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Functional Implications of Intracellular Phase Transitions

Alex S. Holehouse* and Rohit V. Pappu*

Department of Biomedical Engineering and Center for Biological Systems Engineering,
Washington University in St. Louis, St. Louis, Missouri 63130, USA.

Abstract

Intracellular environments are heterogeneous milieus comprising of macromolecules, osmolytes, and a range of assemblies that include membrane-bound organelles and membraneless biomolecular condensates. The latter are non-stoichiometric assemblies of protein and RNA molecules. They represent distinct phases and form via intracellular phase transitions. Here, we present insights from recent studies and provide a perspective on how phase transitions that lead to biomolecular condensates might contribute to cellular functions.

Keywords

Intracellular phase transitions; membraneless organelle; phase behavior; cellular organization

Introduction

Cells may be thought of as the most complete elementary biological systems that process and store information, transduce signals, and generate responses¹. In this “middle out” view² the cell is seen as the fundamental unit. The cellular milieu and spatial/temporal aspects of intracellular organization combine with the dynamics of macromolecular production, functions, and clearance to determine how an individual cell responds to cues, processes signals, and makes decisions. Cellular phenotypes are integrated via cell-to-cell communication and an active extracellular matrix to determine outcomes at the tissue level.

There is growing interest in understanding how individual cells coordinate a network of intra- and extracellular regulatory programs that span multiple length and time scales to generate responses, make decisions, and control fates at the cellular- and sub-cellular levels. Of particular interest is the question of how cells exert control over a range of processes in ways that are simultaneously robust and adaptive.

The organization of eukaryotic cellular matter into distinct compartments appears to be essential for assembling the multiplexed circuitry that enables cellular and sub-cellular processing of signals and generation of integrated responses. Well-known cellular organelles include the *nucleus* where genes are transcribed and the protein translation machinery is

* alex.holehouse@wustl.edu, pappu@wustl.edu.

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synthesized, the *mitochondrion*, which serves as the cellular power plant, and the *lysosome* where proteins are hydrolyzed. These organelles share the characteristic of being membrane bound. A variety of receptors, channels, pumps, and motors that are embedded in organellar membranes help regulate the trafficking of matter into and out of membrane-bound organelles. However, long-standing observations have established that there are many more cellular organelles that lack a vesting membrane^{3–10}. Historically, these have been referred to as cellular bodies, granules, puncta or assemblies. These membraneless organelles, bodies, or compartments are important for cellular dynamics, regulation, the generation of high fidelity responses, and the maintenance of cellular homeostasis.

Membraneless organelles and bodies have garnered considerable attention over the past decade. This increased attention originated in seminal observations showing that archetypal membraneless bodies are non-stoichiometric assemblies of protein and RNA molecules that have the properties of liquids and gels^{7, 10–17}. Following recent clarifications, we refer to these nonstoichiometric assemblies of protein and RNA as biomolecular condensates (or just condensates)^{3, 4}. The underlying biomacromolecules – proteins or RNA – are classifiable as scaffolds or clients¹⁸. Scaffolds are biomacromolecules that are essential for the formation of condensates, whereas clients are selectively recruited into condensates after their formation, thus making the condensates compositionally malleable. An important framework for thinking about scaffolds and clients is that of multivalent associative polymers^{19, 20}. In this framework, a scaffold or client molecule can be described in terms of stickers and spacers (Figure 1)^{19, 21}. Stickers mediate attractive inter-molecular interactions, while spacers engender flexibility and conformational heterogeneity. Stickers and spacers could be folded binding domains and disordered linkers respectively^{17, 18, 21}, but could alternatively be short sequence motifs (even single key residues) embedded in an intrinsically disordered region^{9, 15, 22–24}.

Cooperative, non-stoichiometric, homo- or heterotypic associations amongst multivalent proteins / RNA molecules drive phase transitions that give rise to condensates, which are characterized by non-covalent physical crosslinks^{3, 21}. To a first approximation, the timescales over which these crosslinks re-arrange and the extent of crosslinking will determine if the condensates are viscous liquids or viscoelastic gels.

