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Opinion

Mechanisms for Active Regulation of Biomolecular Condensates

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Liquid–liquid phase separation is a key organizational principle in eukaryotic cells, on par with intracellular membranes. It allows cells to concentrate specific proteins into condensates, increasing reaction rates and achieving switch-like regulation. We propose two active mechanisms that can explain how cells regulate condensate formation and size. In both, the cell regulates the activity of an enzyme, often a kinase, that adds post-translational modifications to condensate proteins. In enrichment inhibition, the enzyme enriches in the condensate and weakens interactions, as seen in stress granules (SGs), Cajal bodies, and P granules. In localization-induction, condensates form around immobilized enzymes that strengthen interactions, as observed in DNA repair, transmembrane signaling, and microtubule assembly. These models can guide studies into the many emerging roles of biomolecular condensates.

Biomolecular Condensates Can Be Formed and Dissolved in the Blink of an Enzyme

Eukaryotic cells possess numerous types of **membraneless organelles** (see Glossary). Each contains between tens and several thousands of protein and RNA species that are highly enriched compared with the surrounding nucleoplasm or cytoplasm. These biomolecular **condensates** are held together by weak, multivalent, and highly collaborative interactions, often between intrinsically disordered regions of their constituent proteins [1,2].

In contrast to membrane-bound organelles, biomolecular condensates can easily be formed or dissolved by merely changing the activity of an enzyme, such as a kinase, that post-translationally modifies key condensate proteins [3–5,71]. The modifications usually lie in intrinsically disordered regions and modulate the strength of attractive interactions with other condensate components [6,7]. Due to the highly cooperative nature of **phase transitions**, small changes in interaction strengths can result in the formation or dissolution of condensates, and this switch-like nature makes them ideal for dynamic regulation.

For instance, SGs form on cellular stress and are dissolved when the stress ceases [3]. Also, P-bodies in the cytoplasm and Cajal bodies, nuclear speckles, paraspeckles, and PML bodies in the nucleus have to be dissolved during mitosis and reformed afterwards to ensure a balanced distribution of their contents to daughter cells [4,8].

These droplet organelles are large enough to be visible using simple light microscopy techniques and have long been known. Recently, **liquid–liquid phase separation** has been implicated in multifarious processes in which – often submicrometer-sized – condensates are formed at particular locations in the cell: at sites of DNA repair [9], Polycomb-mediated chromatin silencing [10], transmembrane signaling [11,12], microtubule formation [13–15], actin polymerization [16], endocytosis [17,18], presynaptic active zones [19,20], and ribonucleoprotein (RNP) transport [21–23]. Such localized condensates form on a local stimulus to recruit the required set of proteins and are dissolved once their job is done.

Cells do not only need to regulate the formation and dissolution of each type of condensate. They sometimes also need to regulate their size and with it their numbers, to allow many condensates to form in different locations; for instance, to activate many genes at the same time [24–27]. Here, we propose two active mechanisms used by cells for these purposes.

Highlights

Biomolecular condensates are phase-separated domains in the nucleoplasm or cytoplasm formed by weak and highly multivalent interactions between their protein and RNA components. They allow cells to create compartments strongly enriched or depleted in specific proteins or RNAs, without the need for membranes.

Cells can actively regulate the formation, dissolution, and localization of condensates by phosphorylation and other post-translational modifications of condensate components.

We propose two unifying, generic mechanisms of active regulation. In the first, the protein concentration is above saturation and the modifications limit the condensate size by inhibiting intracondensate interactions. We believe this mechanism regulates most membraneless organelles.

In the second mechanism, the protein concentration is below saturation, but localized enzymes attach modifications that promote interactions and induce condensate formation in the volume around them. This mechanism can explain how a single kinase (e.g., recruited to a double-strand DNA break) can recruit hundreds of specific proteins.

We argue that many types of condensates are actively regulated by one of these two mechanisms.

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Phase Separation and Condensate Size Behavior

To keep the models simple, we consider only one type of condensate protein. In the dilute regime below the saturation protein concentration c_{out}, no condensate can form (Figure 1A). Above c_{out}, in the phase separation regime, condensates can be stable.

However, in a passive system more than one condensate cannot exist in equilibrium because larger condensates will grow at the expense of smaller ones (Figure 1B). The reason is that proteins on the surface of small droplets have fewer favorable interactions among themselves than proteins on the surface of larger droplets, due to the difference in surface curvature. They are therefore more easily lost, resulting in a higher equilibrium concentration outside the droplet (section 1.1 in the supplemental information online). Due to this size dependence, the protein concentration decreases from small to large condensates, and the decrease generates a diffusive flux in the direction of steepest descent. Consequently, there exists a critical radius R_{crit} below which condensates will shrink, while condensates above R_{crit} will grow (Figure 1C and section 1.2 in the supplemental information online). The critical radius increases until a single large condensate survives, a phenomenon called coarsening [28].

