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Mechanobiology of Protein Droplets: Force Arises from Disorder

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The use of optogenetic approaches has revealed new roles for intracellular protein condensates described in two papers in this issue of *Cell* (Bracha et. al., 2018; Shin et al., 2018). These results show that growing condensates are able to exert mechanical forces resulting in chromatin rearrangement, establishing a new role for liquid-liquid phase separation in the mechanobiology of the cell.

Spatial organization of molecular components is crucial for ensuring biological function within the heterogeneous intracellular milieu. Membrane-bound compartments provide spatial control over the localization of biomolecules within them; it has recently become apparent, however, that many biomolecules, including intrinsically disordered proteins, are also able to spontaneously form spatially well-defined compartments (Brangwynne et al. 2009) even without the guidance of lipid membranes but rather as a result of liquid-liquid phase separation (LLPS). This phase transition leads to the conversion of a homogeneous solution within the cytoplasm of the cell into dense liquid droplets and can be modulated by the presence of secondary molecules such as RNA (Maharana et al. 2018). The biological roles of these membraneless organelles are very diverse and range from controlling the transport of molecules within a cell to creating catalytic environments for RNA processing (Shin and Brangwynne, 2017). In addition to the role of LLPS in normal biological function, liquid protein droplets have also been shown to have the propensity to transform further into solid aggregated structures implicated in a range of neurodegenerative diseases (Murakami et al. 2015). As such, liquid-liquid and liquid-solid phase transitions of proteins are increasingly recognized to be at the heart of both biological function and malfunction, which means there is a greater desire to understand the physical principles that define these transitions in a biological context.

In contrast to membrane-bound organelles, membraneless organelles controlled by LLPS are highly dynamic and can form and dissolve rapidly at specific locations within the cell, an aspect that is integral to their biological function. However, the physical basis of how this spatiotemporal control is achieved in cells remains only partially understood. In this issue, a series of methodological and conceptual advances, described in a set of two papers by Brangwynne and colleagues, shed new light on how the cell controls LLPS and uncover new functional roles for liquid droplets within cells. In one paper, Bracha et al. (2018, this issue of *Cell*) make key advances in the understanding of the molecular basis of LLPS phenomena by exploring the influence of polyvalent interactions between low complexity domains in coordinating and directing phase transitions. The other, by Shin et al. (2018, this issue of *Cell*), reveals a fascinating new role for such droplets in chromatin organization. The authors find that the force originating from surface tension in these microscopic droplets is sufficient to exert mechanical forces that can rearrange chromatin. The fascinating discovery that soft liquid droplets are able to generate significant mechanical forces in a cellular context highlights the role of classical soft condensed matter concepts, including wetting and surface tension, in defining the behavior of living matter on the micron scale.

A key step to reduce the complexity associated with LLPS in a cellular context down to molecular mechanisms is the ability to exert spatiotemporal control over the phase-transition within a living cell. Optogenetic approaches have proven to be particularly powerful in this context (Shin et al. 2017) and in their paper, Shin et al. (2018) present an approach that they term CasDrop (Figure 1A), which combines gene targeting and protein activation techniques to direct the initiation of LLPS to specific genomic regions. To this effect, the authors make use of the Cas9 system (Doudna and Charpentier, 2014) to first localize a labeled protein construct to specific genomic loci through the ability to direct an enzymatically inactivated Cas9 construct to bind to specific genes. The second component in the CasDrop system is a photosensitive protein, with the ability to initiate LLPS upon irradiation, conjugated to an antibody with an affinity to the first construct. Co-expression of both proteins thus leads to the ability to initiate LLPS through light irradiation at the location of specific chromatin elements. Using this approach, the authors discover that the propensity to undergo LLPS is significantly enhanced in general in the vicinity of regions of low chromatin density. This finding is likely to originate from the fact that in regions of high chromatin density, the higher mechanical energy required to deform the dense chromatin to create space for a growing protein drop would generate an energetic penalty limiting

