

Networking and Dynamic Switches in Biological Condensates

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Cellular liquid-liquid phase separation (LLPS) plays a key role in the dynamics and function of RNA-protein condensates like stress granules. In this issue of *Cell*, Yang et al., Guillén-Boixet et al., and Sanders et al. use a combination of experiment and modeling to provide an exciting mechanistic insight into the relationship between stress granules and LLPS, for example, in the context of protein disorder, switchable interactions, graph theory, and multiple interacting dense phases.

It has long been recognized that not all compartments within a cell are surrounded by membranes. Membraneless compartments, also called membraneless organelles or more generally biomolecular condensates (Banani et al., 2017), occur in both the nucleus and the cytoplasm and include the nucleolus and various types of RNA-protein condensates (RNP granules). One type of RNP granule is the stress granule (SG), a type of cytoplasmic condensate formed by association of proteins and mRNA released from ribosomes during stress (Protter and Parker, 2016). Work in recent years has revealed the importance of the physical process of liquid-liquid phase separation (LLPS) in the formation of SGs and several other RNP granules in cells. Nonetheless, much remains to be understood about the molecular mechanisms underlying RNP granule function. For example, how do these compartments dynamically form and disintegrate, and what are the molecular determinants of their composition, substructuring, and interactions? Along these lines, in the current issue of *Cell*, a trio of papers probes important mechanistic questions related to SGs and LLPS (Figure 1) by combining experiments in cells and *in vitro* with computation and development of conceptual models.

All three papers focus on the central role of the RNA binding protein G3BP in LLPS during the formation of SGs. This protein has several interesting features that may be important in its function in

SG formation. Intrinsically disordered regions (IDRs) of proteins are thought to play a major role in cellular LLPS by way of multivalent interactions (Banani et al., 2017; Bentley et al., 2019). In the case of G3BP, however, the story appears to be more nuanced. The IDRs within the protein interact with each other, and one of them interacts with RNA to promote SG formation. Yang et al. (2020) and Guillén-Boixet et al. (2020) show that these competing interactions of the IDRs result in a molecular switch: RNA concentrations above a certain threshold result in conformational switching to allow higher G3BP-RNA interaction, thus resulting in SG formation in response to increased free cytoplasmic RNA levels. The central IDRs also appear to determine what additional proteins are recruited to the SGs, which sheds some light on the interesting question of how specific macromolecules selectively partition into different molecular condensates (Yang et al., 2020). Additionally, the network of interactions leading to LLPS requires a multivalent RNA binding protein system (more on this below). G3BP dimerizes through its N-terminal domain, which by itself increases its valency to two, but at least one additional valence is required. Each of these three papers proposes a mechanism by which this can be accomplished.

The new papers explore SGs, and biomolecular condensates more generally, from the perspective of graph theory, which has been used to understand a wide variety of problems ranging from

the well-known Königsberg bridge problem (solved by Euler in the 18th century) to questions related to chemical structures, computer science, and bioinformatics. In the work by Yang et al. (2020), protein-protein interaction network analysis reveals that G3BP is the major hub in the SG interaction network. Sanders et al. (2020) cast the ideas of (poly)valency and linking in a simple graph theoretic framework to understand, predict, and test rules for SG formation. The basic idea is that LLPS is a manifestation of the formation of molecular networks. Thus, species with three or more valences can act as network nodes and form cross-links (and not just linear chains), while species with two valences are spacers (or bridges), and species with one valence lead to capping (and hence limit the network). Aspects of valence matching and capping have been invoked previously in the biological LLPS literature (Banani et al., 2017; Bentley et al., 2019). For example, for a pair of interacting polymers with complementary valences (such as oppositely charged patches in RNP components), phase separation can arise in a window around the ratio of polymer concentrations at which the valences on the species are matched. If the conditions move away from this matching ratio, one can observe droplet dissolution (due to valence capping) or even dynamic layered substructures (Banerjee et al., 2017).