We focus here on condensates whose formation is controlled by the concentrations of scaffold molecules whereas their functions are influenced by the concentrations of clients that are recruited^{3, 4, 18}. The mechanisms of and driving forces for phase transitions of scaffold molecules have been written about extensively^{6, 9, 11, 14, 15, 17, 22, 25–43}. While there are numerous important unanswered questions regarding these topics, an equally important set of questions reflect how nature harnesses condensates that form via spontaneous phase transitions for cellular functions. We address this question by highlighting published examples and considering scenarios that represent the types of behavior that one might expect to see where phase transitions could be used to mediate cellular organization.

Compartmentalization, sequestration, and concentration effects

Many of the first condensates identified were found to be micron-sized assemblies that have been referred to as membraneless organelles, foci, or puncta⁵. These included Cajal bodies, nuclear speckles, nucleoli, paraspeckles, P-bodies (processing bodies), and stress granules^{16, 44–47}. These micron-sized condensates are associated with many different functions that range from stress response to numerous roles in nucleic acid processing, and can be cytoplasmic or nuclear^{3–5}. They can exhibit both internal organization and sub-structure^{10, 48–50} as well as rapid internal dynamics^{7, 10}. Here, rapid dynamics refers to timescales that are on a par with or only a few orders of magnitude slower than molecular processes such as protein / RNA folding, macromolecular dissociation, and diffusion of multimolecular complexes⁵¹.

The chemical environment inside condensates is expected to be significantly different from the bulk cytoplasm^{40, 42, 52}. The rules that determine which proteins and nucleic acids are recruited into or are excluded from specific organelles remain poorly understood^{40, 53}. Here, we approach this problem from purely thermodynamic considerations. From this vantage point, equalization of chemical potentials across a phase boundary will be the main determinant of the extent to which different molecules are partitioned into or excluded from condensates. Chemical potentials are partial molar free energies and are governed by a combination of physico-chemical properties including structural features, the spatial ranges of interactions, the strengths of interactions, and the concentrations of scaffold versus client modules within and outside condensates. Accordingly, condensates could provide a way to concentrate specific types of cellular components (proteins, RNA, small molecules, etc.), thereby enhancing reactions efficiency through a high effective concentrations^{17, 54–56, 57} (Figure 2a).

Condensates have the potential to facilitate distinct types of chemical reactions through a finely tuned microenvironment that may prove to be optimal for certain types of biochemical reactions (Figure 2b). Although the notion that condensates have evolved to act as distinct micro-reactors is often invoked and extremely appealing, it has been challenging to demonstrate this in cellular contexts. *In vitro* studies have shown that condensates formed by the DEAD-box helicase Ddx4 reduce the free energy associated with double stranded DNA melting by creating an environment with the equivalent denaturing effect of 4 M GdmCl^{40, 42}. However, in contrast to non-specific denaturation, the melting of duplex DNA is not accompanied by protein unfolding. Instead, the melting of double stranded DNA appears to be a thermodynamic linkage effect tied to the preferential binding of Ddx4 to single-stranded nucleic acids⁵⁸. Similarly, the pyrenoid matrix forms a hexagonal-packed liquid-crystalline assembly to optimize CO₂ conversion in photosynthetic algae⁵⁹. The local order observed in the pyrenoid matrix may have evolved to allow a high enzyme concentration while ensuring that there is sufficient space for reactants and products to enter and exit. Local substructure and internal demixing also allows for the formation of collections of coexisting condensates, as is the case in the nucleolus, where distinct regions are believed to participate in discrete steps in ribosome biogenesis^{10, 12}.

