ANNUAL REVIEWS

Annual Review of Genetics

Liquid–Liquid Phase Separation in Disease

Simon Alberti[1,2] and Dorothee Dormann[3,4]

[1]Biotechnology Center (BIOTEC), Center for Molecular and Cellular Bioengineering (CMCB), Technische Universität Dresden, 01307 Dresden, Germany; email: Simon.Alberti@tu-dresden.de

[2]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany

[3]BioMedical Center (BMC), Ludwig-Maximilians-University Munich, 82152 Planegg-Martinsried, Germany; email: dorothee.dormann@med.uni-muenchen.de

[4]Munich Cluster for Systems Neurology (SyNergy), Ludwig-Maximilians-University Munich, 81377 Munich, Germany

Annu. Rev. Genet. 2019. 53:171–94

First published as a Review in Advance on August 20, 2019

The *Annual Review of Genetics* is online at genet.annualreviews.org

https://doi.org/10.1146/annurev-genet-112618-043527

Keywords

phase separation, membraneless organelle, biomolecular condensate, cancer, neurodegeneration, infectious disease

Abstract

We have made rapid progress in recent years in identifying the genetic causes of many human diseases. However, despite this recent progress, our mechanistic understanding of these diseases is often incomplete. This is a problem because it limits our ability to develop effective disease treatments. To overcome this limitation, we need new concepts to describe and comprehend the complex mechanisms underlying human diseases. Condensate formation by phase separation emerges as a new principle to explain the organization of living cells. In this review, we present emerging evidence that aberrant forms of condensates are associated with many human diseases, including cancer, neurodegeneration, and infectious diseases. We examine disease mechanisms driven by aberrant condensates, and we point out opportunities for therapeutic interventions. We conclude that phase separation provides a useful new framework to understand and fight some of the most severe human diseases.

1. INTRODUCTION

Cell biology is in the midst of a revolution. Important biological problems, such as the organization of the cytoplasm and nucleoplasm, the compartmentalization of cells, and the mechanisms of gene regulation, are beginning to be understood in increasing detail. Some of this recent progress has been possible through the incorporation of liquid–liquid phase separation into biology. Phase separation is a process by which a homogeneous liquid solution (or phase) of macromolecular components separates (or demixes) into two distinct phases, one phase that is enriched for the macromolecules and another phase that is depleted of the same macromolecules (4, 12, 75, 138). The liquid properties of the dense phase, such as rapid exchange of components and droplet coalescence, are often interpreted as evidence that a particular cellular structure has formed by phase separation. Phase separation is now thought to underlie many biological processes, including heterochromatin formation (93, 141); nucleocytoplasmic transport (136); and the formation of membraneless compartments, such as nucleoli (50, 107). Cellular structures that form by phase separation have been termed biomolecular condensates (12, 138) to reflect their origin through condensation. Altogether, these recent developments have provided a powerful new framework to explain various biological processes, encouraging major efforts across the world to reexamine biological phenomena through the lens of phase separation. Most importantly, these efforts have taught us that aberrant forms of phase separation frequently occur in cells and organisms and may contribute to a large array of complex human diseases.

In this review, we describe our current level of understanding of biomolecular condensates and the various ways in which they impact biology and may cause disease. We hope to demonstrate that phase separation provides a useful concept to understand and ultimately treat complex human diseases, including cancer, neurodegeneration, and infectious diseases.

2. MEMBRANELESS COMPARTMENTS ARE BIOMOLECULAR CONDENSATES

The mechanisms underlying the organization and properties of the cytoplasm have eluded us for many centuries. The cytoplasm contains hundreds of thousands of different constituents. A large volume fraction of the cytoplasm is taken up by macromolecules. For proteins, this fraction amounts to more than 30%, translating to an overall concentration of 150 mg/ml in eukaryotic cells (47). Anyone who has purified a protein knows that few proteins are soluble above 50 mg/ml; so how can all these proteins be soluble inside the highly crowded cytoplasm? What molecular mechanisms regulate protein solubility? Why is the cytoplasm a liquid and not a solid? All these questions are gaining importance given recent evidence that cells tune the solubility of proteins for biological functionality (12, 53, 54). On the one hand, cells have invented mechanisms to maintain proteins in a soluble state to achieve resistance against perturbations (55, 90, 100, 132). On the other hand, proteins can persist in the cytoplasm at concentrations much larger than their solubility, and this appears to be harnessed by cells to assemble compartments with diverse functionality (12, 138). But how can a cell organize itself solely by manipulating the solubility and phase behavior of proteins?

The last ten years have witnessed a deluge of newly described compartments that form by phase separation. These compartments are often termed membraneless compartments or membraneless organelles to distinguish them from compartments that are surrounded by membranes, such as the Golgi apparatus. Examples of membraneless compartments are the nucleolus (50, 107) in the nucleus or centrosomes (155) in the cytoplasm of cells. But what are the properties that allow condensates to function as compartments? Condensates can selectively enrich certain

components while excluding others. This allows them to generate distinct microenvironments that can accelerate or inhibit biochemical reactions. Various other functions have been proposed for condensates, including the buffering of protein concentrations (120); the formation of molecular filters (117, 136); the sensing of environmental changes (132); the formation of signaling compartments (142); the nucleation of cytoskeletal structures (15); and the deformation of membranes, for example, during endocytosis (17). Not all condensates necessarily must have a function. Some condensates may just form without any particular purpose, as may frequently be the case in cells that are exposed to strong environmental perturbations. However, these condensates could still be involved in disease processes; thus, understanding their principles of formation may be highly relevant.

3. MOLECULAR MECHANISMS UNDERLYING CONDENSATE FORMATION

What molecular mechanisms drive the formation of condensates? In the past five years, we have made tremendous progress in understanding the principles that drive condensate formation in vitro and in cells. The most important step toward forming a condensate is the establishment of a dense network of interacting macromolecules. The valence of the interacting components, i.e., the number of interaction domains or motifs, is the key determinant (12). In vitro experiments have shown that there is a critical number of valences of a protein or RNA above which it phase separates (13, 96, 152).

One concept that has been useful for describing how components get enriched in condensates is the concept of scaffolds and clients (13). Scaffold proteins drive the formation of condensates and are critically required for their integrity. Client proteins partition into condensates after they have been formed by a scaffold. The term partition coefficient is often used to describe the selective enrichment of proteins in condensates (13, 155). It indicates how strongly enriched a component is in a condensate and reflects its compatibility with the physicochemical microenvironment of the condensate. Scaffold proteins are often present at high concentrations in cells and they often have many valences. By contrast, client proteins are typically present at lower protein concentrations, and they usually have a lower number of valences. Another important factor that affects condensate formation is the affinity of a given interaction module for its ligand and the reversibility of a binding reaction (12). This is particularly relevant for the physical properties of a condensate. A liquid, for example, must rearrange on short timescales, and this requires that the interactions maintaining the condensate are transient. This can be achieved in two ways: (*a*) through weak interactions or (*b*) through strong interactions that are reversible on short timescales. Reversibility could be achieved through regulation by posttranslational modifications (PTMs). Indeed, there is increasing evidence for regulation of phase separation by PTMs (71), such as phosphorylation (91, 109) and methylation (72). These PTMs frequently tune the valence of scaffold or client proteins to drive condensate formation or client partitioning, respectively.

Many membraneless organelles exhibit significant internal organization. For example, the nucleolus consists of three discrete layers, each of which is defined by specific protein and RNA constituents (50). Recent work suggests that the multilayered topology of the nucleolus could arise through differences in the surface tensions of the distinct liquid phases (50). Thus, it appears that spontaneous (passive) phase separation is often sufficient to explain the formation of a multilayered compartment. However, active processes also play important roles. Indeed, there is increasing evidence that many cellular condensates are regulated by ATP-consuming processes (48). Understanding the interplay between spontaneous phase transitions and driven processes that consume energy is one of the most important challenges in the field.

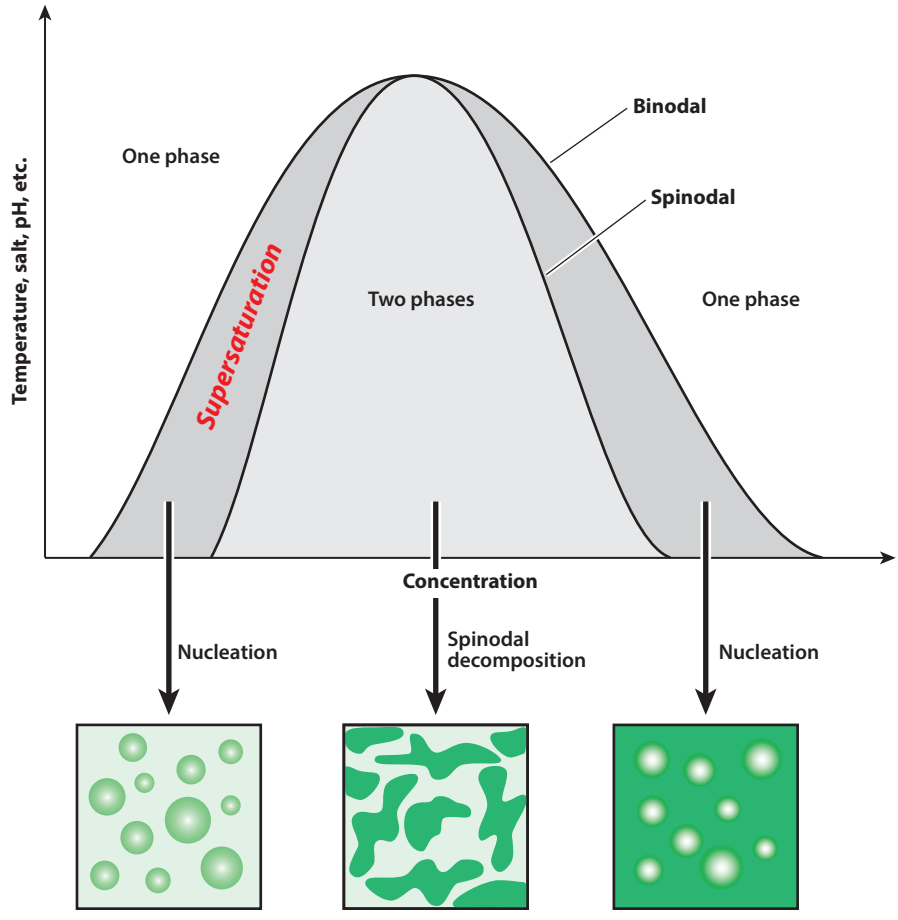

Figure 1

The phase diagram depicted is obtained by systematically changing two conditions (e.g., concentration, salt, pH) and determining the regions in which the system switches from a one-phase state to a two-phase state. In the two-phase state a dense phase and light phase stably coexist. The binodal (coexistence line) separates the one-phase state from the two-phase state, and it describes the region where phase separation is thermodynamically favorable. The spinodal is the line that separates nucleation-limited (discrete) phase separation (the region where nucleation takes place) from diffusion-limited (uniform) phase separation (the region where spinodal decomposition takes place). Light green and dark green indicate the dilute phase and the dense phase of the phase-separated system, respectively.