The graph theory view presented is elegant and proves predictive for the SG cases tested. Future work on cellular



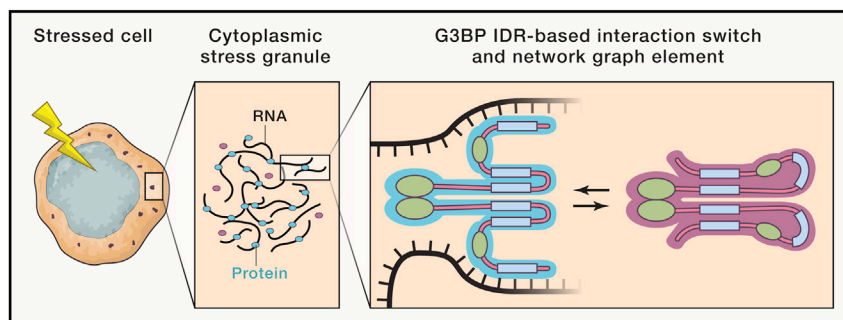


Figure 1. A Cartoon Illustrating Key Points Discussed in This Preview

Cell under stress (left) containing cytoplasmic stress granules. A zoom of an SG in the middle shows the network containing mRNAs, G3BP, and other proteins. Further right is another zoom depicting the G3BP IDR-based interaction switch that constitutes a network graph element and is also involved in other interactions. See text for details.

condensates could investigate additional elements potentially involved. An example is the influence of steric effects (i.e., can valences spatially reach each other?) and valence patterning (such as patterning of side-chain interaction motifs in protein sequences; [Martin et al., 2020](#)). Topology should also be considered, such as the effects of entanglement brought up in [Guillén-Boixet et al. \(2020\)](#), or catenated structures, such as in the kinetoplast mitochondrial DNA ([Klotz et al., 2020](#)) (an “Olympic gel” of the kind envisioned by Pierre-Gilles DeGennes), where topological constraints of linked polymer circles or loops serve as intermolecular connectors. Also along these lines, simulations in the paper by [Guillén-Boixet et al. \(2020\)](#) support a model of G3BP clusters between RNA strands, which provides for another interesting concept: rather than SG LLPS requiring preexisting well-defined valences on molecular nodes in the network, valences could also form spontaneously and dynamically on long polymers to regulate LLPS. As a related note, while cellular LLPS is often considered in a 3D context, phase separation initiated in “2D” (on membranes) and “1D” (along polymers) contexts should also be considered.

A number of other interesting insights emerge from the papers. [Sanders et al. \(2020\)](#) address how the number of dense phases is determined. This aspect is particularly relevant given the cellular links between SGs and P-bodies, another type of RNP granule, which can form on the surface of SGs and remain separate,

and which performs a different function in the cell. While G3BP appears to be the essential node for SG formation, other proteins act as nodes within SGs and other RNP granules. [Sanders et al. \(2020\)](#) propose a mechanism by which the number of different dense phases, as well as whether these phases are associated or separate, is determined by the network distance between different nodes in the phases (i.e., whether the central nodes for the different phases share other node proteins with which they can interact directly or, if not, the degree of separation) and the relative concentrations of the nodes involved. [Yang et al. \(2020\)](#) and [Guillén-Boixet et al. \(2020\)](#) address the effects of phosphorylation in regulation of SGs. They propose a model in which phosphorylation in one of the central IDRs, by increasing its interaction with the terminal IDR, thereby reducing the interaction of that region with RNA, modulates SG formation. Additionally, the specific RNA sequence appears to be less important than its length and single-strandedness. The broad range of techniques employed in these papers, including super-resolution imaging, single-particle analysis of SG dynamics, and the reconstitution of these complex systems, allowed specific perturbations to be applied and tested. Use of more complex methods, including single-molecule methods that have been tailored or developed for such studies, will clearly lead to further insight in the future.