The timescale for droplets to change their size by coarsening scales roughly with their radius cubed (derived in section 1.3 in the supplemental information online). Therefore, small droplets can grow or shrink fast, on a scale of minutes for $R \approx 100$ nm, whereas droplets of a micrometer radius already take days. This explains why, in *in vitro* experiments, droplets of micrometer size and above can coexist for long periods.

We now show that, to actively regulate the formation and size of liquid droplet condensates, two generic mechanisms exist. A protein concentration maintained above saturation leads to the enrichment-inhibition model, in which a regulating enzyme such as a protein kinase inhibits favorable interactions and is enriched in condensates. A concentration maintained below saturation leads to the localization-induction model, in which the enzyme is localized or attached and induces favorable interactions. Although simplified, these models might capture two essential mechanisms for active regulation of cellular condensates.

Both mechanisms modulate interaction strengths of key condensate proteins by an enzyme that adds or removes post-translational modifications. Often, the regulating enzyme will be a kinase that attaches phosphoryl groups to disordered regions of condensate proteins. However, other post-translational modifications could take this role: poly-ADP-ribosylation in DNA repair [9], SUMOylation (e.g., in PML bodies [29]), arginine demethylation of proteins in RNA granules [30,31], lysine acetylation and methylation [15,32], ubiquitination [33], and RNA modifications [34,35]. Even RNA helicase activity can take over this role in RNA-containing condensates [36].

The Enrichment–Inhibition Model

Above the saturation concentration, a mechanism must exist that limits the size of larger condensates to allow the coexistence of multiple condensates. This can be achieved if the loss of proteins from the condensate increases faster with condensate radius R than the gain by net diffusive influx. The influx is proportional to $R-R_{crit}$ (Figure 1C). A loss that scales with the condensate volume $(4\pi/3)R^3$ would grow faster than $R-R_{crit}$. Above a certain radius, the loss would surpass the influx, shrinking condensates that are too large and thereby resulting in a stable condensate size.

We propose the loss mechanism to be the modification of condensate proteins (or RNA) by an antagonistic regulating enzyme (orange) that is itself enriched in the condensate (Figure 2, Key Figure). We use phosphorylation as an example, but the mechanisms work the same for other modifications. Because the concentration of unphosphorylated proteins (blue) is approximately constant in the condensate, the phosphorylation rate scales with the condensate volume. In this model, unphosphorylated proteins as well as the kinases attract each other, while the phosphorylation weakens the interactions with other condensate proteins. It might seem counterintuitive that the droplet-dissolving kinase enriches in the droplet, yet it is this feature that allows the droplet growth to be self-limiting.

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Since the concentration of the unphosphorylated proteins is above saturation, the concentration decreases towards the condensate, leading to a net influx of unphosphorylated proteins (Figure 2A). This influx is compensated by the loss of proteins that become phosphorylated inside the condensate, which diffuse out along the negative concentration gradient. Outside, they are dephosphorylated by phosphatases (green), closing the circle of protein flux.

To avoid wasting energy by a short-circuited phosphorylation–dephosphorylation reaction, the phosphatase and kinase would best be concentrated in different phases. Therefore, we expect the phosphatase to be strongly depleted in the condensates.

For phosphorylation rates k below a certain threshold k_{thr}, all condensates will grow or shrink to the same stable radius R, which is determined by k (Figure 2B and section 2 in the supplemental information online). The dependence of R on the phosphorylation rate k has a switch-like behavior (Figure 2C): above k_{thr}, no condensates can exist.

Evidence Supporting Enrichment-Inhibition

We give five examples of biomolecular condensates that behave as expected from the enrichment-inhibition model: (i) their key condensate proteins are phosphorylated by a kinase, (ii) increased kinase activity dissolves the condensates, and (iii) the kinase is enriched in the condensates. The model also predicts the main phosphatase to be depleted in the condensates. This information appears to be mostly unavailable.

'P granules' are condensates of RNAs and proteins in the one-cell embryo of the worm *Caenorhabditis elegans*. These localize to the posterior end of the cell and after cell division end up in the one cell that will give rise to the germ line. P granules are highly enriched for the intrinsically disordered MEG proteins. (i) They are phosphorylated by MBK-2 and dephosphorylated by the PPTR-1 phosphatase. (ii) Phosphorylation of MEGs promotes granule disassembly and dephosphorylation promotes assembly. Furthermore, (iii) MBK-2 localizes to P granules [37].

The vertebrate ortholog of MBK-2, DYRK3, plays a central role as dissolvase of several types of membraneless organelles during mitosis. Rai *et al.* suggested that, as for P granules, DYRK3 is involved in the size control of many other types of condensates [4], as we would expect from the enrichment-inhibition model.

'SGs' are another example. (i) They are regulated by DYRK3 [3]. However, since DYRK family kinases are constitutively active, it is unclear how the stress signal could be quickly relayed via DYRK3. Wurtz and Lee proposed a plausible mechanism [38]: on stress, ATP levels can fall by 50%, within the same timescale as SG formation. Also, ATP depletion alone is sufficient to induce SG formation. The reduction in DYRK3 activity (k in Figure 2C) by ATP depletion might reduce the level of phosphorylation of its targets, (i) several of which are key SG proteins. (ii) The concomitant increase in favorable interactions then would trigger SG formation. (iii) In accord with the enrichment-inhibition model, DYRK3 localizes to SGs [3].

