Osmotic Stress Triggers Phase Separation

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In this issue of *Molecular Cell*, Jalihal et al. (2020) show that cell volume changes upon osmotic stress result in rapid and reversible condensation of numerous multivalent proteins.

Eukaryotic cells contain many subcellular domains, such as nucleoli, that concentrate specialized biomolecules without an encapsulating membrane. Seminal discoveries nearly a decade ago that these domains behave like phase-separated liquids (Brangwynne et al., 2009) and that their formation can be described using principles from polymer science (Li et al., 2012) have transformed our understanding of cellular organization. These assemblies, collectively called biomolecular condensates, have now been identified to organize enzymes involved in nearly every aspect of cellular biochemistry.

A prevailing model is that these condensates form via multivalence-dependent phase separation. Biomolecular condensates in cells are enriched in multivalent proteins: proteins that contain multiple binding sites for themselves or partner proteins/RNA. Depending on the interaction affinity and concentration, multivalent molecules can form oligomers of various stoichiometries. Beyond a certain threshold concentration, these molecules assemble into large molecular networks that separate out from the cellular milieu. At fixed protein number, an increase in cell size can dilute proteins and result in condensate dissolution (Weber and Brangwynne, 2015). Likewise, reduction in cell size may potentially tip the concentration of certain proteins above the threshold for network formation and phase separation. Bearing this hypothesis to fruition, in this issue of *Molecular Cell*, Jalihal and co-workers (Jalihal et al., 2020) elegantly demonstrate that osmotic stress-associated reduction in cell volume induces condensation of numerous multivalent proteins (Figure 1).

Jalihal et al. use a trimeric protein, DCP1A, a component of mRNA processing bodies (P-bodies), as a model multivalent protein. Upon hypertonic stress, the protein rapidly, within seconds, condensed into numerous *de novo* foci in the cytoplasm. In contrast, G3BP1, a marker for stress granules that forms auto-inhibited dimers (Yang et al., 2020), remained dispersed at these timescales. This condensation of DCP1A occurs substantially faster than the cell's adaptive response to osmotic stress, such as the accumulation of stress granules or induction of integrated stress response, as evidenced by EIF4E phosphorylation. Live-cell imaging revealed that DCP1A bodies appeared concomitantly with a decrease in cell volume. The extent of DCP1A condensation scaled with the osmolyte concentration and the corresponding reduction in cell size. Moreover, similar partitioning of DCP1A to condensates could be achieved by either increasing the osmolyte concentration or by increasing DCP1A expression level, thus buttressing the notion that DCP1A condensation results from an increased effective concentration of the protein in the cell upon osmotic stress.

This hyperosmotic stress-driven phase separation (or HOPS) of DCP1A was reversible: upon restoring the cell culture medium's tonicity, the DCP1A condensates dispersed within minutes. In contrast, oxidative stress-induced P-bodies (that contain DCP1A), as well as stress granules, persist for a substantially longer period after rescue. Cells could be subjected to multiple cycles of osmotic stress and recovery without a noticeable change in the extent of DCP1A condensation or cell viability.

The C-terminal trimerization domain of DCP1A was necessary and sufficient for its condensation in response to osmotic stress. This observation led authors to hypothesize that other cellular proteins with multimerization domains may also undergo HOPS. The authors screened >100 proteins via high-throughput microscopy. It emerged that many multivalent proteins (18 out of 29 that were tested), including several proteins with low-complexity domains, undergo HOPS, while most proteins without an annotated oligomerization domain (65 out of 75) do not. HOPS appeared independent of the cell type. In fact, a recent study reported similar findings in yeast, where many self-interacting proteins, including those with prion-like domains, underwent rapid and reversible condensation upon osmotic stress (Alexandrov et al., 2019).

On a longer timescale, cells adapt to osmolyte imbalances and restore cell volume and ionic strength. Interestingly, several recent studies have discovered HOPS-like phenomena for specific proteins and offer insights on how this rapid condensation may enable cells to mount a nuanced adaptive response. For instance, Cai et al. (Cai et al., 2019) found that condensation of a transcription factor, YAP, allowed it to re-organize the genome and activate the expression of its target genes. Yasuda et al. (Yasuda et al., 2020) reported that osmotic stress resulted in transient phase separation of the proteasome with ubiquitinated proteins that helps clear misassembled ribosomal proteins.