A key parameter that remains poorly quantified in most of the phase transition literature is a direct measure of concentrations of components within condensates. This is of importance given the purported conundrum associated with the micro-reactor model for condensates. Banani *et al.* have noted 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*”³. A recent study shows one way around this conundrum. Direct measurements of protein concentrations within droplets formed by the LAF-1 protein suggest that concentrations of scaffold proteins within condensates can, in some cases, be ultra-low, ~30 μM ⁶⁰. These low concentrations are the direct result of large conformational fluctuations that are the hallmark of certain types of intrinsically disordered regions, which are tethered to folded domains such as helicases and RNA binding domains⁶⁰. This observation highlights one major role for intrinsically disordered regions. They are likely to be determinants of an optimal functionally relevant balance of scaffold densities and intracondensate client concentrations. Comparisons to other condensates suggests the possibility that different types of scaffold molecules might be differently concentrated in their respective condensates^{36, 52, 61}.

The presence of a phase boundary also provides a convenient mechanism for buffering the intracellular concentration of scaffolds in the absence of an active regulatory system (Figure 2c)^{10, 62}. This could be via a direct effect, in which above a saturation concentration excess components partition into and are sequestered within condensates, thus ensuring that the soluble concentration never exceeds a well-defined threshold^{63, 64}. Alternatively, buffering might be realized through an indirect effect whereby a scaffolding molecule that forms condensates binds to or releases a second component in one phase but not the other. An elegant example of this indirect mechanism comes from the yeast RNA binding protein PAB1¹¹. Under non-stress conditions, PAB1 is soluble and binds mRNA transcripts that encode proteins associated with the stress response. Under conditions of stress, PAB1 undergoes self-association to form spherical assemblies, releasing its bound mRNA transcripts *en masse*, and leading to a significant and specific change in the repertoire of soluble cytoplasmic mRNA.

Membraneless organelles that show reversible assembly/disassembly could also provide a convenient mechanism for the partitioning of their components during cell division, as suggested by recent work⁵⁹. If disassembly occurs before mitosis, this could facilitate symmetrical partitioning of organelles by evenly distributing constitutive components across the cytoplasm prior to cell division. Alternatively, if condensates form during cell division they could sequester certain types of cellular components and then be directed into specific daughter cells.

Decision Making and Signal Adaptation

Phase transitions that regulate condensate formation are under the influence of the concentrations of a variety of molecules. These include scaffolding protein and RNA molecules, client proteins and / or RNA, enzymes that catalyze post-translational modifications or nucleic acid processing, osmolytes, hydrotropes, and salts^{18, 33, 34, 42, 60, 65, 66}. In addition, there are control parameters such as pH, pressure, and

temperature that influence the overall phase behavior^{36, 39, 67, 68}. The concentrations of macromolecules and small molecules serve as proxies for their chemical potentials. However, despite an arbitrary level of compositional complexity, phase transitions are governed by switch-like changes along system-specific collective coordinates known as order parameters⁶⁹. For intracellular condensates, the relevant order parameters are the densities of scaffold molecules and the extent of physical crosslinking amongst scaffold molecules²¹.

Two distinct types of boundaries exist for describing the collective self-assembly behaviour of polymers. The sol-gel line reflects a topological transition, while the phase boundary reflects a density transition²¹. In the context of biomolecular condensates, these two transitions are coupled, yielding spherical droplets that are technically gels, although we stress this does not necessarily mean they have material properties consistent with solids^{21, 70}. Importantly, upon crossing the phase boundary, the concentrations in the dispersed and dense phases remain unchanged as the total concentration of the scaffold molecules continues to increase. Thus, the presence of a phase boundary provides a mechanism to quantize a continuous input, namely the concentration of protein, into a binary output – the presence or absence of a condensate. This is because the phase boundary, which leads to phase separation defines a first order phase transition, which is an infinitely cooperative process⁶⁹. Thus, it would appear that phase transitions provide a natural way to quench noise from an analog input signal and commit to a specific cellular program in a digitized manner. Indeed, the use of phase transitions as a mechanism for binary decision making is apparent in the amyloid propagation associated with the RIP1/RIP3 signaling cascade and in the MAVS and ASC inflammatory response, both of which are effectively first order crystallization processes^{71, 72}. In these examples, the phase transition represents an irreversible commitment to a specific fate, an important feature given the cellular context of these processes. In contrast, condensates, especially those with liquid-like characteristics, could offer similar fidelity in decision-making, albeit in a way that is fully reversible in response to changes in the cellular state⁴.