4. MATERIAL PROPERTIES OF BIOMOLECULAR CONDENSATES

In physical terms, liquid–liquid phase separation is a density transition that results in the formation of a dense phase in which the molecules can move about somewhat freely. Such density transitions are typically described with phase diagrams (**Figure 1**). A phase diagram is a graphical representation of the phase behavior of a molecule; it involves systematically changing conditions and determining the regions in which a dense phase is observable. Order parameters frequently used in phase diagrams are temperature, concentration, and salt.

In addition to phase separation, there are other types of phase transitions, such as gelation. Gelation is not a density transition but a connectivity (or topological) transition that involves the

formation of a system-spanning network of interactions (62). Importantly, phase separation and gelation are often coupled. In this case, the system-spanning network involves all the molecules in a given droplet. Consequently, many liquid phases can technically be considered gels. However, many readers may not be familiar with this definition, and gel formation is more commonly thought of as being associated with the acquisition of solid-like properties. Here, we use the term gelation to refer to connectivity transitions that also change the material properties of a condensate (a.k.a. acquisition of solid-like properties).

One important finding in the last four years has been that dense protein solutions have a strong thermodynamic drive to mature into more solid-like states, such as gels (6, 108, 123). This transition can also involve crystal-like states, in which the molecules are highly ordered in lattice-like arrangements. The transition from a liquid-like state to a solid-like state has been referred to as molecular aging (6). In cells under stress or cells that overexpress recombinant proteins, solid-like states of proteins are often observed and manifest as protein aggregates. These protein aggregates are either disordered gels or highly ordered crystal-like amyloid fibrils. There is strong evidence that these solid-like states of proteins are linked to disease (94, 103, 108, 112, 123). How and why phase transitions from a liquid-like state to a solid-like state cause disease are discussed in Section 7.

5. MOLECULAR FEATURES OF PHASE-SEPARATING PROTEINS

In the last seven years there has been extensive work on the molecular features that promote condensate formation by phase separation. This work has delineated two archetypes of phase-separating proteins: (*a*) proteins with multiple folded domains or modules and (*b*) intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) (4, 12). Multidomain proteins often carry several folded protein–protein interaction domains connected by flexible linkers. These interaction domains frequently interact with short linear motifs in other proteins (96, 142). The more modules a protein has, the higher its valence and therefore the higher its propensity to phase separate, as discussed above. While many readers may already be familiar with multidomain proteins, the features of IDPs and IDRs are less well known and thus warrant a more detailed description. In contrast to structured proteins, IDRs and IDPs have no fixed structure and there is no single low-energy state representing the native state. Instead, these proteins are better described as ensembles of interconverting conformations with slightly different energies (40, 80), primarily because IDRs and IDPs lack aliphatic and aromatic residues that drive folding of proteins with globular structure.

IDRs and IDPs are polymers and as such they obey the laws of polymer chemistry. The theory of associative polymers has been applied to understand IDRs and IDPs (133, 137). In this framework, IDRs and IDPs can be described as polymers consisting of two fundamental units: stickers and spacers (152). Stickers provide attractive interactions, while the spacer regions promote conformational flexibility. Indeed, many phase-separating IDRs and IDPs carry distributed weakly interacting motifs. These stickers provide specific amino acid chemistry encoding the driving forces and the specificity for condensate formation. Several different classes of phase-separating IDRs can be classified on the basis of their sequence compositions and motifs. Typical determinants of the physicochemical properties of IDRs are the fraction and patterning of charged residues.

One important class of IDRs are those that contain arginine- and glycine-rich (RGG/RG) repeats (33, 118). RGG/RG-containing IDRs can self-interact, but they can also associate with RNA to promote the formation of RNA-containing condensates or coacervates. Prion-like low-complexity sequences compose another class of IDRs (5). The term low complexity describes

sequences that consist of only a few of the 20 possible amino acids. In the case of a prion-like protein, these are mostly polar amino acids, such as glutamine, asparagine, serine, and tyrosine. Recent work has shown that prion-like RNA-binding proteins use a specific protein-intrinsic molecular grammar to drive the formation of condensates (152). This primarily involves interactions among tyrosine and arginine residues through cation–π interactions, but also π–π interactions mediated by tyrosine residues (the aromatic ring systems of tyrosines or phenylalanines are termed π systems). The tyrosine and arginine residues are the stickers that determine the saturation concentration of a phase-separating protein. These stickers are connected by spacer residues that determine the material properties of condensates, presumably by introducing conformational constraints on the polypeptide chain. Determining the different protein-intrinsic molecular grammar rules underlying the formation of various condensates is one of the most urgent challenges in the field.

In addition to proteins, another macromolecule plays a key structural role in condensates, especially in ribonucleoprotein (RNP) granules: RNA. RNA is an ideal scaffold molecule because it is long, flexible, and multivalent. Recent evidence suggests that RNA molecules can interact through specific intermolecular base-pairing events (76, 92, 149). It appears that such RNA–RNA interactions are particularly important for determining the identity of RNP condensates.

6. HOW PROTEIN SOLUBILITY DETERMINES CONDENSATE FORMATION

A concept important for understanding the driving forces for phase separation is supersaturation. To understand supersaturation, we must take a closer look at a phase diagram (**Figure 1**). A typical phase diagram has two regions: a region where the one-phase state is favored and a region where the two-phase state is favored. The binodal (or coexistence line) describes the barrier that must be crossed for phase separation to be thermodynamically favorable. However, this does not mean that a protein solution that has crossed the binodal immediately enters into a two-phase state. Often a dense phase forms only upon assembly of a rate-limiting nucleus that then seeds condensate formation. In the absence of this nucleus, the protein solution remains in the one-phase state, even though the two-phase state is thermodynamically favored. This requirement for nucleation is not absolute, and phase separation also occurs spontaneously when the protein solution crosses a second line, the spinodal. This transition from nucleation-limited (discrete) phase separation to diffusion-limited (uniform) phase separation is termed spinodal decomposition. This distinction is important because many proteins exist in a state in which they are close to the binodal, or they may have even crossed the binodal, but they have not phase separated (35, 64, 86). Such proteins are termed supersaturated proteins. Supersaturation, therefore, is a metastable state of a solution that contains more protein than the thermodynamic solubility of that solution. Cells have developed mechanisms to break protein supersaturation, for example, through the local production of an RNA molecule, which binds a protein with high affinity and thus increases its local protein concentration beyond the saturation concentration for phase separation (19).

Supersaturated proteins can rapidly respond to physiological stimuli, and this can be harnessed by cells to mount physiological responses. But this also is a problem because supersaturated proteins are sensitive not only to physiological stimuli but also to many nonphysiological perturbations (6, 53, 54). This requires control systems to guard against nonphysiological fluctuations inside cells that could inappropriately perturb the supersaturated state. Indeed, there is emerging evidence that IDRs have been added to proteins to specifically tune and buffer the phase behavior of supersaturated proteins (53–55, 90, 132). Importantly, there appears to be a strong link between protein supersaturation, phase separation, and protein aggregation. The very same motifs

that promote protein phase separation are often mutated in diseases and promote a rapid conversion of dense protein solutions to protein aggregates (37, 101, 105, 108, 123). Here, the sensitivity of supersaturated protein solutions becomes problematic. Once a nucleus that promotes the formation of a protein aggregate has formed, a supersaturated protein solution often undergoes a catastrophic transition into an aggregated state (35, 36, 139). Of note, although protein phase separation and protein aggregation often affect the same proteins, they are different molecular processes with different underlying molecular mechanisms and driving forces.

7. ABERRANT PHASE TRANSITIONS AND HOW THEY MAY CAUSE DISEASE

Evidence is accumulating that aberrant condensates are associated with various diseases, such as cancer and neurodegeneration (1, 3, 6, 52, 138). Before we examine the existing evidence, we consider the theoretical possibilities of how aberrant phase transitions could cause disease. On a general level, there are three possibilities. Genetic mutations or environmental perturbations could compromise condensate formation by (*a*) directly changing the molecular mechanism of condensate assembly, (*b*) altering the activity of a critical regulator of condensation, or (*c*) altering the general physicochemical conditions inside a cell (summarized in **Figure 2**).

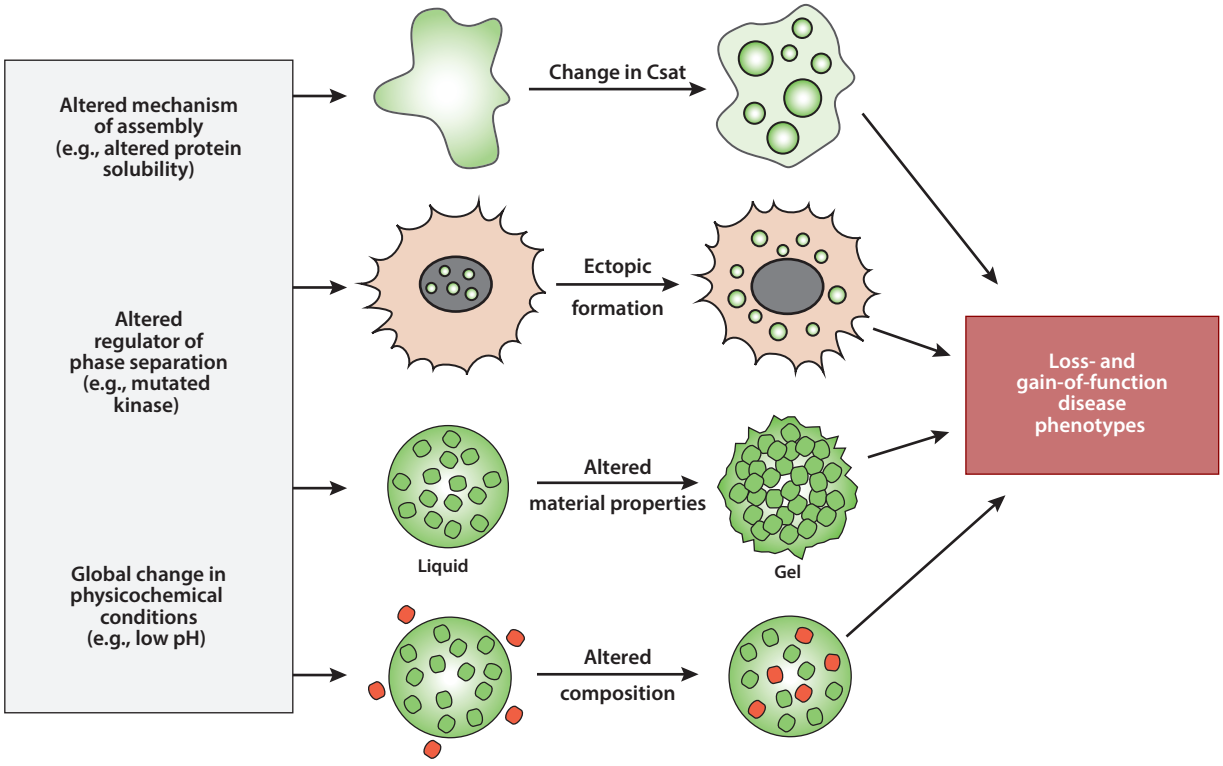

Figure 2

Theoretical possibilities of how disease phenotypes can arise from aberrant phase separation and condensate formation. Diseases can arise because of alterations to the mechanism of assembly, alterations to regulators of phase separation, or global changes that affect the physicochemical conditions in cells. Csat is the saturation concentration for phase separation.

