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STRESS RESPONSES

Reversible sequestration over irreversible aggregation

> the Sup35 IDR mediates the formation of reversible Sup35 gels instead of irreversible aggregates

Following various cellular stresses, proteins with intrinsically disordered regions (IDRs) can undergo liquid–liquid phase separation and form membrane-less assemblies or granules. Prion-like proteins are examples of such proteins that also form amyloid-like, fibrillar aggregates, which are toxic and implicated in age-related neurodegeneration. Franzmann *et al.* now show that in stress conditions in *Saccharomyces cerevisiae*, the IDR of a prion protein promotes reversible protein phase separation instead of the more stable fibrillar aggregation.

Stressed cells arrest their growth, which generally involves inhibition of protein synthesis and the sequestration of translation factors in stress granules. The yeast prion protein Sup35 is a translation termination factor. Its amino-terminal region is an IDR comprising an N-terminal prion domain, which alone can form heritable, fibrillar aggregates, and a charged middle domain.

Depletion of yeast cells of energy resulted in growth arrest and the formation of submicrometre-scale Sup35 assemblies; cells persisted in the arrested state for as long as the assemblies were present. Unlike prion aggregates, the Sup35 assemblies dissolved within a few minutes of removing the stress, were independent of the prion-remodelling enzyme Hsp104 for their formation or dissolution and lacked biochemical features of amyloid-like aggregates. Energy-depleted yeast cells also featured reduced cytosolic pH, and acidification was sufficient to induce the formation of Sup35 assemblies. Thus, stress-mediated pH changes regulate the formation of reversible Sup35 condensates.

The authors purified Sup35 to study its condensation in vitro. When purified Sup35 was incubated in physiological buffer, the protein remained diffuse; upon reducing the pH from 7.5 to 6, Sup35 phase-separated to liquid-like condensates and then solidified into a gel-like state. Cryo-electron tomography revealed that the gel-like droplets comprised a well-defined polymer meshwork. Importantly, the gel-like condensates dissolved when the pH was raised. Fluorescence recovery after photobleaching showed that Sup35 was mobile in growing cells, but became immobile when sequestered into condensates following stress.

The Sup35 middle domain has a cluster of negatively charged Glu and Asp residues. Mutation of these residues to neutral residues yielded a fully functional protein, but with altered phase separation properties manifested as pH-independence of droplet formation. Importantly, whereas the IDR alone was sufficient for phase separation, the carboxy-terminal GTPase domain alone formed irreversible aggregates at all tested pH conditions; in the presence of the middle domain this was strongly reduced in favour of condensate formation, albeit at an order of magnitude lower than for the wild-type protein. Thus, the IDR helps maintain the solubility of Sup35 and provides its pH sensitivity as well as the cohesiveness required for its condensation.

Yeast cells expressing only the C-terminal domain of Sup35 (Sup35C) had no noticeable growth defect in the absence of stress, and, during stress, Sup35C assembled similarly to Sup35. However, following the removal of stress, condensates formed by wild-type Sup35 dissolved within minutes whereas Sup35C aggregates took up to several hours to dissolve, and Sup35C cells took longer to restart growth. Mechanistically, translation shutdown during stress and its resumption upon stress removal coincided with Sup35 condensation and dissolution, respectively. Importantly, translation was impaired in recovering Sup35C cells but not in recovering control cells.

In summary, the Sup35 IDR mediates the formation of reversible Sup35 gels instead of irreversible aggregates during stress, which supports translation resumption and increases cell fitness when the stress ceases. This indicates that promoting the formation of reversible gels rather than prions is the original function of prion domains, and that prion domains could be protein-specific stress sensors and adaptors of cell responses to stress.

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ORIGINAL ARTICLE Franzmann, T. M. *et al.* Phase separation of a yeast prion protein promotes cellular fitness. *Science* **359**, pii: eaao5654 (2018)
FURTHER READING Banani, S. F., Lee, H. O., Hyman, A. A. & Rosen, M. K. Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285-298 (2017)