'Nuclear speckles' concentrate proteins involved in pre-mRNA splicing. These factors possess a terminal low-complexity RS region enriched for arginine and serine, which is required for the multivalent interactions within the speckles [39]. (i) The CLK kinase phosphorylates the RS domains of splicing factors and (ii) phosphorylation by CLK promotes the disassembly of nuclear speckles [40]. Finally, (iii) CLK itself possesses an RS domain that is required and sufficient for its enrichment in the speckles [41].

'Cajal bodies' are nuclear condensates defined by the key architectural self-oligomerizing protein coilin. (i,ii) Hyperphosphorylation of coilin by Cdk2/cyclin E dissolves them. Also, (iii) Cdk2/cyclin is strongly enriched in Cajal bodies [42].

'Synaptic vesicles (SVs)' containing neurotransmitters form dense clusters at synapses. Synapsin, the major constituent of the matrix around SVs, forms condensates under physiological conditions *in vitro* [43]. The condensates enrich small lipid vesicles, explaining SV clustering at synapses. As expected, (i)

Glossary

Condensate: the protein-rich liquid, gel-like, or solid phase in the liquid cytoplasm or nucleoplasm. Liquid condensates are usually spherical to minimize the energetically unfavorable interface with the dilute phase. However, their shape can be influenced by scaffolding structures as in the case of chromatin. Their content is exchanged rapidly with the surroundings by diffusion. The mean retention time can be on the order of seconds or below, even for proteins highly enriched in the condensate.
Liquid–liquid phase separation: in mixtures of two or more components (e.g., proteins and water molecules), it may be energetically favorable for the components to separate into two liquid phases of different relative concentrations. For example, if proteins attract each other but have less favorable interactions with water, they can condense into liquid droplets with high protein concentration in a dilute phase of low protein concentration.
Membraneless organelles: biomolecular condensates that organize cytoplasmic and nucleoplasmic space, such as nucleoli, nuclear speckles, PML bodies, Cajal bodies, paraspeckles, SGs, and P-bodies.
Phase transition: transitions between states of matter determined by their interactions. Examples are the transition of a gas to a liquid state (condensation) or from a solid to a liquid state (melting). When two phases coexist (e.g., ice in water, water droplets in water vapor), we speak of phase separation.