7.1. Changes in the Molecular Mechanism of Condensate Assembly

Genetic mutations could affect the valence of a client or scaffold protein. Mutated sticker residues in a scaffold protein could alter the attractive interactions between molecules and thus could affect the size, number, or morphology of a condensate. If the mutation affects a client protein, there could be changes in the partition coefficient, resulting in protein mislocalization with associated loss-of-function and gain-of-function effects. Another example is a mutation that changes the degree of protein supersaturation. Solubility changes could also render the protein more sensitive to environmental perturbations and stress, thus increasing the probability that the protein will undergo aberrant phase transitions. One can also imagine that a protein becomes more insoluble when it localizes to a different environment. This could occur, for instance, when a nuclear protein is mislocalized to the cytoplasm. In addition to mutations that affect the valence or solubility of a protein, there could be mutations that alter the material properties of a condensate. For example, mutations of spacer residues could change the conformational landscape of a protein and this could affect the material properties of a condensate with potential gain- and loss-of-function defects.

7.2. Altered Activity of a Regulator of Phase Separation

Mutations that inactivate an enzyme regulating condensate formation are an example of this type of disease-causing change. These could be mutations or altered expression levels of a kinase that adapts protein phase behavior to changing conditions in a cell. Altered activity or levels of such a kinase could result in premature or delayed formation of condensates or altered material properties. Another example is the misexpression of a key nucleator of a condensate, which could lead to the formation of ectopic condensates. Similarly, defects in a key suppressor of a particular condensate, e.g., a factor with chaperone-like activity, could also lead to the formation of ectopic condensates.

7.3. Altered General Physicochemical Conditions Inside Cells

Perturbations and genetic changes that affect the general physicochemical conditions in a cell and hence affect condensate formation can also cause disease. For example, mutations in energy metabolism could prevent cells from maintaining normal ATP levels. Because many condensates are regulated by active, energy-consuming processes, this could lead to aberrant condensate formation throughout a cell. More generally, cells must maintain stable internal conditions, such as osmotic pressure, salt concentration, or pH, to ensure normal condensate formation and properties (54, 128). Unstable internal conditions lead to excessive phase separation or altered material states of condensates. Changes in the physicochemical conditions of the cytoplasm are likely to occur in aging cells or tissues (6).

In summary, many possibilities exist in which aberrant phase transitions may cause disease. In the following section, we examine the existing evidence for disease-causing phase behavior and we discuss how our increasing knowledge of physiological phase separation could help us understand mechanisms of disease.

8. PHASE SEPARATION AND NEURODEGENERATION

Neurodegenerative diseases are characterized by progressive loss of cognitive or motor function; for example, cognitive deficits occur in Alzheimer disease (AD) and frontotemporal dementia (FTD), and motor deficits are seen in amyotrophic lateral sclerosis (ALS), Huntington disease

(HD), and Parkinson disease. The specific deficits are caused by the progressive loss of neurons and synapses in distinct brain regions. A key feature of all neurodegenerative disorders is the regional aggregation of cytosolic or nuclear proteins, which are believed to drive neurodegeneration (145). Examples include cytosolic aggregates of the microtubule-associated protein Tau (MAPT) in AD, cytosolic inclusions of the RNA-binding proteins TAR DNA binding protein of 43 kDa (TDP-43) or Fused in Sarcoma (FUS) in ALS and FTD, and poly-glutamine (polyQ) aggregates in HD (56). Many of these proteinaceous assemblies also spread from one brain region to another, consistent with the progressive nature of these diseases (81).

Most neurodegenerative disease cases are sporadic; that is, they have no known genetic cause. In rare cases, neurodegeneration is driven by single gene mutations, causing dominantly inherited forms of the disease, e.g., familial AD or ALS. Often, the genes that are mutated in these familial cases encode the aggregating proteins, e.g., TDP-43 or FUS mutations in ALS patients (144). The mutations are thought to promote pathological aggregation, to cause a partial loss-of-function of these proteins, or both. Cases of sporadic disease show the same pathological protein inclusions, suggesting that familial and sporadic cases have overlapping disease mechanisms and that therapies directed against pathological protein aggregates should be effective for both familial and sporadic disease (56). Exactly how disease-linked protein aggregates arise, as well as how they cause neurodegeneration, is still not well understood. Research in the past four years has provided overwhelming evidence that aberrant phase separation and liquid-to-solid phase transitions are involved in the formation of pathological protein aggregates, opening new avenues for a potential treatment of so far incurable diseases.

8.1. Phase-Separating Proteins in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

The most prominent examples of neurodegenerative diseases that involve aberrant phase separation are the related disorders ALS and FTD, in which aberrant membraneless organelles and phase transitions of RNA-binding proteins appear to play a key role in pathogenesis (**Figure 3**). First, ALS-causing mutations in RNA-binding proteins, such as FUS, TDP-43, HNRNPA1, HNRNPA2, and TIA1, promote accumulation of the respective protein in membraneless compartments called stress granules (SGs) (24, 43, 45, 88, 99). Second, several proteins typically found in SGs, e.g., PABPC1, EIF4G1, and TIA1, are frequent components of pathological FUS and TDP-43 aggregates in postmortem brains of ALS and FTD patients (16, 45, 98). This finding suggests that SGs are precursors to pathological RNA-binding protein aggregates. Third, some ALS-linked mutations in RNA-binding proteins alter dynamics, transport, or size of SGs and neuronal RNP granules (2, 43, 59, 98, 99), supporting the idea of disturbed RNP granule dynamism in ALS and FTD. The physicochemical basis of this impaired RNP granule dynamism was proposed to be an altered phase separation behavior of disease-linked RNA-binding proteins. These proteins phase separate and form liquid-like droplets in vitro but over time transition from a liquid-like state to a solid-like state, a phenomenon that has been termed in vitro aging (123). ALS-linked mutations in the low-complexity domains of FUS, HNRNPA1, and TIA1 accelerate this transition (99, 108, 112, 123) and hence impair RNP granule dynamics.

Besides disease-causing mutations, aberrant PTMs on RNA-binding proteins are associated with pathological phase transitions in ALS and FTD (71). One example is arginine methylation of FUS. In the healthy human brain, the RGG/RG-rich IDRs of FUS are methylated on arginine residues (130, 143), but in brains of FTD patients they are unmethylated and monomethylated (44, 143). Loss of FUS arginine methylation promotes liquid–liquid phase separation and reduces the dynamism of FUS condensates in vitro and in cells (72, 127), most likely by enhancing interactions

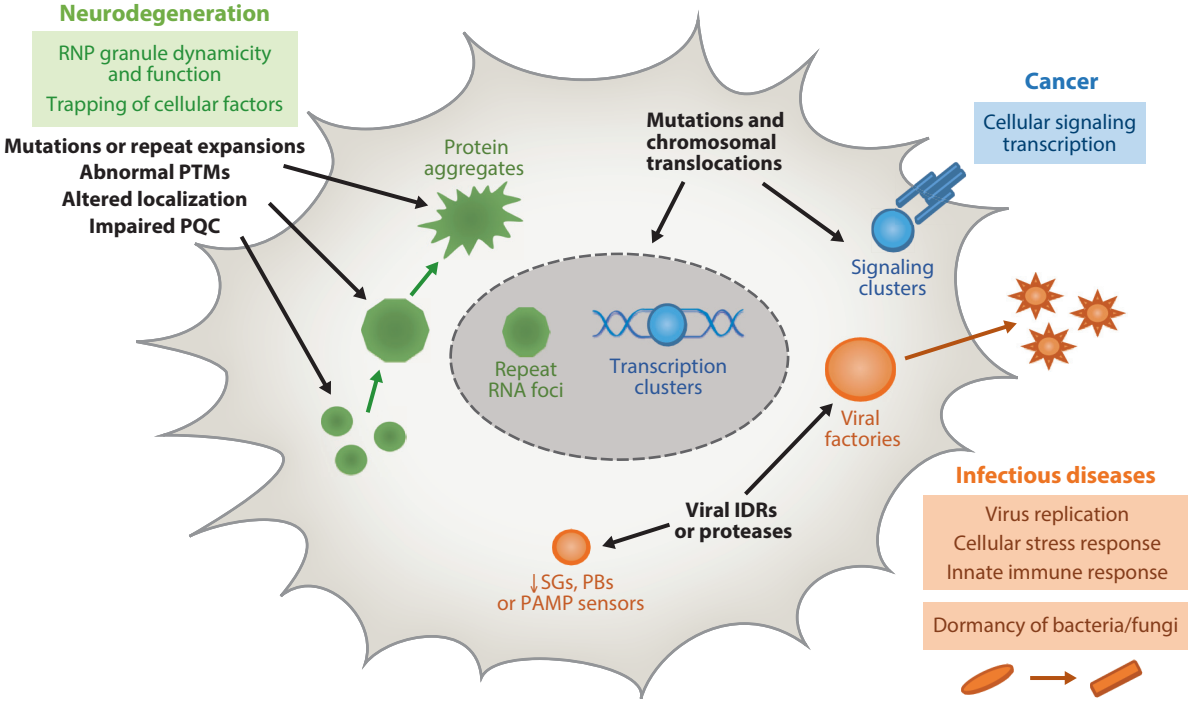

Figure 3

Evidence for disturbed phase separation and condensate formation in neurodegeneration, cancer, and infectious diseases. In neurodegenerative diseases, mutations or repeat expansions, abnormal posttranslational modifications (PTMs), altered subcellular localization, or impaired protein quality control (PQC) can promote the formation of ectopic condensates as well as their transition from a liquid-like state to a solid-like state, leading to pathological protein aggregates. This can impair the dynamism and function of ribonucleoprotein (RNP) granules and trap essential cellular factors, contributing to neuronal dysfunction and eventually neuronal cell death. In cancer, mutations in signaling receptors or chromosomal translocations involving an intrinsically disordered region (IDR) can alter the formation of signaling clusters or condensates at sites of transcription or DNA damage repair. This can alter cellular signaling cascades, drive aberrant transcriptional programs, or impair DNA damage repair, thus promoting a proliferative and malignant state of a cell. In viral infections, liquid-like condensates termed viral factories form de novo through phase separation of viral intrinsically disordered proteins, promoting viral genome replication or altering the antiviral immune response. Some antiviral sensors that detect pathogen-associated molecular patterns (PAMPs) form condensates upon binding to foreign DNA or RNA, thus stimulating an immune response. Some viruses also modulate cellular condensates, e.g., stress granules (SGs) and P bodies (PBs), by viral protease-induced cleavage of essential SG or PB proteins, thus suppressing the cellular stress response and innate immune response. Moreover, dormancy in bacteria and fungi is caused by a solidification of their cytoplasm, giving rise to dormant microbes that are resistant to cytostatic drugs.