Modulation of the concentrations of scaffold molecules by gene expression, overall dilution, or protein degradation will determine whether the molecule of interest is in the one-phase or two-phase regime with respect to the system-specific phase boundary. In addition, the location of the phase boundary and the width of the two-phase regime can also be regulated in three distinct ways (Figure 2d–f)^{25, 65}. First, the saturation concentration, which refers to the low concentration arm of the coexistence curve, can be shifted left or right, to lower or higher concentrations, respectively (Figure 2d). In this way, the concentration at which condensates form can be tuned by a variety of different factors. This provides a framework in which positive or negative feedback can shift the saturation concentration to provide cells with a convenient mechanism for attenuating signals, which involves shifting a phase boundary to higher concentrations, or for hypersensitivity, which involves shifting a phase boundary to lower concentrations. As an example, the presence of specific RNA molecules has a significant impact on the low-concentration arm associated with the formation of condensates by the protein Whi3, shifting the phase boundary by several orders of magnitude⁸. Second, the critical point on a phase diagram can move up or down as a function of binding partners, amino acid sequence^{52, 73}, pH^{11, 67}, post-translational

modifications³⁴, temperature^{11, 74–76} or other control parameters. Depending on how close to the critical point the normal cellular concentration is, this can provide a mechanism by which the cell can toggle between robust condensate formation and no condensate formation (Figure 2e). As a result, the cell can exist in two fundamentally different regimes with respect to some scaffold component; one in which the soluble concentration of the scaffold can increase and decrease continuously, which happens above the critical point, or one where a phase boundary sets a maximum concentration threshold, above which excess protein is sequestered into condensates. This happens below the critical point. Third, as shown in a surprising recent study⁶⁰, the high concentration arm of the coexistence curve can move independently to the left or right, thus adjusting the protein concentration inside the condensate (Figure 2f). As an example of this independence, for the DEAD-box helicase LAF-1 it was discovered that the presence of RNA shifts the high concentration arm while leaving the low concentration arm and critical point fixed⁶⁰. In effect, RNA molecules tune the concentration of LAF-1 inside condensates. The functional implications of this discovery remain an open question, but we speculate that this might be a mechanism to alter the accessibilities of sites on scaffold molecules, the compositions of clients, or enzymatic efficiencies within condensates. As a final comment, although we have described these three types of changes to phase diagrams as distinct events, in many cases we should expect them to be coupled, although this need not necessarily be the case.

Signal Amplification, Integration, and Homeostasis

Condensate formation has been observed across a range of eukaryotic cells, and in many cases it appears to be involved in enabling complex cellular processes^{8, 11, 13, 66, 77–85}. Accordingly, there are several features associated with phase transitions that make them attractive from the standpoint of cellular information processing. If condensate formation is controlled by a single key scaffold component, this provides a mechanism for signal amplification (Figure 2g). As an example, the phosphorylation of T-cell receptors facilitates a downstream phase separation of signaling output, allowing simple input to drive a complex output⁵⁵. The presence or absence of a single protein above some threshold concentration could dictate the spatial assembly of an arbitrarily complex cellular body. This mechanism could be used in the context of micron-scale assemblies for RNA processing or the stress response, or on a smaller scale, such as through transcriptional initiation or membrane signaling^{7, 11, 48, 55, 85–88}.