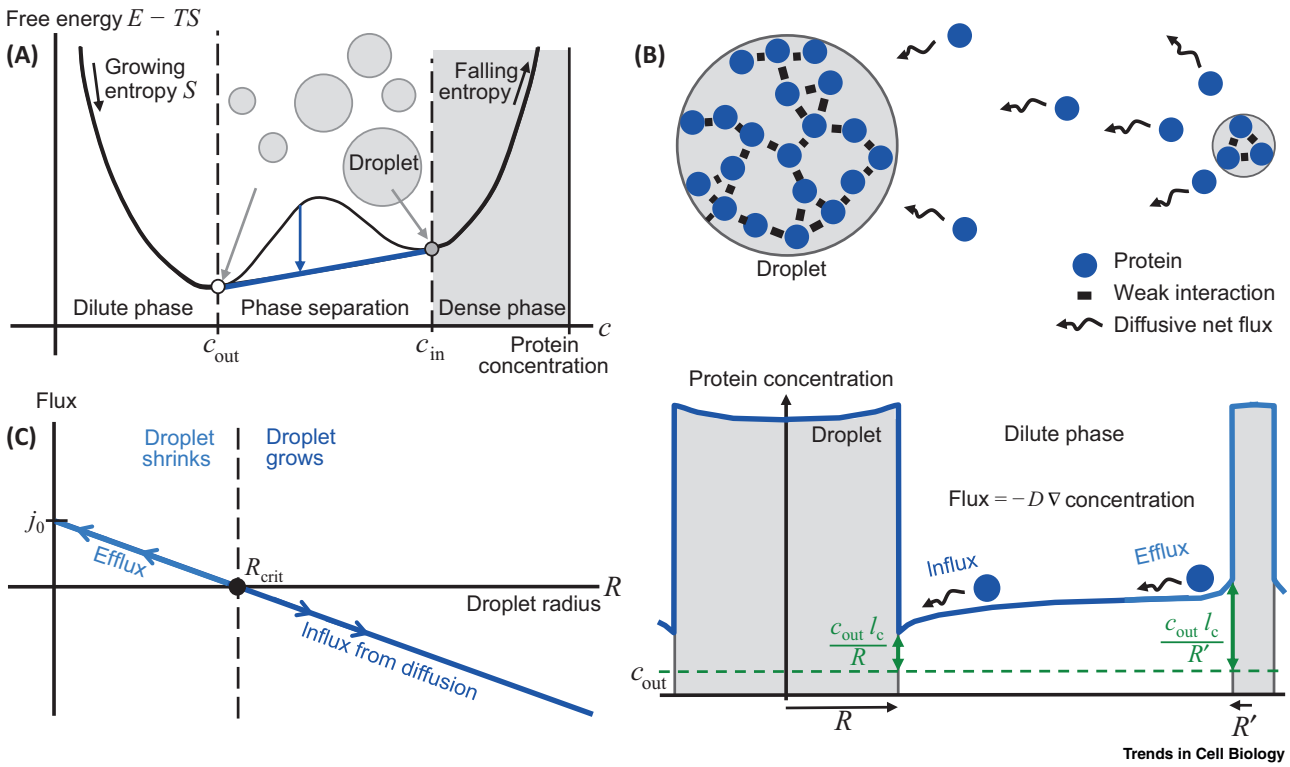

Figure 1. Phase Separation and Condensate Droplet Size Behavior.

(A) When protein–protein and solvent–solvent interactions are more favorable than protein–solvent interactions, demixing into two phases can occur: a dilute phase with low protein concentration c_{out} and a dense phase with high concentration c_{in}. This happens when the sum of the free energies of the two phases is lower (tip of blue arrow) than the energy of the single phase (base of arrow). T, temperature. (B) c_{out} is the limiting concentration for infinite condensate droplet radius. The concentration just outside a condensate increases with decreasing droplet radius (green double-headed arrows), as small droplets cannot hold on to their proteins as well as large ones. This creates a concentration gradient (r), which fuels a diffusive flux from small to large droplets (wiggly arrows). D, diffusion coefficient; l_c, protein–protein interaction strength. (C) As a result, condensates below a radius R_{crit} will shrink and larger ones will grow.

CaMKII kinase phosphorylates synapsin, (ii) its activity dissolves SV clusters *in vivo* in the presence of ATP, and (iii) CaMKII localizes to the condensates [43].

The Localization-Induction Model

Below the saturation concentration, no condensates can form. However, it is possible to locally push the concentration of proteins above saturation. This could be achieved if, in contrast to the previous model, the modifying enzyme acts agonistically and the modified proteins are attracted to each other through multivalent interactions, while the unmodified proteins have little or no affinity for each other and other condensate proteins. With that assumption, the locally confined addition of modifications represents a source of condensate proteins. This replenishment can compensate the diffusive loss that, below saturation, would otherwise cause the fast shrinkage and disappearance of any condensate.

Figure 3A illustrates the model with the example of phosphorylation. Proteins are phosphorylated at the site where the kinases are attached or bound, near the center of the condensate. From there, phosphorylated proteins diffuse out along the negative gradient of the concentration, which drops towards the outside. Outside the condensate, the phosphorylated proteins are dephosphorylated by phosphatases. The dephosphorylated proteins diffuse in towards the center of the condensate to the point of lowest concentration, where the kinase activity depletes them, closing the circle of protein flux.

