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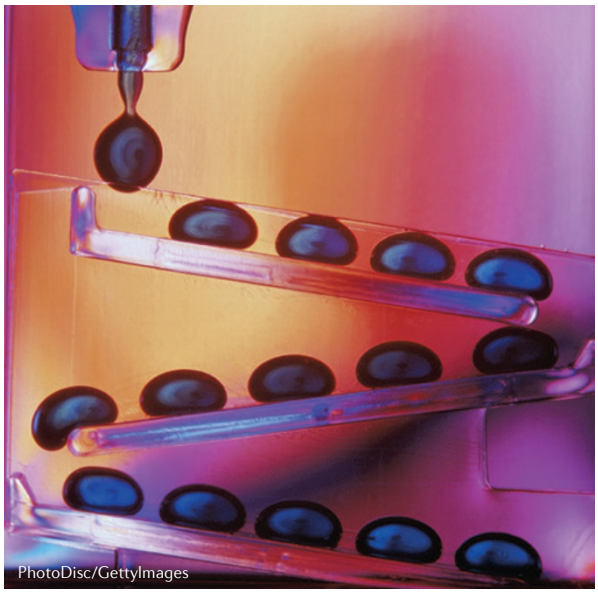
MOLECULAR NETWORKS

Protein droplets in the spotlight

> how various proteins, implicated in both physiological and pathological processes, can undergo phase separation

Previous studies revealed that both *in vitro* and *in vivo* proteins can undergo liquid–liquid phase transition to form liquid droplets. This phase separation may serve to locally concentrate proteins, thereby influencing their functions and activities. Current research from three independent laboratories provides novel insights into the importance of this phenomenon in fundamental biological processes.

Jiang *et al.* report that phase transition is important for microtubule polymerization and bundling during spindle assembly. The authors studied a vertebrate protein, BuGZ (also known as ZNF207), which was previously identified as a component of the spindle matrix, an ill-defined structure incorporating various proteins involved in spindle formation. They found that, with the exception of the amino-terminal end of the protein, which has been shown to bind microtubules (as well as free tubulin), the majority of the BuGZ sequence has low amino acid complexity and was predicted to be unstructured. In accordance with previous studies suggesting that proteins containing unstructured regions could undergo phase transition leading to droplet formation (a process known as coacervation), BuGZ formed droplets *in vitro*. Coacervation was dependent on the presence of hydrophobic and aromatic residues in the unstructured region of the protein. Microtubule binding by BuGZ was not essential for coacervation but facilitated phase transition at low protein concentrations. Furthermore, BuGZ was found to promote the assembly of the spindle apparatus together with the spindle matrix. Importantly, *in vitro* and in mammalian cells, both microtubule binding and phase transition were essential for BuGZ to function in spindle assembly. The authors suggest that microtubule binding prevents BuGZ from acquiring a semi-folded state, which would inhibit intermolecular hydrophobic interactions and thus efficient phase transition. Consequently, microtubule binding promotes BuGZ droplet formation, and this allows local concentration of BuGZ molecules and their tubulin-binding domains. This results in efficient tubulin recruitment, which promotes microtubule polymerization and bundling, thereby facilitating spindle assembly.



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The studies by Lin *et al.* and Molliex *et al.* focused on the role of protein phase separation during the assembly of RNA–protein (RNP) granules, which include many important non-membrane-bound organelles such as the nucleolus, Cajal bodies and stress granules. Many RNP proteins contain RNA-binding domains and unstructured, low-complexity regions. Both groups, primarily using *in vitro* assays, demonstrated that RNP proteins can undergo efficient droplet formation. The unstructured regions are sufficient for coacervation, but RNA binding largely stimulates this process, allowing phase separation to occur at biologically relevant concentrations. The authors suggest that RNA acts as a crosslinker, locally increasing protein levels. Thus, RNA binding in RNP proteins has a similar function to microtubule binding in BuGZ, allowing efficient interaction between proteins in order to drive droplet formation. Also, similar to BuGZ, coacervation of RNP proteins appears to depend on the presence of aromatic residues and is additionally enhanced by electrostatic interactions. Furthermore, these studies reveal that droplet formation by RNP proteins augments their potency to undergo fibrillization, which when enhanced has been associated with severe degenerative human diseases.

Together, these studies illustrate how various proteins, implicated in both physiological and pathological processes, can undergo phase separation to form liquid droplets. It would be interesting to investigate how widespread coacervation is in the broader, cellular context and how it is regulated, as well as how it might be modified to prevent disease.

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ORIGINAL RESEARCH PAPERS Jiang, H. *et al.* Phase transitions of spindle-associated protein regulate spindle apparatus assembly. *Cell* http://dx.doi.org/10.1016/j.cell.2015.08.010 (2015) | Molliex, A. *et al.* Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell* http://dx.doi.org/10.1016/j.cell.2015.09.015 (2015) | Lin, Y. *et al.* Formation and maturation of phase-separated liquid droplets by RNA-binding proteins. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2015.08.018 (2015)