Paraspeckles: Paragons of functional aggregation

Edward Courchaine and Karla M. Neugebauer

Yale University, New Haven, CT 06511

Low-complexity proteins undergo phase separation in vitro, forming hydrogels or liquid droplets. Whether these form in vivo, and under what conditions, is still unclear. In this issue, Hennig et al. (2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201504117) show that formation of the paraspeckle, a nuclear body that regulates gene expression, requires low-complexity prion-like domains (PLDs) within paraspeckle proteins. The same proteins were shown to form hydrogels, shedding light on the role of "functional aggregation" in nuclear substructure.

Cellular compartments that are not bound by lipid bilayers are most often referred to as "bodies," and have been observed for more than a century. Yet our understanding of how they form lacks significant depth. Nucleoli and Cajal bodies (CBs) manifest properties of liquid droplets by splitting to form smaller bodies and fusing to make larger ones (Platani et al., 2000; Brangwynne et al., 2011). In characterizing their physical properties, Gall and colleagues suggested that nucleoplasm undergoes liquid phase separation to generate nucleoli and CBs (Handwerger et al., 2005). Cytoplasmic P-granules also display characteristics of liquid droplets, indicating the generality of this concept (Brangwynne et al., 2009). The present work establishes the nuclear paraspeckle as another compartment that likely forms through phase separation (see Hennig et al. in this issue). Thus, we can think of cellular bodies as the consequence of a phase transition that creates a liquid, water-permeable compartment that condenses from the bulk solution.

Recently, in vitro studies of liquid phase separation have addressed how cellular bodies may behave in physiological and pathological circumstances (Han et al., 2012; Kato et al., 2012; Li et al., 2012). The hydrogels formed in these experiments are stable, de-mixed droplets that can fuse extensively and are even susceptible to enzymatic modification such as phosphorylation (Kwon et al., 2013, 2014). Furthermore, proteinaceous gels can be manipulated by changing salinity and temperature, altering their size and the solubility of nucleic acids inside of them (Elbaum-Garfinkle et al., 2015; Nott et al., 2015). These observations implicate multivalent binding, disordered protein structure, and locally variable conditions in the phase separation process.

In this issue, Hennig et al. (2015) directly address how phase separation might occur in the nuclear paraspeckle, using both in vivo and in vitro approaches on the same protein components. Paraspeckles were previously shown to form on the lncRNA NEAT1, concentrating at the site of transcription (Mao et al., 2011). While analyzing the protein interaction network of paraspeckles, the authors noticed a concentration of proteins that contain low-complexity prion-like domains (PLDs) in these bodies. They then focused on two of these proteins: FUS and RBM14. These PLDs were necessary for proper paraspeckle formation in cells. When removed from the cellular environment, FUS and RBM14 formed hydrogels reminiscent of the in vitro hydrogels discussed earlier. We begin to see that the repeated amino acid sequences of low-complexity proteins, exemplified in the PLDs of these paraspeckle proteins, are fundamentally involved in cellular body formation.

The significance of this work on FUS and RBM14 goes beyond an observation of phase separation behavior, as many of the proteins in the paraspeckle have direct links to a variety of neurological diseases like amyotrophic lateral sclerosis (ALS). These often manifest with the emergence of cytotoxic aggregates formed from constituent proteins (Shelkovnikova et al., 2014). Other low-complexity and/or RNA-binding proteins are implicated in diseases, such as myotonic dystrophy type 1 and ALS, that exhibit abnormal body formation (Ranum and Cooper, 2006; King et al., 2012). Huntingtin is a classic example of a protein that forms a toxic aggregate upon repeat expansion. Interestingly, Hennig et al. (2015) show that PLDs can form fibers that are quite similar to those of huntingtin, even though they lack expanded repeats and characteristic resistance to SDS denaturation. The authors invoke "functional aggregation," in which proteins with stretches of repeated amino acids are able to dynamically concentrate themselves and other molecules under normal conditions (Fig. 1). If true, cellular bodies could provide a mechanism for localizing or sequestering certain molecular species, possibly to promote reactions or prevent damaged and unassembled machines from causing harm. Abnormal function accompanies unwanted morphologies, such as toxic fibers (Fig. 1).