Key Figure

Enrichment-Inhibition Model

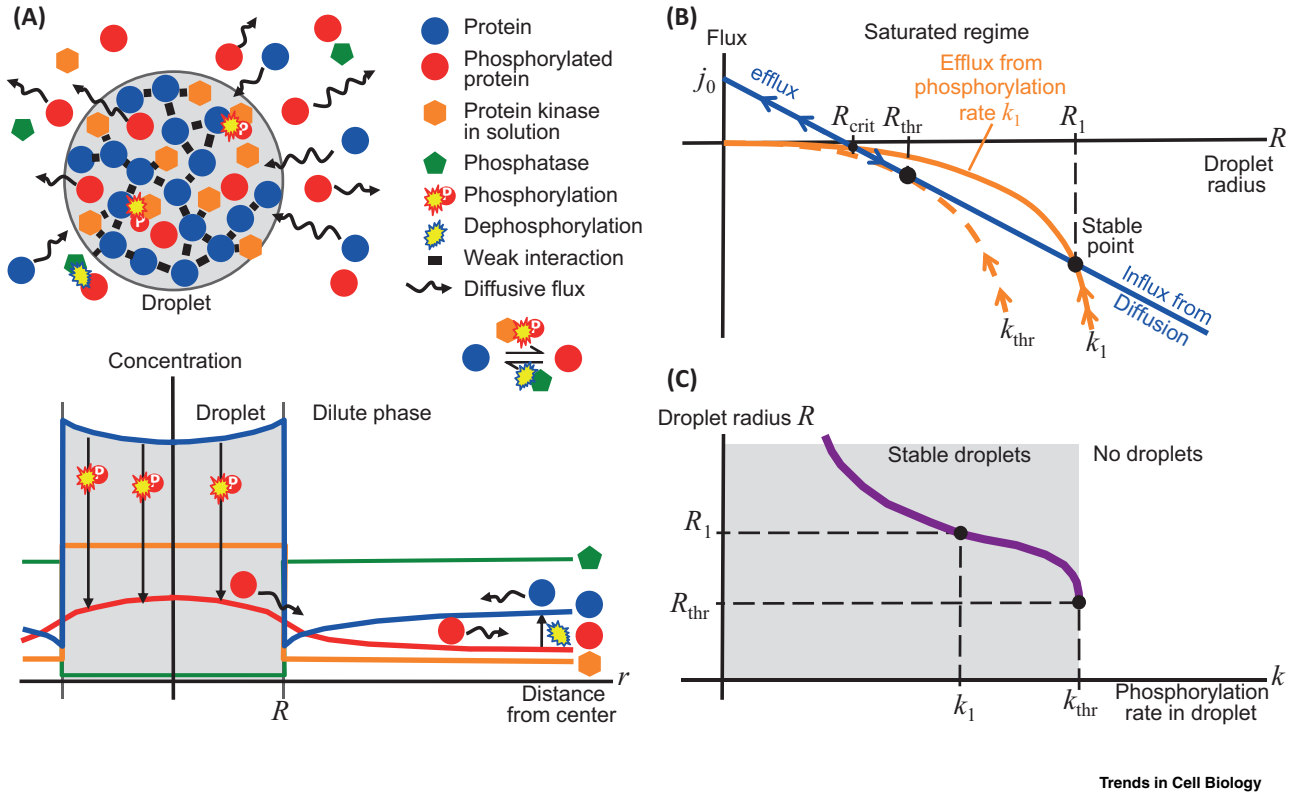

Figure 2. Condensate droplets can form when the concentration of unphosphorylated proteins is above the saturation concentration c_{out}. Phosphorylation weakens protein interactions, so condensates can be dissolved by increasing kinase activity. (A) The unphosphorylated proteins (blue) and kinase (orange) become concentrated in the condensates via multivalent, attractive interactions. There, the kinase phosphorylates condensate proteins (red bursts). The noninteracting phosphorylated proteins (red) diffuse out of the condensate. Outside, they are dephosphorylated (blue burst). Unphosphorylated proteins diffuse back into the condensate along the gradient of concentration (blue), compensating the outward flux of phosphorylated proteins. (B) Without losses from phosphorylation, condensates in the phase separation regime grow by diffusive influx if their radius R is above a critical value R_{crit}, whereas small condensates shrink (blue arrows). Because the influx grows linearly with condensate radius R whereas loss through phosphorylation grows with the condensate volume $(4\pi/3)R^3$ (orange), a stable radius R_1 results. (C) This radius depends on the phosphorylation rate k and shows a switch-like response.

For phosphorylation rates Q below a certain threshold Q_{thr}, no condensates can form (Figure 3B) because the efflux from a tiny drop is larger than the rate with which the kinases generate phosphorylated proteins. Above Q_{thr}, the condensate radius R depends linearly on kinase activity (Figure 3C and section 3 in the supplemental information online). As before, we expect the phosphatase to be depleted in the condensates.

In summary, the model provides a possible explanation for how condensates can be created and dissolved at specific cellular locations by regulating the activity of enzymes that add post-translational modifications to condensate components that enhance their interactions with other components.

Analogous to three dimensions, phase separation can occur in two dimensions above a certain saturation surface density, with the formation of clusters of high density surrounded by low-density regions [11,12].