Protein disorder has a history of revealing surprises when viewed through traditional mechanistic lenses, and this

will no doubt continue to be the case for RNP granules. The disordered and polymeric nature of granule components could reveal interesting elements related to complex non-equilibrium effects that result in rapid responses to changing cellular conditions, or perhaps correlated fluctuations across granules (the droplet equivalent of well-known allosteric effects at the molecular scale where conformational changes are propagated over a distance in proteins or other macromolecules). Finally, the rules found to encode the formation and dynamics of SGs and other RNP granules could also be used to implement new cellular functions ([Reinkemeier et al., 2019](#)) or synthesize novel materials.

Altogether, the three new papers provide important insight into the mechanistic basis for SG formation, regulation, interactions, and function, while also affording conceptual advances for understanding RNP granules in general and their roles in cellular function.

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SLeuthing Tuberculous Cough

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Cough, a hallmark of tuberculosis, transmits the disease. Ruhl et al. find that a *Mycobacterium tuberculosis* (Mtb)-specific lipid, SL-1, stimulates human nociceptive neurons and makes guinea pigs cough. Mtb extract, but not SL-1, also stimulates non-nociceptive neurons that participate in the cough reflex, suggesting additional cough-inducing mechanisms.

Cough, a sudden expulsion of air through the airways, is an essential protective reflex that enables the extrusion of foreign bodies, mucus, irritants, and infectious particles (Canning et al., 2014). The cough reflex is complex. It is initiated through two types of afferent nerves in the airways: nociceptors that sense inflammatory mediators and irritants, and mechanoreceptors that sense mechanical triggers, e.g., particulate matter (Canning et al., 2014). Diverse lung diseases cause cough: bacterial and viral pneumonias, asthma, chronic obstructive lung disease, and cancer, suggesting that lung damage and the resultant inflammatory mediators trigger the common final pathway leading to cough (Canning et al., 2014). Tantalizingly, in this issue of *Cell*, Ruhl et al. (2020) find that a *Mycobacterium tuberculosis* surface lipid, sulfolipid-1 (SL-1), can produce cough in guinea pigs and can activate nociceptive neurons in culture.

Cough is a major feature of tuberculosis (TB), and epidemiologic studies suggest that it is a predictor of TB transmission (Turner et al., 2018). In contrast to some

lung infections in which cough lasts for weeks after infection is cleared, cough in tuberculosis decreases rapidly after initiation of treatment, as does contagion (Acuña-Villaorduña et al., 2019; Proaño et al., 2017). Yet, lung damage and live *M. tuberculosis* can persist for weeks to months. So, the authors set out to determine if a specific bacterial product was responsible for TB cough. They took advantage of age-old findings that guinea pigs can both transmit and acquire TB through the respiratory route (Lurie, 1930). Guinea pigs are also used to study the cough reflex in response to irritants like capsaicin. But do guinea pigs cough when they get TB? Ruhl et al. found that they do and that an *M. tuberculosis* organic phase lipid extract could induce cough (Figure 1). Moreover, the lipid extract induced calcium fluxes in mouse nodose and jugular ganglia of the vagus nerve that initiate cough, and in other nociceptive neurons, such as mouse and human dorsal root ganglion cells (Figure 1). How did they get to SL-1? Extracts from virulent *M. tuberculosis* isolates induced the fluxes, but not the

extract from the avirulent derivative of a common lab strain. The avirulent strain lacks a functional two-component regulator and has global changes in gene expression. One of its missing determinants is a mystery lipid that is generally present in clinical isolates, speaking to its essential nature for *M. tuberculosis* survival. Perplexingly, the SL-1-deficient bacterium is not attenuated in animal models, suggesting that it is not a classic virulence factor. Honing in on SL-1, the authors found that it triggered both neuronal calcium fluxes and guinea pig cough (Figure 1). Moreover, extracts from a genetic SL-1 mutant triggered neither neuronal calcium fluxes nor guinea pig cough. Finally, the authors showed that the SL-1 mutant failed to increase cough in guinea pigs despite the mutation having no effect on bacterial burden or the usual pathology of TB in guinea pigs.

This rigorous study is nevertheless perplexing because of the relationship between SL-1 production and disease transmission caused by other mycobacterial infections. While a close pathogenic