To understand how phase separation organizes cells, we must consider what mechanisms give rise to these structures. Individual proteins involved in actin nucleation (N-WASP) and nuclear transport (FG domains) have inspired some of the in vitro work on phase separation (Frey et al., 2006; Frey and Görlich, 2007; Li et al., 2012). Hydrogels formed by FG domains form the basis for the selective permeability of nuclear pores (Schmidt and Görlich, 2015). Knowledge of how PLDs act alone strengthens our understanding of nuclear bodies. In addition, Hennig et al. (2015) present an impressive yeast two-hybrid screen that reveals the complexity of the paraspeckle interaction network, achieved through the presence of PLDs within multiple components. The resulting added complexity among interacting proteins is an important step toward physiological relevance.

Hennig et al. (2015) are open about neglecting the RNA component NEAT1 in their experiments. It is striking that meaningful phase separation is obtained without NEAT1, but this may not be possible in the context of other bodies. The nucleolus, the

Correspondence to Karla M. Neugebauer: karla.neugebauer@yale.edu

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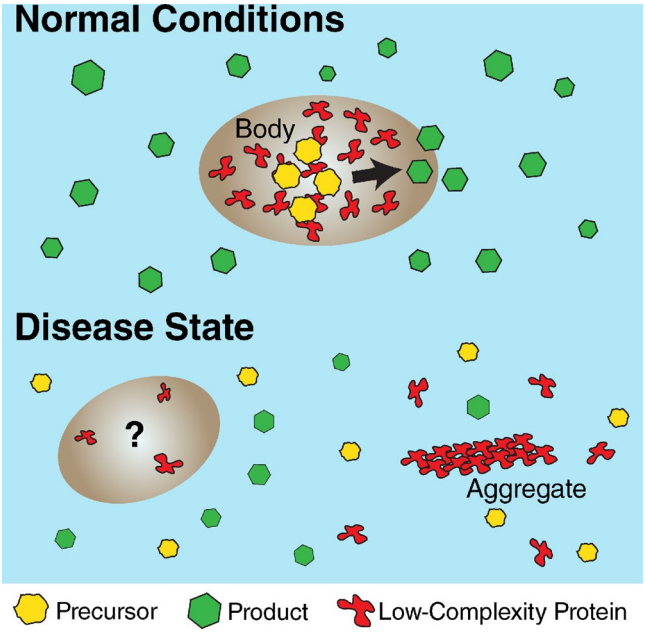

Figure 1. **Low-complexity proteins and the formation of cellular bodies in health and disease.** In normal body function (top), low-complexity proteins coordinate the sequestration of precursor molecules, which can be either RNA or proteins. Within the body, these components are assembled into functional complexes, which then leave the body for a function, such as product generation (green). In disease (bottom), low-complexity proteins can form aggregates that are toxic and may prevent bodies from functioning normally. This results in a depletion of functional complexes and the release of precursor into the nucleoplasm and/or cytoplasm.

site of rRNA processing, contains hundreds of RNA–protein and RNA–RNA interactions (Pederson, 2011). The CB, where snRNPs are assembled, is scaffolded by coilin, a low-complexity protein that binds RNA through a noncanonical mechanism (Machyna et al., 2014, 2015). This raises the possibility that other low-complexity proteins also interact with RNA in cellular bodies, suggesting that RNA should be introduced into the mix.

Does assembly of all bodies depend on the high complexity of networks of low-complexity proteins? Or are there really a small number of key interactions that give rise to a platform for all the others? Protein–protein and protein–RNA interaction maps are a critical starting point for a better understanding of how diverse molecular species may be involved, making this and other recent studies important steps forward. By establishing direct links between the behavior of low-complexity proteins in and out of the cell, Hennig et al. (2015) challenge the field to further investigate the many facets of functional aggregation and what influences dysfunction in disease.

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