Complex phase behavior, in which condensates consist of multiple types of proteins and RNA, also provide a mechanism for signal multiplexing. The input signal may depend on a single component, while the output is an emergent property that depends on the characteristics of condensates as a whole, and less on the characteristics of the individual components. In this way, condensates provide an ideal mechanism for signal integration, whereby the concentration and/or ratio of different types of species that partition into the condensate can directly influence the internal properties of the condensate and hence function (Figure 2h). The enrichment of specific client components in a condensate can also undergo a sharp and mutually exclusive rearrangement, suggestive of a mechanism through which condensate composition can rapidly reset in response to one or more external signals¹⁸.

Finally, the dynamics of condensate formation need not necessarily match the dynamics of disassembly. For example, while assembly may occur rapidly in response to some input signal (pH, temperature, phosphorylation, *etc.*) even after this signal is removed, slow or even glassy intra-condensate dynamics could introduce a lag time for disassembly (Figure 2i). As a simple tangible example, honey is water soluble, yet a single drop of honey placed in a beaker of water can remain spherical and distinct from the bulk solution for hours to days, depending on the temperature of water. Of course, stirring will accelerate the dissolution of honey into water. Why might this example be relevant? The material properties of condensates are determined by a combination of the intrinsic sequence-encoded properties of its constitutive components and the interaction among those components⁸⁹. These material properties will in turn govern the disassembly dynamics. In this way, cells could in principle tune condensate lifetime, allowing for spatial and temporal regulation. The tunability of condensate disassembly could provide a route for the gradual release of molecular components, for encoding short-term cellular memory by providing distinct markers of cellular state, and could act as an internal timekeeping mechanism. Of course, in analogy with the stirring of honey-water mixtures, energy dependent processes could catalyze the dissolution / disassembly process, or could actively suppress condensate breakdown^{16, 87}.

If additional nucleation processes occur *within* condensates (e.g., liquid-to-solid transitions), then this provides another timescale that the cell may be able to use to its advantage. In this manner, a more complex logical circuit such as – IF condensate for n time units, THEN form solid – could be constructed. Liquid-to-solid transitions are involved in signaling pathways critical to the inflammatory response and necrotic cell death^{71, 72, 90}. Therefore, it seems plausible that the liquid-to-solid transitions associated with stress granules that are typically considered aberrant could be an adaptive cellular mechanism to trigger apoptosis or necrosis⁹¹, in response to constitutive cellular stress. The balance between nucleation limited and diffusion limited condensate formation remains poorly understood within the cellular context, but new approaches are beginning to query the dynamics of assembly^{88, 92–95}. It seems reasonable to expect that as new methodological advances appear, the dynamics of condensate formation and disassembly will provide further insight into their function.

Conclusion

In principle, the phase behavior associated with many distinct components provides a versatile way to control information processing at the cellular level. Critically, this offers a mechanism for information transfer across wildly different length scales and time scales. However, given that cells are far from equilibrium, the types of mechanisms outlined in this perspective are likely to be augmented by sophisticated thermodynamic linkages with other spontaneous processes and / or driven processes^{95–98}. Understanding the interplay between spontaneous phase transitions and driven processes that require energy sources and sinks remains a challenge and necessitates novel approaches that enable using the cell as a test-tube^{94, 99}. Without such advances, a true realization of how intracellular phase transitions impact cellular and tissue-level emergent properties will remain opaque. It is also possible that in some cases, these condensates are simply an unavoidable consequence of a high concentration of cytoplasmic species, and represent labile “dumping grounds” for cellular

components. We see no reason to assume that all assemblies are necessarily associated with a specific biological function. However, driven by our own intellectual biases, we propose that condensates realized via spontaneous or driven phase transitions offer a route for assembling complex multiplexed circuits for processing biochemical signals, controlling cellular decisions and responses, managing cellular fates, and determining how cells are integrated into tissues.