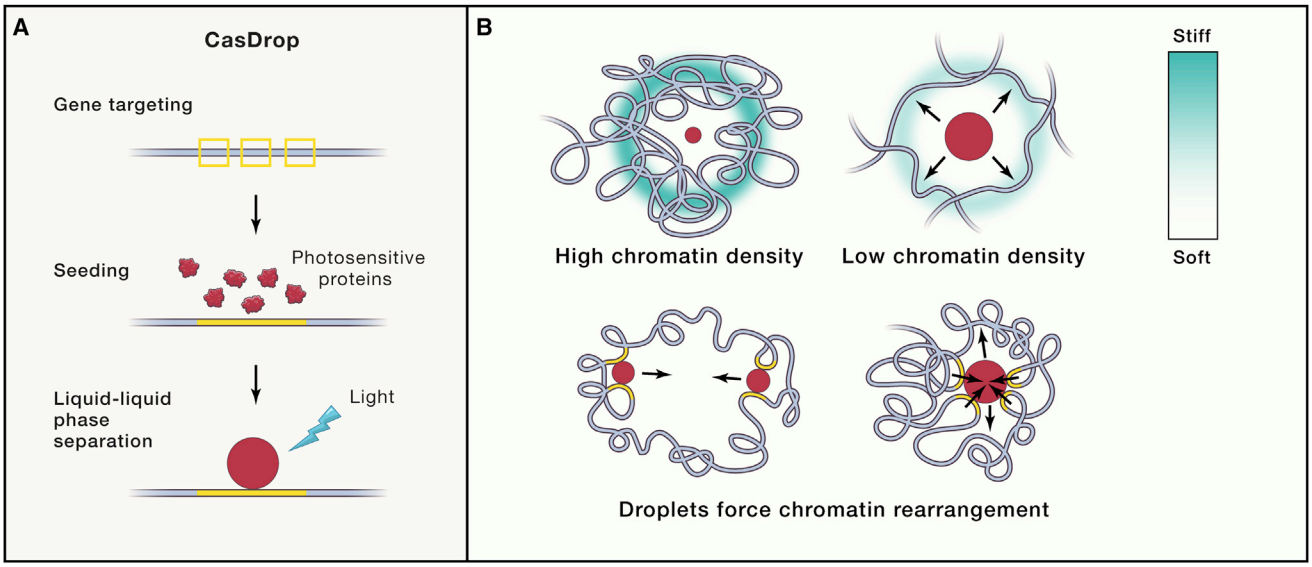

Figure 1. Controlled Liquid-Liquid Phase Separation Mechanically Alters the Spatial Arrangement of Chromatin

(A) CasDrop is designed to control liquid-liquid phase separation by targeting specific genomic loci to localize a photosensitive protein that can initiate phase separation when activated with light.

(B) Protein condensates form in low chromatin density areas and their formation is inhibited in high chromatin density regions due to mechanical forces generated by the elastic chromatin network. Merging of droplets can bring together different regions in the chromatin structure resulting in structural rearrangements.

the probability of nucleating a protein droplet in such areas.

Strikingly, the authors further observe that the growth of liquid droplets within the chromatin matrix is associated with two distinct mechanical effects (Figure 1B). First, chromatin is expelled as the drops grow, creating an effective repulsive interaction. However, the tendency of the droplets to merge to minimize their surface energy can lead to a second effect, where regions of chromatin initially far apart are brought into closer proximity through the growth and subsequent merging of the droplets that creates an effective attractive interaction. The interplay between LLPS and chromatin is thus able to generate significant forces that can both push chromatin regions away from each other as well as bring them together. This phenomenon demonstrates a new highly versatile potential mechanism for controlling the 3D arrangement of regulatory elements, aspects which are likely to be key for chromosomal communication and thus cell differentiation and gene expression (Dekker and Mirny, 2016).

The paper in this issue by Bracha et al. (2018) describes the development and application of a related optogenetic technology allowing the authors to optically trigger the formation of multivalent constructs bringing together a well-defined number of intrinsically disordered proteins into a "corelet" oligomer. These oligomers can then interact with each other more strongly than the monomeric proteins leading to LLPS. This work highlights the role of interaction between disordered proteins in allowing liquid droplets to nucleate and grow and provides further insights into the role of multivalent interactions in providing spatiotemporal control in cells.

