechanisms that underlie the formation, regulation and function of biomolecular condensates. Many of these structures appear to form through liquid–liquid phase separation, driven by interactions of multivalent molecules. This mechanism naturally leads to routes to control the assembly and disassembly, composition and physical properties of condensates. These routes in turn have implications for the biochemistry that occurs within them and consequently their cellular functions.

The phase boundary allows molecules to be concentrated within condensates while continuously exchanging with the surroundings, without the complications of transport through a membranous barrier. Therefore, composition of condensates can be regulated in a more flexible manner than that of classic organelles and without specialized molecules and signals for import and export. For example, a more general mechanism, such as charge, can be used to target certain molecules to specific phases[27] even in the absence of high-affinity binding interactions. Furthermore, components within condensates can freely diffuse, providing ideal conditions to regulate the rates of biochemical reactions while spatially constraining them.

Given that cells can form compartments by phase separation, why would cells need intracellular membranes at all? Membrane-bound compartments can provide long-term stability that may be difficult to maintain with condensates because the local environment of a condensate is constantly changing owing to fluctuations in, for example, gene expression and molecule turnover. For instance, homeostatic reactions that are ongoing require long-term separation from the bulk cytoplasm. In addition to long-term storage, cytotoxic reactions need to be kept structurally separate to protect the integrity of the surrounding cytoplasm or nucleoplasm. Finally, very small molecules, such as ions, will be difficult to retain inside condensate. For example, a pH gradient could not be stably maintained without a membrane. Thus, these two ways of organizing a cell — membranes or phase separation — are complementary and allow maximal possibilities in organizing cellular contents.

Many important questions remain to be addressed regarding biomolecular condensates. Most importantly, we do not understand in most cases what biochemical or cellular functions uniquely emerge from organizing molecules into such structures. In many cases we can infer function from the collection of condensate components, but we do not understand how the activities of those components change by virtue of being in the structure rather than being more uniformly distributed in the cell. Where examined, the phenotypes resulting from the disruption of condensates are relatively subtle and the structures do not appear to be essential for the viability of cells or organisms[112–115]. However, biomolecular condensates are evolutionarily conserved, suggesting that they do have important functional roles, perhaps in response to particular stimuli or stresses.

We also do not understand the relationship between the microscopic properties of the component molecules and the macroscopic properties of condensates. Furthermore, it is not known how the latter relate to biochemical and cellular functions, or if cells regulate these properties to functional effect.

Although at low resolution many biomolecular condensates appear to be homogeneous, as described above, electron microscopy and super-resolution light microscopy have both indicated that many of these condensates contain internal organization at multiple scales[52,92–94]. Does this organization occur in other condensates, and, in general, how does it arise? Is it dynamically controlled? Is it functionally important?

Partition coefficients
Measures the enrichment of chemical species into the condensed phase of a two-phase system. Mathematically, the partition coefficient is defined as the ratio of concentration of the species in the condensed phase to that in the dilute phase.

What are all of the factors that control the composition of a given biomolecular condensate? We have discussed the importance of direct binding interactions and electrostatic effects, but are there other considerations, perhaps related to active processes? What do we need to know about a condensate (or even a simplified phase-separated droplet) to quantitatively predict how other molecules will partition into it? How is composition finely tuned so that distinct condensate can coexist in a cell with shared components but functional differences? Is there a sequence- or structure-based code for recruiting IDRs into phase-separated droplets?

Does the idea that condensates are generated through phase separation and multivalent assemblies have implications for disease, and could this knowledge enable novel clinical opportunities? Existing data suggest that condensates may lie across a continuum of material and compositional states. Moreover, aberrations in this natural spectrum, some of which may involve misregulation of fibre formation, are implicated in neurodegeneration. How do these aberrations affect cell physiology? This instance is probably only one of many whereby a mechanistic understanding of biomolecular condensates could have medical implications.

Finally, what other cellular structures might be organized by phase separation? In principle, any system composed of interactions between multivalent entities has the propensity to phase separate under appropriate solvent conditions. Chromatin biology is an intriguing area of cell biology that is enriched in multivalent interactions. Chromatin can be considered as long arrays of nucleosomes modified with specific marks on their component histones. These marks are read by specific modular domains that also often appear in multivalent arrays in chromatin-binding proteins. Thus, it seems reasonable to infer that modified nucleosomes and histone tail readers may phase separate and that this process could affect aspects of chromatin organization and function.

Addressing these questions is likely to require new technologies and new conceptual approaches, drawing on disciplines ranging from genetics to biochemistry to physics. The answers to these questions promise to explain how nanometre-scale molecules can give rise to micron-scale cellular organization and the function of this organization in biology.

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