As a final speculative comment, phase separation and biomolecular condensates could play a role in emergent properties at the cellular level^{98, 100–102}. In the same way that the material properties of condensates are governed by the organization and dynamics of their constituents, the material properties of tissues control morphogenesis, and these properties are in turn governed by the organization and dynamics of cells at the tissue interface. Although morphogenesis is regulated by gene expression, the transduction of information from genes to tissues involves distinct physical transformations that occur along different scales. Phase transitions also occur at the tissue-level and the interactions that drive these transitions are amongst collections of cells¹⁰³. According to Steinberg's differential adhesion hypothesis, morphogenesis is akin to liquid-liquid phase separation at the tissue level as it is driven by the spontaneous demixing or wetting of liquids, where the liquids in this case are tissues made up of cells¹⁰⁴. Similarly, metastasis may be thought of as a topological solid-to-liquid transition that occurs without a change in packing fractions of the underlying cellular matter¹⁰³. These observations raise the intriguing possibility of a yet to be discovered role for biomolecular condensate formation and dissolution in the hierarchical chain of phase transitions that ultimately governs morphogenesis. Discerning the flow of information across distinct scales will likely require a framework for connecting distinct types of phase transitions and uncovering the coupling between distinct types of collective coordinates. A first step will be to connect the regulation of biomolecular condensates to the control of cellular level processes, and these efforts are currently underway.

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Figure 1. Schematic showing how multivalency could be manifest in biomacromolecules.
a) Linear multivalent proteins with folded binding domains connected by flexible intrinsically disordered linkers. A similar polymer architecture could in principle also be formed by an RNA molecule. **b)** A fully disordered polymer (disordered protein region or an RNA molecule) composed of regions that mediate intermolecular interactions (stickers) and flexible non-adhesive spacers. The relative proportion of sticker and spacer, the strength of sticker-mediated interactions, and the intrinsic dynamics of spacers, will all contribute to material properties of a condensate.

Figure 2. Summary of a subset of putative mechanistic and regulatory features that phase transitions might support.

For convenience, all phase diagrams drawn here are in the inverse χ (χ^{-1}) versus concentration plane, but the ordinate could alternatively be one of any number of parameters such as salt concentration, pH, concentrations of binding partners *etc.* The parameter χ^{-1} provides a measure of the strength of interaction between constitutive components (high values reflect weaker interactions). These diagrams are meant to be illustrative of the types of phenomena one might expect to see. **a)** Condensates facilitate a high local concentration of scaffold and client components. **b)** Through the partitioning of distinct components, the condensate interior can provide a specific repertoire of macromolecules. **c)** A phase boundary provides a passive mechanism to fix the bulk concentration of scaffold

components. At concentrations above the low concentration arm of the coexistence line, additional components partition into condensates, while the concentration in the soluble phase remains unchanged. In this way, the volumes of the two phases will change, but their concentrations will not. **d)** The low concentration arm of the coexistence curve can move right (top) or left (bottom), leading to an increase or decrease in the saturation concentration, respectively. **e)** The critical point can move up or down, such that for a system where the position on the ordinate was already close to the critical temperature a shifting of the critical point down could eliminate the ability to form condensates (bottom). **f)** The high concentration arm of the coexistence curve can move left (top) or right (bottom) indicating a decrease or an increase in the concentration of components inside the condensate, respectively. **g)** For multicomponent assemblies, the presence or absence of a single component can trigger the formation of an arbitrarily complex assembly with multi-phase behaviour. **h)** Condensates with similar components may have very different functional outputs in response to the presence of one specific component versus another. **i)** Condensate dissolution and disassembly dynamics may be complex and could depend on multiple different factors, leading to rates that are quite different to those predicted by simple mean-field models. A condensate could persist for long timescales even after the bulk concentration is below the saturation concentration if the droplet was kinetically trapped (top row). If the components contain multiple distinct interacting domains, once condensate formation has been driven by one set of domains, additional interactions may now occur via an orthogonal mechanism (middle row). A secondary nucleation-dependent process (such as solid formation, e.g. spherulite or amyloid growth, as depicted in the bottom row) could occur within the condensate, allowing a liquid-like droplet to transition into an alternative state according to some characteristic and sequence/composition dependent timescale.