Both papers published in this issue of Cell contribute in an important manner to the recent paradigm shift revealing the central role of physical phase separation in biology. The coupling between LLPS and mechanobiology demonstrated elegantly by Shin et al. (2018) adds a new dimension to the connections between physical phase behavior and biological function. It is interesting to speculate that this connection could also play a role in the onset and development of disease states that are triggered by the conversion of LLPS droplets into solid aggregates Qamar et al. (2018). By understanding the biophysics underlying LLPS events, we may in the future be able to correct improper function or even engineer new systems undergoing LLPS in a controlled manner for functional purposes.

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Secretin: An Old Hormone with a Burning Secret

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Most theories of meal-induced thermogenesis involve a gut-brain-brown adipose tissue axis driving sympathetic nervous system-mediated thermogenesis. Li et al. demonstrate that secretin released by the gut after a meal binds to abundant receptors in brown adipose tissue to stimulate thermogenesis, inhibiting food intake and thereby suggesting a novel role for secretin regulating satiety.

Secretin is a 27-amino-acid polypeptide secreted by the duodenum that inhibits gastric acid secretion and stimulates pancreatic bicarbonate production. In this issue of *Cell*, Li et al. (Li et al., 2018) now propose a new function for this digestive hormone, the first hormone ever discovered in 1902 (Bayliss and Starling, 1902). The authors performed an impressive series of experiments in cells, mice, and humans and propose secretin as a key player in a gut-brown adipose tissue-brain axis. Indeed, in response to a meal, serum secretin increases and binds to its abundant receptors in brown adipose tissue (BAT), which subsequently produces heat (and probably BATokines) that the brain senses. Consequently, the brain hypothalamus generates a satiation response with inhibition of orexigenic (neuropeptide Y [NPY] and agouti-related peptide [AgRP]) neurons and stimulation of anorexic signals via pro-opiomelanocortin (POMC) neurons (Figure 1). In short, the secretin released after a meal causes an increase in energy expenditure (meal-induced thermogenesis) with a subsequent suppression of hunger and enhancement of fullness, leading to meal termination. It almost sounds too good to be true since such mechanisms of action for secretin would most likely lead to negative energy balance and weight loss not only in rodents but probably also in humans.

Meal-induced thermogenesis is the increase in energy expenditure caused by the obligatory cost of digesting, absorbing, and storing the ingested food and a facultative component in response to sympathetic nervous system stimulation (Acheson et al., 1984). On a standard eucaloric and equilibrated diet, meal-induced thermogenesis accounts for approximately 10% of daily energy expenditure, half of which is facultative. Notably, secretin seems to increase facultative thermogenesis by stimulating brown adipose tissue metabolism, thus producing heat with an elevation of temperature not only in this tissue but in the whole body. The brain senses these rises in temperature and/or the proteins secreted by brown adipose tissue (BATokines) and triggers a response leading to meal termination.

In a series of well-designed experiments, Li et al. (Li et al., 2018) first show that the secretin receptor (a G_s-protein-coupled receptor that activates lipolysis) is highly expressed in interscapular brown adipose tissue, and its expression increases if differentiating brown adipocytes are incubated in the presence of secretin. Furthermore, the addition of secretin to primary brown adipocytes increases oxygen consumption with a potency 50-fold higher than isoproterenol by a mechanism independent of sympathetic stimulation. In addition to the robust *in vitro* data, the authors report that secretin injection increases *in vivo* heat production in control mice but not in mice deficient in uncoupling protein 1 (Ucp1), i.e., without active BAT. In addition, humans exhibit increased serum secretin concentrations following a single meal, which is associated with increased energy expenditure. The authors provide more evidence for the thermogenic action of secretin in brown adipose tissue by FDG-PET-CT, showing that secretin administration significantly increases glucose uptake in human brown adipose tissue.

Surprisingly, and in parallel to the induction of heat production in brown adipose tissue, secretin injection reduces