between arginines and aromatic residues, which drive condensation of FUS (127, 152). Aberrant condensation caused by abnormal phosphorylation may also contribute to disease, as TDP-43 is hyperphosphorylated in ALS and FTD brains (63, 82). Moreover, PTMs on binding partners of phase-separating proteins may also play an important role; for example, binding of TDP-43 and FUS to polyADP ribose (PAR), which is present on many RNA-binding proteins, promotes phase separation of FUS and TDP-43 (8, 105, 123). Thus, PTMs can alter phase separation and aggregation of disease-linked proteins in *cis* and in *trans*. Targeting the respective PTM-modifying enzymes, e.g., with small molecules, is a promising therapeutic strategy, as recently exemplified by the neuroprotective effects of PAR polymerase inhibitors in cell and animal models of TDP-43-associated toxicity (105, 106).

Finally, a key concept that has become clear from ALS and FTD research is that altered subcellular localization can change the phase behavior of a protein and thus cause disease. This paradigm has been established for ALS-causing mutations in the nuclear localization signal (NLS) of FUS: They reduce binding of FUS to the nuclear import receptor Transportin, leading to impaired nuclear import and to cytosolic accumulation of FUS (45). The shift from the nucleus to the cytoplasm brings about several changes that affect the phase behavior of FUS. First, the concentration of FUS in the cytoplasm is elevated, favoring phase separation and liquid-to-solid transition of FUS (123). Second, the concentration of RNA is much lower in the cytoplasm than in the nucleus. Because RNA acts as a buffer and suppresses phase separation of RNA-binding proteins (100), the reduced RNA/protein levels in the cytoplasm further promote phase separation and solidification of mislocalized FUS. Altered phase behavior due to changes in protein localization is likely, as every subcellular compartment has its own protein quality control (PQC) system (20). In the cytoplasm, for example, nuclear import receptors fulfill an important PQC function, as they bind with high affinity to the NLSs in their cargo proteins and suppress their phase separation and aggregation (60, 72, 79, 156). Disturbances in such PQC mechanisms can cause disease; for instance, chaperoning of FUS by Transportin is disturbed both in ALS patients that carry FUS-NLS mutations and in FTD patients in whom Transportin is aggregated and functionally disabled (27, 41, 60, 72). One can envision that similar mechanisms operate in other diseases that are associated with the accumulation of a phase-separating protein in a specific subcellular compartment, e.g., the nucleus, cytoplasm, or endoplasmic reticulum (20). Thus, correcting protein mislocalization and boosting compartment-specific PQC mechanisms are also promising strategies for therapeutic intervention.

8.2. Phase-Separating Proteins in Alzheimer Disease and Tauopathies

Besides ALS and FTD, other prominent neurodegenerative diseases have been linked to aberrant phase transitions of proteins. For example, the microtubule-binding protein Tau, which forms neurofibrillary tangles in AD and other tauopathies, also undergoes liquid–liquid phase separation in vitro (9, 67, 153, 157). In a healthy neuron, Tau phase separation may have an important physiological function, namely locally concentrating tubulin and thus nucleating microtubule bundles (67). Disease-linked Tau mutations or abnormal PTMs shift the equilibrium toward the condensed state and promote subsequent hardening and aggregation: Genetic mutations in the Tau-encoding *MAPT* gene on chromosome 17 cause FTD with parkinsonism (FTDP-17) and promote phase separation of Tau (153). Similarly, hyperphosphorylation of Tau, as seen in AD and FTDP-17 patients, promotes Tau phase separation (9, 153). There is an intimate connection between Tau and phase-separating RNA-binding proteins, suggesting that aberrant RNP granules and disturbed phase behavior of RNA-binding proteins may play a wider role in neurodegenerative diseases: TIA1 and other RNA-binding proteins interact with Tau and accumulate together with Tau in AD and FTDP-17 patients or tauopathy mouse models (104, 150, 151). Phase separation provides a new framework to interpret these observations and allows us to pose new testable hypotheses to dissect the underlying molecular mechanisms.

8.3. Phase Separation and Repeat Expansion Disorders

Repeat expansion disorders, e.g., HD, and other $(CAG)_n$ expansion disorders, or the most common genetic form of ALS/FTD linked to a $(GGGGCC)_n$ expansion in the *C9ORF72* gene (42, 131), further exemplify aberrant phase separation linked to neurodegeneration. In all these disorders, the disease manifests only beyond a critical number of repeats within the gene (58) and

phase separation may provide an explanation for this (**Figure 3**). Two principal types of phase-separating molecules arise in repeat expansion disorders: (*a*) repeat RNAs and (*b*) repeat proteins that arise through repeat-associated noncanonical translation in all three reading frames (158, 159). Because of their repetitive nature, these sequences can engage in multivalent intermolecular interactions and therefore can form condensates by phase separation. Repeat-containing RNAs undergo gelation in vitro beyond a critical number of repeats, and in cells they form nuclear RNA foci that sequester essential cellular proteins, e.g., splicing factors (76). Blocking the formation of such aberrant condensates by inhibitors that disrupt RNA gelation (76) may be a viable strategy for treating repeat expansion disorders.

Toxic repeat proteins, such as polyQ and additional homopolymeric expansion proteins in HD (14) or dipeptide repeat (DPR) proteins, e.g., polyGR and polyPR, in *C9ORF72*-linked ALS/FTD (11, 110), may also contribute to disease by aberrant phase separation. PolyQ-expanded Huntingtin N-terminal fragment forms liquid-like assemblies that convert to solid-like assemblies over time, resembling the pathological aggregates in HD patients (125). Arginine-rich DPR proteins polyGR and polyPR undergo liquid–liquid phase separation with RNA (22) and interact with low-complexity domains of numerous cellular proteins, including disease-linked RNA-binding proteins (22, 94, 97). This perturbs their phase separation behavior and impairs the assembly of diverse membraneless organelles, such as stress granules and nucleoli (94). Thus, disturbed RNP granule dynamism due to altered phase behavior of RNA-binding proteins may be a common pathomechanism that contributes to diverse neurodegenerative diseases.

8.4. Quality Control of Phase-Separating Proteins and Membraneless Organelles

The examples described above illustrate that failure to control phase separation is at the heart of several neurodegenerative diseases. This raises the questions of how neurons normally control phase separation and the dynamism of membraneless organelles, and why neurons are especially vulnerable to aberrant phase transitions of biomolecules. Research in the last years has uncovered several quality control mechanisms that govern phase separation and ensure dynamism of condensates. Chaperones, such as the HspB8-Bag3-Hsp70 chaperone complex and Hsp27, which remodel RNP granules and keep them in a dynamic and fluid-like state (57, 103), are an important class of phase separation regulators. PQC factors in the ubiquitin–proteasome and autophagy pathways, e.g., VCP and UBQLN2 (7, 29, 39), compose another class. The dynamics of RNP granules are also controlled by ATP-consuming enzymes, such as RNA helicases (74, 76, 78), by nuclear import receptors (60, 72), and by microtubules and motor proteins (113). Not only ATP-driven molecular machines but also ATP itself control protein phase separation. At physiological concentrations of 5–10 mM, ATP acts as a biological hydrotrope (i.e., solubilizing agent) and prevents phase separation of RNA-binding proteins and even dissolves protein aggregates (65, 124). In summary, multiple ATP-dependent PQC mechanisms are in place to control phase separation and ensure dynamism of membraneless organelles. These mechanisms are especially important in neurons, as neurons have several special features.

8.5. The Vulnerability of Neurons to Aberrant Phase Transitions

Neurons are a special cell type. First, they are postmitotic; that is, they do not undergo cell division and therefore are unable to remove protein aggregates with cell division. Thus, neurons are especially dependent on efficient PQC systems, including the ones controlling phase separation and RNP granule dynamics described above. Impairment of one or several of these PQC mechanisms

can result in aberrant phase transitions and hence accumulation of protein aggregates inside neurons. Second, neurons are highly polarized cells with extensive, complex protrusions—usually one extended axon and numerous branched dendrites. Consequently, they have an enormous surface area and need to transport macromolecular complexes, such as RNP granules, ribosomes, and organelles, along microtubules into axons or dendrites. Third, neurons are electrically excitable and propagate action potentials to transmit information through electrical and chemical signals. These transport and signaling processes are highly energy consuming; hence, neurons have a high metabolic activity and consume large amounts of oxygen to produce large amounts of ATP. In fact, the brain consumes >20% of our baseline energy, even though it makes up only 2% of our body weight (129). Thus, neurons are especially dependent on ATP, and a drop in cellular ATP levels impairs the above-described ATP-dependent mechanisms controlling phase separation and RNP granule dynamics. Finally, a remarkable feature of neurons is their constant ability to undergo molecular, morphological, and functional changes. This is achieved through highly complex posttranscriptional gene regulation, including high levels of alternative splicing, RNA editing, messenger RNA (mRNA) transport, and local translation in axons and dendrites (73, 87). For example, in mature neurons, certain mRNAs are transported in neuronal RNP granules into axons and dendrites in a translationally repressed state but then are released from RNP granules at stimulated synapses and locally translated. This process is thought to be crucial for learning and memory (83), and it appears to be regulated by phase separation, as recently suggested for the translational repressor FMRP (fragile X mental retardation protein) (148). Thus, translational control through phase separation, including local translation at synapses, is a crucial process in neurons that most likely becomes impaired when cells are unable to control phase separation and RNP granule dynamism.

In most diseases, including neurodegenerative diseases, aging is the most common risk factor (115). Conspicuously, many of the PQC mechanisms described above that control phase separation decline with aging. Aging has numerous effects on cell homeostasis, including the decline of mitochondrial activity and, consequently, a drop in ATP levels. The latter is expected to impair the activity of the ATP-dependent molecular machines that control phase separation and RNP granule dynamics (e.g., chaperones and RNA helicases), as well as the activity of ATP as a biological hydrotrope. On the one hand, this causes aberrant phase transitions of RNA-binding proteins and formation of pathological protein aggregates as seen in diverse neurodegenerative diseases. On the other hand, this impairs the dynamism, and potentially the key biological functions, of RNP granules. Interfering with the age-related decline in PQC mechanisms and ATP levels could therefore be a promising strategy to reverse aberrant phase transitions and treat neurodegenerative diseases and age-related cognitive decline.