Could HOPS also underlie the cellular defects resulting from osmotic stress? Hypertonic stress induces widespread readthrough transcription, and aberrant transcripts containing regions downstream of genes are observable within minutes after stress induction (Vilborg et al., 2017). The authors found that a pre-mRNA cleavage factor, CPSF6,

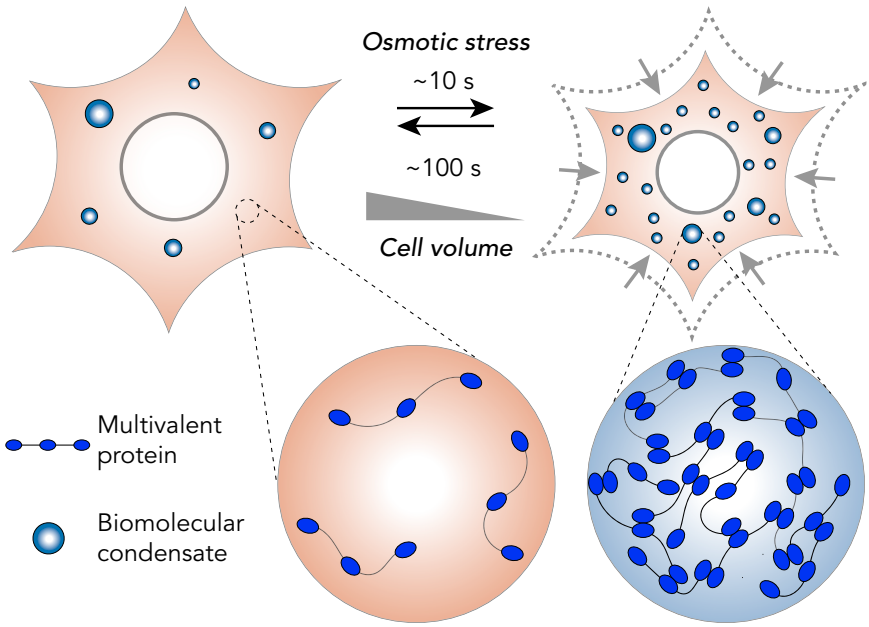

Figure 1. Schematic for Hyperosmotic Stress-Induced Phase Separation
Increased osmotic pressure reduces cell volume and results in an increased concentration of ions and macromolecules. Jalihal et al. show that several multivalent proteins that remain dispersed at physiological conditions reversibly condense to microscopic granules under hyperosmotic stress.

underwent HOPS. CPSF6 condensation correlated with its reduced occupancy on the transcription termination sites and an increased residence of RNA Polymerase II in the regions 3′ to the canonical termination sites. Sequestration of transcription termination factors may provide a cogent mechanistic explanation for the rapid onset of RNA processing defects.

Sustained hyperosmotic stress results in an accumulation of P-bodies. One peculiarity is that HOPS-induced DCP1A foci are not bona fide P-bodies and do not recruit other P-body components such as EDC4 and DDX6. Whether and how these HOPS-induced foci relate to P-bodies and other known membraneless organelles remains to be explored. Hyperosmotic stress is also associated with amyloidogenesis. HOPS appears to be reversible on short timescales, but prolonged condensation may potentiate irreversible aggregation, as has been observed for several proteins present in stress granules. Besides changing cell volume, osmotic stress alters ionic strength and membrane morphology and reduces molecular mobility due to crowding. Moreover, the choice of osmolyte may also affect the cell's immediate and adaptive responses. Further work will be required to mechanistically understand the interplay of these factors with HOPS.

This exciting work by Jalihal et al. uncovers a facile biophysical mechanism by which cells can sense extracellular stimuli. Future studies may reveal additional routes through which HOPS may disrupt cellular functions or enable cells to respond to environmental cues. Changes in cell volume also occur during cell cycle and cell migration. One tantalizing possibility is that HOPS-like phenomena may help buffer the concentration of soluble proteins (Klosin et al., 2020). On the other hand, this process may pose a fundamental biochemical constraint on the abundance and interaction affinities of multivalent proteins. This insightful study adds to the expanding list of cellular processes that are modulated by biomolecular condensates and will inspire future discoveries on its role in stress response.

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