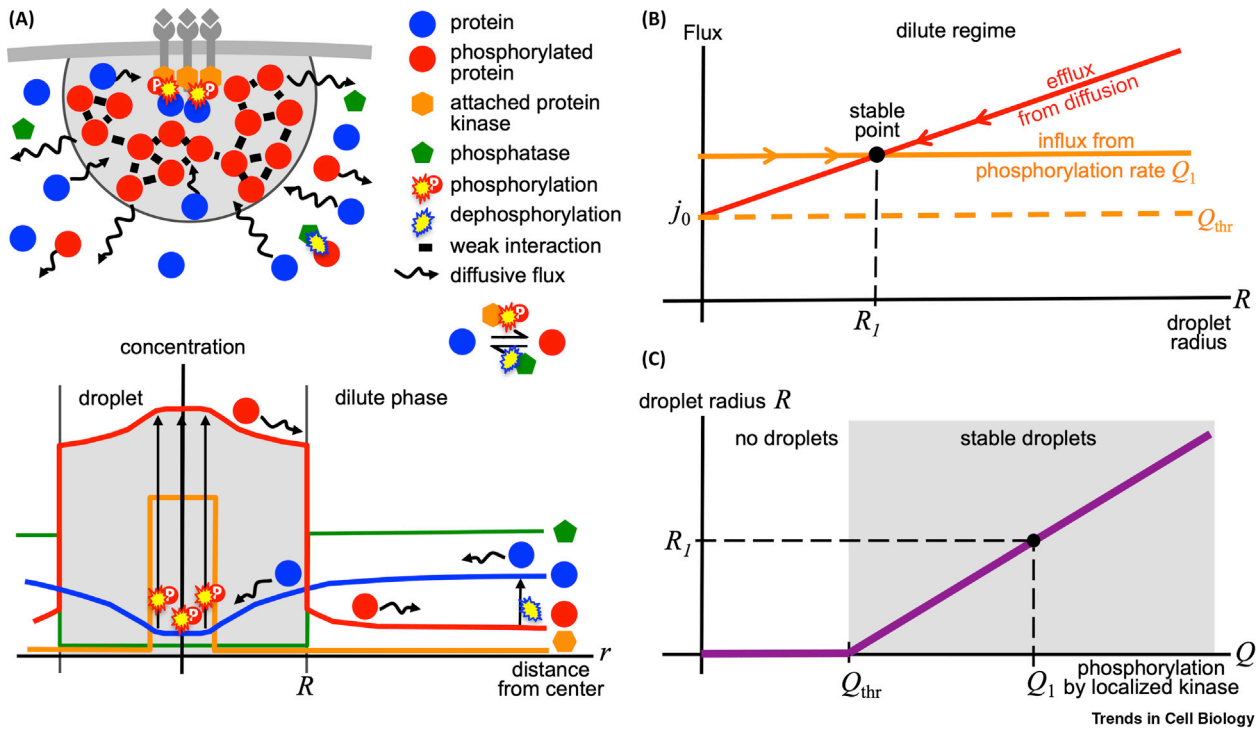

Figure 3. Localization-Induction Model.

(A) Condensates can form when kinases (orange), bound or attached to a cellular structure such as a membrane, locally phosphorylate proteins (blue). This raises the local concentration of the phosphorylated proteins (red), which bind to each other through multivalent interactions, above the threshold for phase separation. Because the concentration of phosphorylated proteins outside the condensate is below saturation, the condensate loses phosphorylated proteins through diffusive flux (wiggly arrows). Outside, they are dephosphorylated (blue burst). Unphosphorylated proteins diffuse back into the condensate, compensating the outward flux of phosphorylated proteins. (B) Without kinase activity (orange), condensates in the dilute regime would shrink rapidly by diffusive efflux. Because the kinase activity supplies phosphorylated proteins at a constant rate Q_1, a stable equilibrium is reached at radius R_1. Below rate Q_{thr}, no condensates can form. (C) Above Q_{thr}, stable condensates form, whose radius depends linearly on Q.

However, analogous to the 3D case, large clusters would grow at the expense of small ones in an unregulated fashion. A 2D extension of the localization-induction model could explain transmembrane receptor clustering upon binding to extracellular ligands and why some types of receptors organize into clusters of fixed size [12,44–46] (section 4 in the supplemental information online).

Evidence Supporting Localization-Induction

The localization-induction model makes three predictions. (i) The key regulating enzyme (e.g. a kinase) is targeted or attached to a specific cellular location, (ii) where it post-translationally modifies key condensate proteins. (iii) The modifications promote attractive interactions, leading to condensate formation around the site of the enzyme. Here we discuss examples of processes that behave as expected if they were regulated by the localization-induction model.

Upon the 'formation of a DNA double-strand break', (i) a poly(ADP-ribose) (PAR) polymerase-1 (PARP-1) protein will bind to the DNA break site, which allosterically triggers its autoactivation [47]. (ii) PARP-1 starts adding PAR to itself and target proteins. (iii) These recruit proteins with positively charged RGG-rich disordered regions, particularly FET proteins such as Fused in Sarcoma (FUS), via multivalent interactions with the negatively charged ADP-ribose subunits. Within seconds, a liquid condensate forms, which enriches the first-response factors at the damage site [9]. As expected, knockdown of the PAR-degrading enzyme PARG enhances the formation of DNA repair foci [48].

'T cell signal transduction' exemplifies localization induction in two dimensions. The T cell receptor kinase is phosphorylated and can then recruit and bind the membrane-bound ZAP70 kinase, which thereby becomes localized (i). The phosphorylation of T cell receptors also turns on the activity of its kinase domain, which phosphorylates and thereby activates the bound ZAP70 kinase. (ii) Activated ZAP70 phosphorylates the key membrane-bound protein LAT. (iii) Phosphorylation enables favorable interactions with several other proteins, with which LAT then forms a quasi-2D condensate at the inner plasma membrane surface. The condensate excludes the LAT-dephosphorylating phosphatase CD45 but recruits the machinery for actin assembly, which can form condensates of its own to induce cytoskeletal changes [49].

Many transmembrane signaling processes start by the formation of transmembrane receptor clusters. Case and colleagues [12] argue that many other transmembrane signaling processes (using glycosylated receptors, immune receptors, cell adhesion receptors, Wnt receptors, and receptor tyrosine kinases) possess features that suggest they too involve liquid–liquid phase separation.

The 'assembly of microtubules' is a further example. Metaphase centrosomes comprise a core structure called the centriole pair, surrounded by a condensed phase, the pericentriolar matrix [50]. (i) Condensate formation depends on the kinase PLK-1, which is concentrated at the centrioles and (ii) phosphorylates the key protein Cnn. Phosphorylated Cnn phase separates in *Drosophila* and enhances the nucleation of microtubules during mitosis. In accord with localization-induction (Figure 3C), the phosphorylation rate determines centrosome size [50], and Cnn proteins are continuously added to the condensate at the centrioles, where PLK-1 is bound [51].