9. PHASE SEPARATION AND CANCER

Cancer is a complex disease with many different genetic causes. In recent years, there has been remarkable progress in our understanding of the molecular underpinnings of cancer. Malignant cells exhibit several key hallmarks that allow these cells to survive and multiply; they must sustain their ability to proliferate, evade growth suppressors, enable replicative immortality, resist cell death, induce angiogenesis, and activate invasion and metastasis (61). Cancer mutations typically introduce an aberrant activity that drives these processes or they reduce the activity of an inhibitor of these processes. However, we often do not understand mechanistically why a certain mutation causes cancer. Phase separation provides a new framework to interpret and understand cancer phenotypes, with potentially new avenues for treatment (**Figure 3**).

The key alteration that every cancer cell must accomplish is to enter into and maintain a highly proliferative state. There is ample genetic evidence that diverse growth factor signaling pathways are hyperactive in cancer cells (61). However, why these signaling pathways are constitutively active is often unclear. Recent studies have highlighted the role of phase separation and condensate formation in regulating cellular signaling. One example is the T cell receptor (TCR) signaling pathway that operates in immune cells. When the TCR is activated, downstream effector proteins spontaneously phase separate into dynamic membrane-associated clusters (142). These signaling clusters are interesting for various reasons. First, they facilitate specific signaling outputs at the membrane where the signal was received. Second, they provide a specialized microenvironment that allows access of certain proteins while excluding others. In the specific case of the TCR, kinases such as ZAP70 can enter the clusters, whereas phosphatases, such as CD45, cannot. This ability enables signaling clusters to amplify an incoming signal. These findings are highly relevant for cancer because the TCR is similar to ligand-activated receptor tyrosine kinases (RTKs) that are often mutated in cancer cells (95). One can envision that cancer mutations promote the formation of aberrant RTK-associated signaling clusters with abnormal composition or properties, which activate oncogenic downstream signaling events. Blocking the formation of such aberrant condensates by targeting scaffold proteins or upstream regulators of condensate assembly may be a viable strategy to stop such oncogenic signaling.

Another example in which a specific signaling outcome is achieved by phase separation involves the cyclic GMP-AMP synthase (cGAS). Phase separation activates cGAS and this leads to the production of the second messenger cyclic GMP-AMP (cGAMP) and the induction of the innate immune response (46). Activation of cGAS requires binding of foreign DNA to the DNA-binding domain of cGAS to unleash multivalent interactions that promote phase separation. To allow for rapid activation in the presence of foreign DNA, the cGAS scaffold protein must persist in a supersaturated state in the cytoplasm. Many other signaling pathways appear to employ similar mechanisms for which activation depends on binding of a ligand to a supersaturated scaffold protein. Cancer mutations could change the properties of such signaling scaffolds, so that they become independent of ligand binding and constitutively assemble into condensates. This could occur, for example, through mutations that alter the saturation concentration or multivalence of these proteins. Manipulating the phase behavior of such oncogenic proteins through specific drugs could be a promising approach for treatment.

Only one study so far has directly investigated the link between protein phase separation and cancer (25). In this study, cancer mutations in the tumor suppressor SPOP (speckle-type BTB/POZ protein) were linked to specific phase separation defects. SPOP is a substrate adaptor of the cullin 3 RING ubiquitin ligase (CRL3) that targets various proto-oncogenic proteins for ubiquitination and subsequent proteasomal degradation. SPOP self-associates into large higher-order oligomers, and this self-association is required for SPOP to localize to nuclear membraneless organelles (102). However, self-association is not sufficient to drive SPOP phase separation; substrate binding to SPOP is also required for SPOP phase separation (25). Importantly, cancer mutations disrupt SPOP substrate interactions and consequently SPOP phase separation and localization to membraneless organelles. This has dramatic consequences because cells accumulate oncogenic SPOP substrate proteins that then promote cell growth. A potential avenue for treatment is to restore SPOP localization to membraneless organelles, for example, by decreasing the saturation concentration of SPOP.

One key hallmark of cancer is the dysregulation of transcription. Numerous cancer phenotypes, including those affecting the clinical progression and responsiveness to treatments, are likely to be driven by dysregulated transcriptional programs. Many human cancers, for example, exhibit defects that lead to transcriptional dysregulation of the MYC oncoprotein (18, 49, 77). Targeting

transcription factors such as MYC appears to be a viable option to treat these types of cancers (140), but these efforts have failed so far because of our limited mechanistic understanding of how MYC oncogenes regulate gene expression.

The emergence of phase separation provides a new opportunity for understanding the molecular underpinnings of transcriptional dysregulation by MYC and other oncogenes. Indeed, a series of recent studies have raised the possibility that condensate formation is critical for regulation of transcription (21, 23, 32, 34, 70, 134). The research focus so far has been on clusters of enhancers known as superenhancers. Superenhancers regulate genes that play prominent roles in cell identity or specialized cellular functions (31, 68, 69, 154). Coactivator proteins such as MED1 and BRD4 form phase-separated condensates at superenhancers (134). Condensates of in vitro–reconstituted superenhancers enriched key components of the transcriptional machinery, such as the mediator complex, suggesting that condensate formation at superenhancers may be associated with gene activation. In support of this view, activation domains of transcription factors such as Oct4 and Gcn4 often carry long IDRs (23). IDR-driven phase separation may therefore be a general mechanism for how transcription factors promote gene expression at active genes.

In this context, many malignancies arise because of chromosomal translocations that result in new gene products. These gene products are fusion proteins in which a DNA- or chromatin-binding domain is connected to an IDR of a different protein. One example is the fusion between the IDR of EWS and the FLI protein in Ewing's sarcoma (26); another example is the fusion between the IDR of FUS and various DNA-binding domains in a specific type of liposarcoma (38). Phase separation provides a mechanism by which these fusion proteins could drive aberrant gene expression programs. Blocking the formation of such aberrant condensates with specific drugs could lead to new therapeutic treatments.

10. PHASE SEPARATION AND INFECTIOUS DISEASES

There is emerging evidence that condensates are involved in infectious diseases (**Figure 3**). Condensates are frequently used by pathogens to maximize their ability to propagate, but they are also often employed by host cells in connection with defense mechanisms that detect and neutralize pathogens. In fact, many viruses induce the formation of compartments upon infection of their host cell (114, 119, 135). These compartments are termed viral factories, viroplasm, or viral replication centers, and they often contain factors for viral genome replication and gene expression as well as components of the antiviral immune response. This has led to the proposal that these compartments promote the production of virus components and allow viruses to evade the immune system. For a long time, we did not know how viral compartments are formed, but recent evidence suggests that they are condensates that form by phase separation. For instance, the compartments formed upon infection by vesicular stomatitis virus (VSV) have liquid-like properties, and expression of three proteins of the VSV replication machinery in cells is sufficient to drive the formation of viral compartments by phase separation (66). Similarly, inclusions called Negri bodies that form in the cytoplasm of rabies virus–infected cells are liquid-like organelles, and their formation is driven by an IDR in the rabies virus P protein, promoting viral genome replication (116). Targeting viral factors that promote condensate formation therefore appears to be a promising therapeutic approach to treat viral infections.

Viral infections cause many cellular changes. Infected cells turn on the production of interferons that drive an antiviral gene expression program; they also frequently form cytoplasmic RNA condensates, suggesting a link between condensate formation and the antiviral response (121, 126, 147). Indeed, many viruses modulate the formation of RNA condensates in cells. Most of the evidence collected thus far has been for stress granules (SGs) and processing bodies (PBs), two

compartments involved in the regulation of mRNAs. PBs are constitutive compartments, whereas SGs form upon exposure of cells to various forms of stress (10, 30). SG formation involves phosphorylation of the translation initiation factor eIF2α, which leads to translational arrest (85). SGs and PBs can be differentiated on the basis of the components residing in them. For instance, SGs contain mRNAs and translation initiation factors such as eIF4G and eIF3, whereas PBs contain factors of the mRNA decapping machinery such as Dcp1 and Dcp2, as well as deadenylation factors such as Ccr1. Accordingly, SGs have been proposed to store mRNAs, while PBs are thought to promote mRNA decay and processing (10, 28).

Viruses use various strategies to maximize the number of produced viral particles, and this frequently involves mechanisms that manipulate SG formation. For instance, many viruses produce factors that inhibit SG formation or that promote changes in SG composition (121, 126, 147). These virus-induced changes often suppress the cellular stress response and promote viral replication. The molecular pathways that are targeted are diverse. Some viruses prevent the phosphorylation of eIF2α; others manipulate the SG proteins G3BP1 and G3BP2 (hereafter called G3BP) (126). G3BP is an RNA-binding protein that is absolutely required for SG formation (146); it promotes the assembly of SGs by binding to free RNA that accumulates upon stress and viral infections (84). G3BP is often cleaved by virus-encoded proteases or sequestered in an inactive state by viral proteins. Similar mechanisms appear to apply to PBs. For example, there have been reports of virus-encoded proteases that cleave key PB proteins such as Dcp1a (126).

As already hinted at above, there are interesting links between RNA condensates and the innate immune response. For example, several components of the innate immune system, such as the double-stranded RNA-dependent protein kinase R, RIG-I (retinoic acid inducible gene I), and RNase L, localize to SGs (126). Moreover, there is a strong correlation between SG formation and interferon production. This suggests that SGs may have an antiviral role by functioning as platforms for signaling and innate immune responses. Indeed, SG formation is reminiscent of the mechanism of condensation of the antiviral cGAS. cGAS is a member of a large family of sensors that detect viral invasion and replication (121). These sensors recognize pathogen-associated molecular patterns (PAMPs), such as double-stranded DNA or RNA, in the cytoplasm. Condensate formation by phase separation appears to be an ideal mechanism to detect PAMPs and then mount adaptive cellular responses. Thus, we predict that phase separation is a frequently used mechanism to detect nonself molecules in infected cells and activate the innate immune response. Modulating the formation of these immune response condensates through drugs may be a new avenue to bolster the antiviral defense of cells.

