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Gel or Die: Phase Separation as a Survival Strategy

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Stress conditions trigger protein assembly by demixing from the cytoplasm, but the biological significance is still unclear. In this issue of *Cell*, Riback et al. report that the yeast poly(A)-binding protein 1 (Pab1) is a phase-separating stress sensor that boosts organismal fitness under physiological stress conditions.

Life is stressful. Organisms have to continually adjust their internal conditions to changes in the external environment. Cells exposed to stress stop the cell cycle, modify their metabolism, downregulate house-keeping functions, and ramp up stress-protective pathways. The best-understood stress response is that to heat stress. Heat shock causes widespread protein misfolding and aggregation. This, in turn, leads to the activation of transcription factors that upregulate protective heat shock proteins, which prevent aberrant behavior of misfolded proteins, thus ensuring stress adaptation (Richter et al., 2010). But is this really the whole picture? In this issue of *Cell*, Riback et al. (2017) make the remarkable discovery that stress-triggered aggregation of Pab1 is an adaptive mechanism that provides a fitness advantage to cells (Figure 1A). The authors show that in vitro, under physiological stress conditions, Pab1 demixes from solution to form gel-like structures. They conclude that Pab1 senses stress through an ultra-sensitive phase separation reaction.

The work suggests that Pab1 phase separation is part of a larger adaptive response that results in the formation of so-called stress granules. Stress granules are microscopically visible structures that form in eukaryotic cells under various stress conditions, such as starvation or temperature fluctuations (Protter and Parker 2016). They contain many translationally silenced house-keeping mRNAs and translation factors, which has led to the proposal that they regulate protein synthesis. However, reliable evidence that stress granules have adaptive value is still scarce.

The authors employ a range of biochemical, biophysical, and cell biological approaches, as well as yeast genetics, to investigate the stress granule protein Pab1. They find that upon changes in temperature or pH, purified Pab1 phase separates into structures that have the appearance of clusters of sticky balls. Photobleaching shows that these sticky balls have solid properties and are reminiscent of colloidal gels. Heat- and pH-induced structures show different morphologies, implying differences in material properties, which may be physiologically important. Surprisingly, in contrast to previous findings, in which RNAs cooperatively promote phase separation (Elbaum-Garfinkle et al., 2015), RNA inhibits demixing and is released from phase-separating Pab1. Thus, phase separation also affects the RNA-binding activity of Pab1. One open question concerns the specific molecular function of Pab1 phase separation. The authors propose that phase separation releases stress-protective mRNAs from Pab1 repression, thus allowing the cell to quickly mount a stress response. However, others have described a role of Pab1 in facilitating translation initiation (Kessler and Sachs 1998). Thus, it remains to be tested why phase separation of Pab1 promotes the survival of stressed cells.

Pab1 not only forms assemblies in response to thermal stress but also in response to starvation conditions. Starvation has been shown to acidify the cytosol, and this leads to the formation of various assemblies, such as filaments of metabolic enzymes (Petrovska et al., 2014) and additional, more amorphous structures (Munder et al., 2016). It has been proposed that these assemblies are adaptive and promote entry into a dormancy-like state (Munder et al., 2016; Petrovska et al., 2014). In fact, preventing cytosolic acidification during starvation causes cell death. This suggests that besides Pab1, many additional proteins can sense physiological stresses and then form liquids, gels, and filaments (Figure 1B). Remarkably, stressed yeast form so many assemblies that they become stiff and keep their shape when the cell wall is removed (Munder et al., 2016). This indicates a massive change in the material properties of the cytoplasm and is reminiscent of conditions found in dormant spores.

One problem has been the lack of appropriate nomenclature to describe the richness of assemblies that form during stress. Unlike quaternary structures, Pab1 assemblies or metabolic enzyme filaments have no fixed stoichiometry. The term "quinary assembly" has been proposed to refer to such structures (Edelstein 1980; Chien and Gierasch 2014). This term has two important advantages: it expands the highly successful concept of four structural levels (primary, secondary, tertiary, and quaternary structure), and it implies that these structures are subject to evolutionary selection. This distinguishes quinary assemblies from randomly formed, misfolding-based protein aggregates.