Other Mechanisms of Size Control

Besides the two presented active mechanisms, a slower but fundamental way to regulate the formation of condensates is via transcriptional or translational control of the concentration of key components. For instance the formation and size of enhancer condensates [24,26,52,53] depends critically on the concentration of the transcription factors binding the enhancer.

In Figure 4, we propose the scaffolded condensate model, a passive mechanism that can keep condensates at a fixed size. For instance, the size of condensates formed at active enhancers might simply be confined by the space probed by the disordered regions of the transcription factors bound at the enhancer. The model might also explain the size control of paraspeckles and other nuclear condensates that assemble around long intergenic noncoding RNAs (lincRNAs) to recruit or sequester specific proteins [54]. Their lincRNAs act as a scaffold around which a condensate can form and their formation and size could be controlled via regulation of the lincRNA concentration.

In centrosomes of *C. elegans*, size is limited by the exhaustion of centrosome material [55,56]. Similarly, exhaustion of material might explain the size control of condensates formed around cytosolic dsDNA to launch an antiviral immune response [57]. Protein aggregation appears to be regulated by an active de-aggregation mechanism that kicks in only at large aggregate sizes, much above the critical radius [58]. Finally, condensates can be limited in size by mechanically preventing their coalescence [59].

The Models Make Testable Predictions

The presented models supply unifying principles to the often seemingly incoherent behavior of various types of biomolecular condensates. The enrichment-inhibition model predicts freely floating condensates in the cytoplasm or nucleoplasm to be size regulated by an antagonistic post-translationally modifying enzyme (e.g., a kinase) enriched in the condensates. The localization-induction model predicts spatially and temporally localized condensates such as those formed at sites of DNA repair to be regulated by an agonistic modifying enzyme at their center.

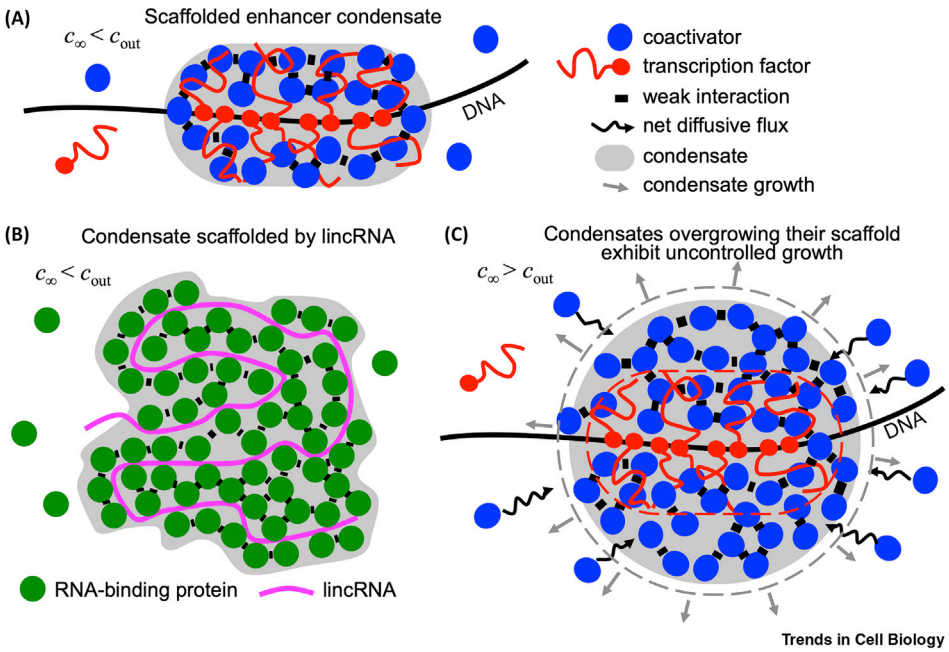

Figure 4. Scaffolded Condensate Model for Passive Size Control.
Without active mechanisms, when the concentration of proteins [blue in (A,C), green in (B)] is below saturation ($c_\infty < c_{out}$) condensates cannot form without help. However, their interaction with a localized scaffold [red in (A,C), pink in (B)] creates an attractive mean field potential $U(x)$ that, by Boltzmann's law, increases the local concentration at position x to $c_\infty e^{-U(x)kBT}$. If it surpasses c_{out}, a condensate forms whose spatial extent is confined by the mean field. (A) The disordered regions of transcription factors bound at an enhancer could interact with coactivators and thereby create an attractive mean field in which the coactivators can condense. (B) Analogously, long intergenic noncoding RNAs (lincRNAs) might be able to create an attractive mean field for RNA-binding proteins to condense in. (C) Below saturation, a passively regulated condensate cannot grow beyond its scaffold, because the protein fluxes through a condensate surface that has grown beyond the scaffold are independent of the scaffold and can therefore be stable only above saturation. Above saturation, the condensate location and size cannot be controlled.

To quantitatively test the enrichment-inhibition model *in vivo*, one could artificially induce the formation of membraneless organelles by partially inhibiting the antagonistic kinase enriched in them with a small-molecule inhibitor. The size of the condensates could be measured as a function of kinase activity and compared with the predictions from our theory (Figure 2C).