Beyond viral infections, condensates are also associated with diseases caused by bacteria and fungi. One problem with treating bacterial and fungal infections is the presence of persisting or dormant cells that are resistant to treatment with cytostatic drugs (51). There now is increasing evidence that dormancy in bacteria and fungi is associated with a phase transition in which large parts of the cytoplasm solidify (111, 122). Importantly, solidification of the cytoplasm appears to be required for survival in stress conditions, but why the solid-like state of the cytoplasm is protective is still unclear. It has been proposed that condensate formation may protect macromolecules from damage or may help modulate the activity of macromolecules (128). There are important differences between higher and lower organisms in terms of their ability to regulate the material properties of the cytoplasm. Whereas bacteria and fungi appear to be able to reversibly solidify their cytoplasm, mammalian cells prefer to keep their cytoplasm in a liquid-like state (89, 111, 128). Targeting the molecular pathways that regulate dormancy-promoting phase transitions in bacteria and fungi therefore presents exciting new opportunities for therapeutic interventions.

11. CONCLUDING REMARKS

We have made rapid progress in recent years in identifying the genetic causes of various human diseases. However, it is often unclear why a genetic mutation causes a specific disease phenotype. This inability to mechanistically explain complex disease phenotypes may be due in part to our current focus on individual molecules. The dominant paradigm of molecular biology has tried to explain complex disease phenotypes through alterations in the structure or function of single proteins or nucleic acids. However, there is increasing evidence that many cellular phenomena cannot be explained through the action of single molecules. Rather, many cellular structures, processes, and functions emerge from the multiple interactions within a collective of molecules. This generates an urgent need for new concepts that appropriately describe the behavior of biological multicomponent systems.

In this review, we have laid out the arguments that phase separation provides a useful new framework to describe and comprehend emergent phenomena in living cells. The abundance of cellular structures that form by phase separation is astounding (12); it is possible that more than 30% of the human proteome is associated with condensates. A mechanism that affects so many different proteins is guaranteed to play fundamental roles in normal physiology and also in disease. By investigating human diseases through the lens of phase separation, we are likely to uncover many new pathomechanisms that have so far been elusive.

Phase separation not only will help us understand complex disease mechanisms but also promises to open up exciting new avenues for therapeutic intervention. The rapid identification of condensate-altering drugs seems realistic given that condensate formation is a phenotype that is readily screenable by imaging technologies. We are optimistic that such screening campaigns will soon lead to the identification of new classes of therapeutics that will help us treat some of the toughest human diseases, including cancer, neurodegeneration, and infectious diseases.

DISCLOSURE STATEMENT

Simon Alberti is a scientific advisor of Dewpoint Therapeutics.

ACKNOWLEDGMENTS

We thank Saskia Hutten and Lara Silva for helpful discussions and comments on an earlier draft of the article. Dorothee Dormann acknowledges funding by the Deutsche Forschungsgemeinschaft (Emmy Noether grants DO1804/1-1 and DO1804/1-2, DO1804/3-1, DO1804/4-1) and under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy – ID 390857198), as well as funding by the Fritz Thyssen Foundation and the Paul Ehrlich Foundation. Simon Alberti acknowledges funding by the Max-Planck Society, the BIOTEC (TU Dresden), the European Research Council (no. 725836), the Bundesministerium für Bildung und Forschung (BMBF) (01ED1601A, 031A359A), the EU Joint Programme – Neurodegenerative Disease Research (JPND) (CureALS), the Human Frontier Science Program (RGP0034/2017), and the Volkswagen Life? Initiative.

LITERATURE CITED

1. Aguzzi A, Altmeyer M. 2016. Phase separation: linking cellular compartmentalization to disease. *Trends Cell Biol*. 26:547–58
2. Alami NH, Smith RB, Carrasco MA, Williams LA, Winborn CS, et al. 2014. Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron* 81:536–43

3. Alberti S, Carra S. 2018. Quality control of membraneless organelles. *J. Mol. Biol.* 430:4711–29
4. Alberti S, Gladfelter A, Mittag T. 2019. Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. *Cell* 176:419–34
5. Alberti S, Halfmann R, King O, Kapila A, Lindquist S. 2009. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. *Cell* 137:146–58
6. Alberti S, Hyman AA. 2016. Are aberrant phase transitions a driver of cellular aging? *Bioessays* 38:959–68
7. Alexander EJ, Ghanbari Niaki A, Zhang T, Sarkar J, Liu Y, et al. 2018. Ubiquilin 2 modulates ALS/FTD-linked FUS-RNA complex dynamics and stress granule formation. *PNAS* 115:E11485–94
8. Altmeyer M, Neelsen KJ, Teloni F, Pozdnyakova I, Pellegrino S, et al. 2015. Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). *Nat. Commun.* 6:8088
9. Ambadipudi S, Biernat J, Riedel D, Mandelkow E, Zweckstetter M. 2017. Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. *Nat. Commun.* 8:275
10. Anderson P, Kedersha N. 2009. RNA granules: post-transcriptional and epigenetic modulators of gene expression. *Nat. Rev. Mol. Cell Biol.* 10:430–36
11. Ash PEA, Bieniek KF, Gendron TF, Caulfield T, Lin W-L, et al. 2013. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron* 77:639–46
12. Banani SF, Lee HO, Hyman AA, Rosen MK. 2017. Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* 18:285–98
13. Banani SF, Rice AM, Peeples WB, Lin Y, Jain S, et al. 2016. Compositional control of phase-separated cellular bodies. *Cell* 166:651–63
14. Bañez-Coronel M, Ayhan F, Tarabochia AD, Zu T, Perez BA, et al. 2015. RAN translation in Huntington disease. *Neuron* 88:667–77
15. Banjade S, Rosen MK. 2014. Phase transitions of multivalent proteins can promote clustering of membrane receptors. *eLife* 3:e04123
16. Bentmann E, Neumann M, Tahirovic S, Rodde R, Dormann D, Haass C. 2012. Requirements for stress granule recruitment of fused in sarcoma (FUS) and TAR DNA-binding protein of 43 kDa (TDP-43). *J. Biol. Chem.* 287:23079–94
17. Bergeron-Sandoval LP, Heris HK, Hendricks AG. 2017. Endocytosis caused by liquid-liquid phase separation of proteins. bioRxiv 145664. **https://doi.org/10.1101/145664**
18. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, et al. 2010. The landscape of somatic copy-number alteration across human cancers. *Nature* 463:899–905
19. Berry J, Weber SC, Vaidya N, Haataja M, Brangwynne CP. 2015. RNA transcription modulates phase transition-driven nuclear body assembly. *PNAS* 112:E5237–45
20. Bertolotti A. 2018. Importance of the subcellular location of protein deposits in neurodegenerative diseases. *Curr. Opin. Neurobiol.* 51:127–33
21. Boehning M, Dugast-Darzacq C, Rankovic M, Hansen AS, Yu T-K, et al. 2018. RNA polymerase II clustering through carboxy-terminal domain phase separation. *Nat. Struct. Mol. Biol.* 25:833–40
22. Boeynaems S, Bogaert E, Kovacs D, Konijnenberg A, Timmerman E, et al. 2017. Phase separation of *C9orf72* dipeptide repeats perturbs stress granule dynamics. *Mol. Cell* 65:1044–55.e5
23. Boija A, Klein IA, Sabari BR, Dall'Agnese A, Coffey EL, et al. 2018. Transcription factors activate genes through the phase-separation capacity of their activation domains. *Cell* 175:1842–55.e16
24. Bosco DA, Lemay N, Ko HK, Zhou H, Burke C, et al. 2010. Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. *Hum. Mol. Genet.* 19:4160–75
25. Bouchard JJ, Otero JH, Scott DC, Szulc E, Martin EW, et al. 2018. Cancer mutations of the tumor suppressor SPOP disrupt the formation of active, phase-separated compartments. *Mol. Cell* 72:19–36.e8
26. Boulay G, Sandoval GJ, Riggi N, Iyer S, Buisson R, et al. 2017. Cancer-specific retargeting of BAF complexes by a prion-like domain. *Cell* 171:163–78.e19
27. Brelstaff J, Lashley T, Holton JL, Lees AJ, Rossor MN, et al. 2011. Transportin1: a marker of FTLD-FUS. *Acta Neuropathol.* 122:591–600
28. Brengues M, Teixeira D, Parker R. 2005. Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. *Science* 310:486–89

29. Buchan JR, Kolaitis R-M, Taylor JP, Parker R. 2013. Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. *Cell* 153:1461–74
30. Buchan JR, Parker R. 2009. Eukaryotic stress granules: the ins and outs of translation. *Mol. Cell* 36:932–41
31. Chapuy B, McKeown MR, Lin CY, Monti S, Roemer MGM, et al. 2013. Discovery and characterization of super-enhancer-associated dependencies in diffuse large B cell lymphoma. *Cancer Cell* 24:777–90
32. Cho W-K, Spille J-H, Hecht M, Lee C, Li C, et al. 2018. Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. *Science* 361:412–15
33. Chong PA, Vernon RM, Forman-Kay JD. 2018. RGG/RG motif regions in RNA binding and phase separation. *J. Mol. Biol.* 430:4650–65
34. Chong S, Dugast-Darzacq C, Liu Z, Dong P, Dailey GM, et al. 2018. Imaging dynamic and selective low-complexity domain interactions that control gene transcription. *Science* 361:eaar2555
35. Ciryam P, Kundra R, Morimoto RI, Dobson CM, Vendruscolo M. 2015. Supersaturation is a major driving force for protein aggregation in neurodegenerative diseases. *Trends Pharmacol. Sci.* 36:72–77
36. Ciryam P, Tartaglia GG, Morimoto RI, Dobson CM, Vendruscolo M. 2013. Widespread aggregation and neurodegenerative diseases are associated with supersaturated proteins. *Cell Rep.* 5:781–90
37. Conicella AE, Zerze GH, Mittal J, Fawzi NL. 2016. ALS mutations disrupt phase separation mediated by α-helical structure in the TDP-43 low-complexity C-terminal domain. *Structure* 24:1537–49
38. Crozat A, Aman P, Mandahl N, Ron D. 1993. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature* 363:640–44
39. Dao TP, Kolaitis R-M, Kim HJ, O'Donovan K, Martyniak B, et al. 2018. Ubiquitin modulates liquid-liquid phase separation of UBQLN2 via disruption of multivalent interactions. *Mol. Cell* 69:965–78.e6
40. Das RK, Ruff KM, Pappu RV. 2015. Relating sequence encoded information to form and function of intrinsically disordered proteins. *Curr. Opin. Struct. Biol.* 32:102–12
41. Davidson YS, Robinson AC, Hu Q, Mishra M, Baborie A, et al. 2013. Nuclear carrier and RNA-binding proteins in frontotemporal lobar degeneration associated with fused in sarcoma (FUS) pathological changes. *Neuropathol. Appl. Neurobiol.* 39:157–65
42. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, et al. 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of *C9ORF72* causes chromosome 9p-linked FTD and ALS. *Neuron* 72:245–56
43. Dewey CM, Cenik B, Sephton CF, Dries DR, Mayer P 3rd, et al. 2011. TDP-43 is directed to stress granules by sorbitol, a novel physiological osmotic and oxidative stressor. *Mol. Cell. Biol.* 31:1098–108
44. Dormann D, Madl T, Valori CF, Bentmann E, Tahirovic S, et al. 2012. Arginine methylation next to the PY-NLS modulates transportin binding and nuclear import of FUS. *EMBO J.* 31:4258–75
45. Dormann D, Rodde R, Edbauer D, Bentmann E, Fischer I, et al. 2010. ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. *EMBO J.* 29:2841–57
46. Du M, Chen ZJ. 2018. DNA-induced liquid phase condensation of cGAS activates innate immune signaling. *Science* 361:704–9
47. Ellis RJ. 2001. Macromolecular crowding: an important but neglected aspect of the intracellular environment. *Curr. Opin. Struct. Biol.* 11:114–19
48. Falahati H, Wieschaus E. 2017. Independent active and thermodynamic processes govern the nucleolus assembly in vivo. *PNAS* 114:1335–40
49. Felsher DW, Bishop JM. 1999. Reversible tumorigenesis by MYC in hematopoietic lineages. *Mol. Cell* 4:199–207
50. Feric M, Vaidya N, Harmon TS, Mitrea DM, Zhu L, et al. 2016. Coexisting liquid phases underlie nucleolar subcompartments. *Cell* 165:1686–97
51. Fisher RA, Gollan B, Helaine S. 2017. Persistent bacterial infections and persister cells. *Nat. Rev. Microbiol.* 15:453–64
52. Forman-Kay JD, Kriwacki RW, Seydoux G. 2018. Phase separation in biology and disease. *J. Mol. Biol.* 430:4603–6
53. Franzmann T, Alberti S. 2018. Prion-like low-complexity sequences: key regulators of protein solubility and phase behavior. *J. Biol. Chem.* **https://doi.org/10.1074/jbc.TM118.001190**