The authors next perform a multisequence alignment of Pab1. While certain regions, such as the folded RRM domains, align nicely, others, like the proline-rich P domain, show extensive variation. However, the P domain is functionally important because mutations that tune its hydrophobicity generate variants with higher or lower stress sensitivity. This suggests that natural selection shapes the phase behavior of Pab1 by altering the sequence composition of the P domain. It further suggests that

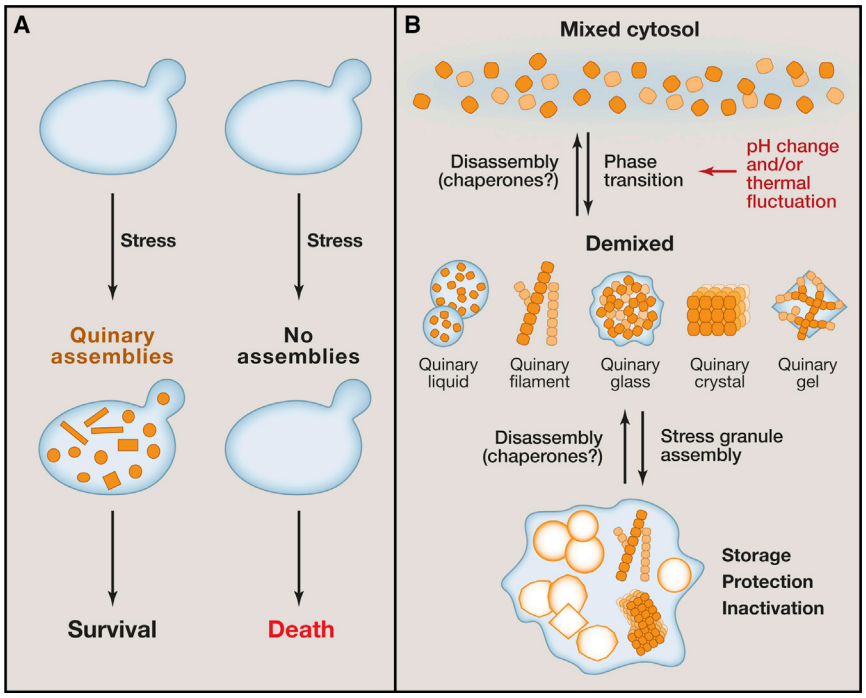

Figure 1. Phases Separation as a Survival Strategy

(A) Stressed yeast cells form quinary assemblies that promote survival.

(B) Changes in pH and/or temperature can cause proteins to demix into reversible quinary liquids, filaments, glasses, crystals, or gels. These quinary assemblies have diverse molecular functions (storage, protection, and inactivation) and form microscopically visible granules with time. Dissolution of some quinary assemblies may be facilitated by chaperones.

P-domain-like sequences could provide a rich source of information about quinary assemblies and their environmental triggers. More generally, there seems to be an opportunity to build a molecular evolutionary theory by combining sequence analysis with mutational perturbations and targeted experiments to probe the biophysics and biology of quinary assemblies.

Having established that Pab1 acts as a phase-separating stress sensor, the authors next set out to unravel the molecular basis of phase separation. They find, surprisingly, that phase separation is not driven by the P domain, but by the RRM domains. However, how the RRM domains mediate phase separation remains unclear. Interestingly, although the P domain is not the main driver of phase separation, it sets the boundaries of phase separation. Mutating hydrophobic residues in the P domain cause dramatic shifts in the critical temperature of demixing, suggesting that the P domain is a modifier of phase separation. The authors further find that the same hydrophobic residues also drive a collapse of the disordered P domain on itself. Together, these findings suggest that there is a complex interplay between disordered and folded domains. However, the situation in cells may be even more complex because during stress, many different proteins phase-separate simultaneously. Indeed, other yeast proteins, such as Pub1, carry P-domain-like sequences (Alberti et al., 2009), suggesting that these findings are generalizable.

The work by Riback et al. (2017) shows that phase separation can mediate environmental sensing. However, what remains unclear is how stress is detected on the molecular level. Mechanisms proposed involve temperature-dependent desolvation, charge-patch interactions, or protonation of residues upon pH fluctuations. More importantly, what applies to Pab1 may also be true for other proteins. These proteins may have specific sensitivities to stress, thus establishing a system of phase-separating proteins that responds to different types of stresses, stress intensities, and rates (Figure 1B).

Many additional questions remain for the future. How are stress granules organized in vivo, and what are their material properties? Do different stresses lead to assemblies with different material properties, and does this affect the rate of adaptation or the duration of the recovery phase? Are chaperones such as Hsp104 required for disassembly? How do modifier sequences regulate the phase behavior of folded domains, and is there cooperativity between different phase-separating proteins? Ultimately, this is a fantastic paper that shows a uniquely complete story from the molecular details of phase separation to biological significance, raising many intriguing questions for future research.

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