To quantitatively test the localization-induction model *in vivo*, one could apply corelet technology [69] to optically trigger the formation of 24-mer oligomers of the kinase suspected to induce the formation of condensates. The activity of the corelet-bound kinases can be modulated either by a small-molecular inhibitor or by expressing an inactive kinase from a controllable promoter. The resulting size of condensates can be compared with the model predictions (Figure 3C).

The localization-induction and the scaffolding models predict condensates to be stable even at very small radii (Figures 3C and 4A), at which the characteristic properties of phase separation are hard to observe by microscopy [70]. It is attractive to speculate that many previously detected protein clusters or foci might actually be tiny phase-separated liquid droplet condensates stabilized by the mechanisms in Figures 3 and 4. Recent molecular dynamics simulations of enhancers and experimental results indicate, for example, that the transcriptional minihubs observed in [24,26] might be true condensates [52]. Whether to call these clusters (e.g., the scaffolded condensates in Figure 4) bona fide condensates or not, their functional relevance might lie in their ability to achieve switch-like regulation through strong cooperatively.

Concluding Remarks

Zwicker *et al*. [60] and Wurtz and Lee [61] described how biochemically driven processes can be utilized for active size regulation of condensates. The enrichment-inhibition and localization-induction models correspond to externally and internally maintained condensates in Weber *et al*. [62], respectively. We clarified a crucial assumption, the differential enrichment of the kinase, phosphatase, or ATP [63] inside and outside condensates for efficient size regulation. Without this enrichment, the chemical potential of phosphorylated and unphosphorylated proteins would be almost constant in space, the net fluxes would be minimal, and size regulation would be impossible (section 5 in the supplemental information online).

The regulation of many biomolecular condensates may be more complex than the simplified models presented here. First, condensates will usually be regulated by several kinases or post-translational modifiers and they exert a combined effect on interactions within the condensate. For instance, at least two kinases, CDK2-cyclinE and VRK1, regulate the formation of Cajal bodies, but VRK1 phosphorylation of coilin at Ser184 stabilizes it against proteasomal degradation and therefore has an opposing effects on Cajal body stability [42,64]. In DNA double-strand break repair, condensate formation is regulated by both poly(ADP-ribose)ylation and phosphorylation of key proteins such as FUS [9,65,66]. Second, some processes will be regulated in a multistep fashion. For example, in signaling cascades, different types of condensate subdomains might form at each cascade step, each regulated by a different kinase and each depending on the previous one for the activation of its condensate-regulating kinase. Despite such regulatory complexity, we surmise that the regulation of condensates can often be understood by combining the two simple models.

The enrichment-inhibition model offers an explanation for the relatively low kinase specificity that is frequently observed in *in vitro* phosphorylation experiments and often deviates from the kinase specificity *in vivo* [67]. Kinases might attain most of their specificity not from their catalytic domain but from their enrichment in specific types of biocondensates. The latter is likely to be mostly determined by their disordered regions and peptide-binding modules. This hypothesis is supported by the high fraction of disorder in protein kinases and cyclins.

A crucial property of biocondensates is their ability to switch processes or biochemical reactions on or off in response to a signal comprising only a few molecules, such as a DNA double-strand break. The extremely cooperative behavior of phase transitions (even for very small condensates) explains how such weak signals can be amplified through condensate formation to recruit a multitude of factors required to react to the signal. To understand the magnitude by which condensates can accelerate reaction kinetics [68], assume that n proteins are required to form an oligomeric complex. If each component is enriched tenfold in the condensate, by mass action the oligomerization rate would be $\sim 10^n$-fold increased. Another advantage of active regulation is the thresholding behavior it produces (Figures 2C and 3C), which can suppress low-intensity noise and improve the robustness of cellular decisions.

It is becoming clear that liquid–liquid phase separation is a fundamental concept underlying most aspects of cellular regulation in eukaryotes. We hope the presented models can help to guide experiments in elucidating the functions of biocondensates and to understand their manifold roles in human diseases [66] (see Outstanding Questions).

Outstanding Questions

Which phosphatase antagonizes DYRK3 in the dissolution of membraneless organelles during mitosis and is it depleted there? Is the phosphatase PPTR-1, which antagonized MBK-2, indeed depleted in P granules?

Does the scaffolded condensate model describe enhancer and promoter condensates? What mechanism causes the observed, intermittent formation and dissolution [26]?

Neuronal granules are RNA–protein condensates serving as containers for transportation to dendrites and axons [21,22,23]. Are their assembly, disassembly, and size regulated by localization induction? What is the inducing enzyme and how is it activated and inactivated at the loading and discharge sites?

Given the involvement of the condensate-forming proteins FUS, TDP-43, and tau in neurodegenerative diseases [2,22], what are their mechanisms of condensate size control and what kinases and phosphatases regulate their condensate-forming potential?

What sequence characteristics distinguish target proteins in condensates regulated by enrichment inhibition from those in localization-inhibition-regulated ones – net charge?

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Supplemental Information

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