54. Franzmann TM, Alberti S. 2019. Protein phase separation as a stress survival strategy. *Cold Spring Harb. Perspect. Biol.* **https://doi.org/10.1101/cshperspect.a034058**
55. Franzmann TM, Jahnel M, Pozniakovsky A, Mahamid J, Holehouse AS, et al. 2018. Phase separation of a yeast prion protein promotes cellular fitness. *Science* 359:eaao5654
56. Gan L, Cookson MR, Petrucelli L, La Spada AR. 2018. Converging pathways in neurodegeneration, from genetics to mechanisms. *Nat. Neurosci.* 21:1300–9
57. Ganassi M, Mateju D, Bigi I, Mediani L, Poser I, et al. 2016. A surveillance function of the HSPB8-BAG3-HSP70 chaperone complex ensures stress granule integrity and dynamism. *Mol. Cell* 63:796–810
58. Gatchel JR, Zoghbi HY. 2005. Diseases of unstable repeat expansion: mechanisms and common principles. *Nat. Rev. Genet.* 6:743–55
59. Gopal PP, Nirschl JJ, Klinman E, Holzbaur ELF. 2017. Amyotrophic lateral sclerosis-linked mutations increase the viscosity of liquid-like TDP-43 RNP granules in neurons. *PNAS* 114:E2466–75
60. Guo L, Kim HJ, Wang H, Monaghan J, Freyermuth F, et al. 2018. Nuclear-import receptors reverse aberrant phase transitions of RNA-binding proteins with prion-like domains. *Cell* 173:677–92.e20
61. Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. *Cell* 144:646–74
62. Harmon TS, Holehouse AS, Rosen MK, Pappu RV. 2017. Intrinsically disordered linkers determine the interplay between phase separation and gelation in multivalent proteins. *eLife* 6:e30294
63. Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, et al. 2008. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann. Neurol.* 64:60–70
64. Hayden E, Cone A, Ju S. 2017. Supersaturated proteins in ALS. *PNAS* 114:5065–66
65. Hayes MH, Peuchen EH, Dovichi NJ, Weeks DL. 2018. Dual roles for ATP in the regulation of phase separated protein aggregates in *Xenopus* oocyte nucleoli. *eLife* 7:e35224
66. Heinrich BS, Maliga Z, Stein DA, Hyman AA, Whelan SPJ. 2018. Phase transitions drive the formation of vesicular stomatitis virus replication compartments. *mBio* 9:e02290-17
67. Hernández-Vega A, Braun M, Scharrel L, Jahnel M, Wegmann S, et al. 2017. Local nucleation of microtubule bundles through tubulin concentration into a condensed Tau phase. *Cell Rep.* 20:2304–12
68. Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-André V, et al. 2013. Super-enhancers in the control of cell identity and disease. *Cell* 155:934–47
69. Hnisz D, Schuijers J, Lin CY, Weintraub AS, Abraham BJ, et al. 2015. Convergence of developmental and oncogenic signaling pathways at transcriptional super-enhancers. *Mol. Cell* 58:362–70
70. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA. 2017. A phase separation model for transcriptional control. *Cell* 169:13–23
71. Hofweber M, Dormann D. 2018. Friend or foe—post-translational modifications as regulators of phase separation and RNP granule dynamics. *J. Biol. Chem.* **https://doi.org/10.1074/jbc.TM118.001189**
72. Hofweber M, Hutten S, Bourgeois B, Spreitzer E, Niedner-Boblenz A, et al. 2018. Phase separation of FUS is suppressed by its nuclear import receptor and arginine methylation. *Cell* 173:706–19.e13
73. Holt CE, Schuman EM. 2013. The central dogma decentralized: new perspectives on RNA function and local translation in neurons. *Neuron* 80:648–57
74. Hubstenberger A, Noble SL, Cameron C, Evans TC. 2013. Translation repressors, an RNA helicase, and developmental cues control RNP phase transitions during early development. *Dev. Cell* 27:161–73
75. Hyman AA, Weber CA, Jülicher F. 2014. Liquid-liquid phase separation in biology. *Annu. Rev. Cell Dev. Biol.* 30:39–58
76. Jain A, Vale RD. 2017. RNA phase transitions in repeat expansion disorders. *Nature* 546:243–47
77. Jain M, Arvanitis C, Chu K, Dewey W, Leonhardt E, et al. 2002. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. *Science* 297:102–4
78. Jain S, Wheeler JR, Walters RW, Agrawal A, Barsic A, Parker R. 2016. ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* 164:487–98
79. Jäkel S, Mingot J-M, Schwarzmaier P, Hartmann E, Görlich D. 2002. Importins fulfill a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains. *EMBO J.* 21:377–86
80. Jensen MR, Ruigrok RWH, Blackledge M. 2013. Describing intrinsically disordered proteins at atomic resolution by NMR. *Curr. Opin. Struct. Biol.* 23:426–35

81. Jucker M, Walker LC. 2018. Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases. *Nat. Neurosci.* 21:1341–49
82. Kametani F, Obi T, Shishido T, Akatsu H, Murayama S, et al. 2016. Mass spectrometric analysis of accumulated TDP-43 in amyotrophic lateral sclerosis brains. *Sci. Rep.* 6:23281
83. Kandel ER, Dudai Y, Mayford MR. 2014. The molecular and systems biology of memory. *Cell* 157:163–86
84. Kedersha N, Panas MD, Achorn CA, Lyons S, Tisdale S, et al. 2016. G3BP–Caprin1–USP10 complexes mediate stress granule condensation and associate with 40S subunits. *J. Cell Biol.* 212:845–60
85. Kedersha NL, Gupta M, Li W, Miller I, Anderson P. 1999. RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2α to the assembly of mammalian stress granules. *J. Cell Biol.* 147:1431–42
86. Khan T, Kandola TS, Wu J, Venkatesan S, Ketter E, et al. 2018. Quantifying nucleation in vivo reveals the physical basis of prion-like phase behavior. *Mol. Cell* 71:155–68.e7
87. Kiebler MA, Scheiffele P, Ule J. 2013. What, where, and when: the importance of post-transcriptional regulation in the brain. *Front. Neurosci.* 7:192
88. Kim HJ, Kim NC, Wang Y-D, Scarborough EA, Moore J, et al. 2013. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature* 495:467–73
89. Kroschwald S, Maharana S, Mateju D, Malinovska L, Nüske E, et al. 2015. Promiscuous interactions and protein disaggregases determine the material state of stress-inducible RNP granules. *eLife* 4:e06807
90. Kroschwald S, Munder MC, Maharana S, Franzmann TM, Richter D, et al. 2018. Different material states of Pub1 condensates define distinct modes of stress adaptation and recovery. *Cell Rep.* 23:3327–39
91. Kwon I, Kato M, Xiang S, Wu L, Theodoropoulos P, et al. 2013. Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains. *Cell* 155:1049–60
92. Langdon EM, Qiu Y, Ghanbari Niaki A, McLaughlin GA, Weidmann C, et al. 2018. mRNA structure determines specificity of a polyQ-driven phase separation. *Science* 360:922–27
93. Larson AG, Elnatan D, Keenen MM, Trnka MJ, Johnston JB, et al. 2017. Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. *Nature* 547:236–40
94. Lee K-H, Zhang P, Kim HJ, Mitrea DM, Sarkar M, et al. 2016. C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. *Cell* 167:774–88.e17
95. Lemmon MA, Schlessinger J. 2010. Cell signaling by receptor tyrosine kinases. *Cell* 141:1117–34
96. Li P, Banjade S, Cheng H-C, Kim S, Chen B, et al. 2012. Phase transitions in the assembly of multivalent signalling proteins. *Nature* 483:336–40
97. Lin Y, Mori E, Kato M, Xiang S, Wu L, et al. 2016. Toxic PR poly-dipeptides encoded by the C9orf72 repeat expansion target LC domain polymers. *Cell* 167:789–802.e12
98. Liu-Yesucevitz L, Bilgutay A, Zhang Y-J, Vanderwyde T, Citro A, et al. 2010. Tar DNA binding protein-43 (TDP-43) associates with stress granules: analysis of cultured cells and pathological brain tissue. *PLOS ONE* 5:e13250
99. Mackenzie IR, Nicholson AM, Sarkar M, Messing J, Purice MD, et al. 2017. TIA1 mutations in amyotrophic lateral sclerosis and frontotemporal dementia promote phase separation and alter stress granule dynamics. *Neuron* 95:808–16.e9
100. Maharana S, Wang J, Papadopoulos DK, Richter D, Pozniakovsky A, et al. 2018. RNA buffers the phase separation behavior of prion-like RNA binding proteins. *Science* 360:918–21
101. March ZM, King OD, Shorter J. 2016. Prion-like domains as epigenetic regulators, scaffolds for subcellular organization, and drivers of neurodegenerative disease. *Brain Res.* 1647:9–18
102. Marzahn MR, Marada S, Lee J, Nourse A, Kenrick S, et al. 2016. Higher-order oligomerization promotes localization of SPOP to liquid nuclear speckles. *EMBO J.* 35:1254–75
103. Mateju D, Franzmann TM, Patel A, Kopach A, Boczek EE, et al. 2017. An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *EMBO J.* 36:1669–87
104. Maziuk BF, Apicco DJ, Cruz AL, Jiang L, Ash PEA, et al. 2018. RNA binding proteins co-localize with small tau inclusions in tauopathy. *Acta Neuropathol. Commun.* 6:71

105. McGurk L, Gomes E, Guo L, Mojsilovic-Petrovic J, Tran V, et al. 2018. Poly(ADP-ribose) prevents pathological phase separation of TDP-43 by promoting liquid demixing and stress granule localization. *Mol. Cell* 71:703–17.e9
106. McGurk L, Mojsilovic-Petrovic J, Van Deerlin VM, Shorter J, Kalb RG, et al. 2018. Nuclear poly(ADP-ribose) activity is a therapeutic target in amyotrophic lateral sclerosis. *Acta Neuropathol. Commun.* 6:84
107. Mitrea DM, Cika JA, Guy CS, Ban D, Banerjee PR, et al. 2016. Nucleophosmin integrates within the nucleolus via multi-modal interactions with proteins displaying R-rich linear motifs and rRNA. *eLife* 5:e13571
108. Molliex A, Temirov J, Lee J, Coughlin M, Kanagaraj AP, et al. 2015. Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell* 163:123–33
109. Monahan Z, Ryan VH, Janke AM, Burke KA, Rhoads SN, et al. 2017. Phosphorylation of the FUS low-complexity domain disrupts phase separation, aggregation, and toxicity. *EMBO J.* 36:2951–67
110. Mori K, Weng S-M, Arzberger T, May S, Rentzsch K, et al. 2013. The *C9orf72* GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science* 339:1335–38
111. Munder MC, Midtvedt D, Franzmann T, Nüske E, Otto O, et al. 2016. A pH-driven transition of the cytoplasm from a fluid- to a solid-like state promotes entry into dormancy. *eLife* 5:e09347
112. Murakami T, Qamar S, Lin JQ, Schierle GSK, Rees E, et al. 2015. ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron* 88:678–90
113. Nadezhdina ES, Lomakin AJ, Shpilman AA, Chudinova EM, Ivanov PA. 2010. Microtubules govern stress granule mobility and dynamics. *Biochim. Biophys. Acta Mol. Cell Res.* 1803:361–71
114. Netherton CL, Wileman T. 2011. Virus factories, double membrane vesicles and viroplasm generated in animal cells. *Curr. Opin. Virol.* 1:381–87
115. Niccoli T, Partridge L. 2012. Ageing as a risk factor for disease. *Curr. Biol.* 22:R741–52
116. Nikolic J, Le Bars R, Lama Z, Scrima N, Lagaudrière-Gesbert C, et al. 2017. Negri bodies are viral factories with properties of liquid organelles. *Nat. Commun.* 8:58
117. Nott TJ, Craggs TD, Baldwin AJ. 2016. Membraneless organelles can melt nucleic acid duplexes and act as biomolecular filters. *Nat. Chem.* 8:569–75
118. Nott TJ, Petsalaki E, Farber P, Jervis D, Fussner E, et al. 2015. Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. *Mol. Cell* 57:936–47
119. Novoa RR, Calderita G, Arranz R, Fontana J, Granzow H, Risco C. 2005. Virus factories: associations of cell organelles for viral replication and morphogenesis. *Biol. Cell* 97:147–72
120. Oltsch F, Klosin A, Julicher F, Hyman AA, Zechner C. 2019. Phase separation provides a mechanism to reduce noise in cells. bioRxiv 524231. **https://doi.org/10.1101/524231**
121. Onomoto K, Yoneyama M, Fung G, Kato H, Fujita T. 2014. Antiviral innate immunity and stress granule responses. *Trends Immunol.* 35:420–28
122. Parry BR, Surovtsev IV, Cabeen MT, O'Hern CS, Dufresne ER, Jacobs-Wagner C. 2014. The bacterial cytoplasm has glass-like properties and is fluidized by metabolic activity. *Cell* 156:183–94
123. Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, et al. 2015. A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162:1066–77
124. Patel A, Malinovska L, Saha S, Wang J, Alberti S, et al. 2017. ATP as a biological hydrotrope. *Science* 356:753–56
125. Peskett TR, Rau F, O'Driscoll J, Patani R, Lowe AR, Saibil HR. 2018. A liquid to solid phase transition underlying pathological huntingtin exon1 aggregation. *Mol. Cell* 70:588–601.e6
126. Poblete-Durán N, Prades-Pérez Y, Vera-Otarola J, Soto-Rifo R, Valiente-Echeverría F. 2016. Who regulates whom? An overview of RNA granules and viral infections. *Viruses* 8:E180
127. Qamar S, Wang G, Randle SJ, Ruggeri FS, Varela JA, et al. 2018. FUS phase separation is modulated by a molecular chaperone and methylation of arginine cation-π interactions. *Cell* 173:720–34.e15
128. Rabouille C, Alberti S. 2017. Cell adaptation upon stress: the emerging role of membrane-less compartments. *Curr. Opin. Cell Biol.* 47:34–42
129. Raichle ME, Gusnard DA. 2002. Appraising the brain's energy budget. *PNAS* 99:10237–39

130. Rappsilber J, Friesen WJ, Paushkin S, Dreyfuss G, Mann M. 2003. Detection of arginine dimethylated peptides by parallel precursor ion scanning mass spectrometry in positive ion mode. *Anal. Chem.* 75:3107–14
131. Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, et al. 2011. A hexanucleotide repeat expansion in *C9ORF72* is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72:257–68
132. Riback JA, Katanski CD, Kear-Scott JL, Pilipenko EV, Rojek AE, et al. 2017. Stress-triggered phase separation is an adaptive, evolutionarily tuned response. *Cell* 168:1028–40.e19
133. Rubinstein M, Dobrynin AV. 1997. Solutions of associative polymers. *Trends Polymer Sci.* 5:181–86
134. Sabari BR, Dall'Agnese A, Boija A, Klein IA, Coffey EL, et al. 2018. Coactivator condensation at super-enhancers links phase separation and gene control. *Science* 361:eaar3958
135. Schmid M, Speiseder T, Dobner T, Gonzalez RA. 2014. DNA virus replication compartments. *J. Virol.* 88:1404–20
136. Schmidt HB, Görlich D. 2016. Transport selectivity of nuclear pores, phase separation, and membraneless organelles. *Trends Biochem. Sci.* 41:46–61
137. Semenov AN, Rubinstein M. 1998. Thermoreversible gelation in solutions of associative polymers. 1. Statics. *Macromolecules* 31:1373–85
138. Shin Y, Brangwynne CP. 2017. Liquid phase condensation in cell physiology and disease. *Science* 357:eaaf4382
139. So M, Hall D, Goto Y. 2016. Revisiting supersaturation as a factor determining amyloid fibrillation. *Curr. Opin. Struct. Biol.* 36:32–39
140. Soucek L, Whitfield J, Martins CP, Finch AJ, Murphy DJ, et al. 2008. Modelling Myc inhibition as a cancer therapy. *Nature* 455:679–83
141. Strom AR, Emelyanov AV, Mir M, Fyodorov DV, Darzacq X, Karpen GH. 2017. Phase separation drives heterochromatin domain formation. *Nature* 547:241–45
142. Su X, Ditlev JA, Hui E, Xing W, Banjade S, et al. 2016. Phase separation of signaling molecules promotes T cell receptor signal transduction. *Science* 352:595–99
143. Suárez-Calvet M, Neumann M, Arzberger T, Abou-Ajram C, Funk E, et al. 2016. Monomethylated and unmethylated FUS exhibit increased binding to Transportin and distinguish FTLD-FUS from ALS-*FUS*. *Acta Neuropathol.* 131:587–604
144. Taylor JP, Brown RH Jr., Cleveland DW. 2016. Decoding ALS: from genes to mechanism. *Nature* 539:197–206
145. Taylor JP, Hardy J, Fischbeck KH. 2002. Toxic proteins in neurodegenerative disease. *Science* 296:1991–95
146. Tourrière H, Chebli K, Zekri L, Courselaud B, Blanchard JM, et al. 2003. The RasGAP-associated endoribonuclease G3BP assembles stress granules. *J. Cell Biol.* 160:823–31
147. Tsai W-C, Lloyd RE. 2014. Cytoplasmic RNA granules and viral infection. *Annu. Rev. Virol.* 1:147–70
148. Tsang B, Arsenault J, Vernon RM, Lin H, Sonenberg N, et al. 2019. Phosphoregulated FMRP phase separation models activity-dependent translation through bidirectional control of mRNA granule formation. *PNAS* 116:4218–27
149. Van Treeck B, Protter DSW, Matheny T, Khong A, Link CD, Parker R. 2018. RNA self-assembly contributes to stress granule formation and defining the stress granule transcriptome. *PNAS* 115:2734–39
150. Vanderweyde T, Apicco DJ, Youmans-Kidder K, Ash PEA, Cook C, et al. 2016. Interaction of tau with the RNA-binding protein TIA1 regulates tau pathophysiology and toxicity. *Cell Rep.* 15:1455–66
151. Vanderweyde T, Yu H, Varnum M, Liu-Yesucevitz L, Citro A, et al. 2012. Contrasting pathology of the stress granule proteins TIA-1 and G3BP in tauopathies. *J. Neurosci.* 32:8270–83
152. Wang J, Choi J-M, Holehouse AS, Lee HO, Zhang X, et al. 2018. A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. *Cell* 174:688–99.e16
153. Wegmann S, Eftekharzadeh B, Tepper K, Zoltowska KM, Bennett RE, et al. 2018. Tau protein liquid-liquid phase separation can initiate tau aggregation. *EMBO J.* 37:e98049
154. Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, et al. 2013. Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* 153:307–19

155. Woodruff JB, Ferreira Gomes B, Widlund PO, Mahamid J, Honigmann A, Hyman AA. 2017. The centrosome is a selective condensate that nucleates microtubules by concentrating tubulin. *Cell* 169:1066–77.e10
156. Yoshizawa T, Ali R, Jiou J, Fung HYJ, Burke KA, et al. 2018. Nuclear import receptor inhibits phase separation of FUS through binding to multiple sites. *Cell* 173:693–705.e22
157. Zhang X, Lin Y, Eschmann NA, Zhou H, Rauch JN, et al. 2017. RNA stores tau reversibly in complex coacervates. *PLOS Biol*. 15:e2002183
158. Zu T, Gibbens B, Doty NS, Gomes-Pereira M, Huguet A, et al. 2011. Non-ATG–initiated translation directed by microsatellite expansions. *PNAS* 108:260–65
159. Zu T, Pattamatta A, Ranum LPW. 2018. Repeat-associated non-ATG translation in neurological diseases. *Cold Spring Harb. Perspect. Biol.* 10:a033019