frontiers | Frontiers in Biophysics

TYPE Mini Review
PUBLISHED 14 June 2023
DOI 10.3389/frbis.2023.1208763

OPEN ACCESS

EDITED BY
David Alsteens,
Université Catholique de Louvain,
Belgium

REVIEWED BY
Charles S. Bond,
University of Western Australia, Australia
Davit Potoyan,
Iowa State University, United States

*CORRESPONDENCE
Danny M. Hatters,
dhatters@unimelb.edu.au

RECEIVED 19 April 2023
ACCEPTED 06 June 2023
PUBLISHED 14 June 2023

CITATION
Hatters DM (2023), Grand challenges in biomolecular condensates: structure, function, and formation.
Front. Biophys. 1:1208763.
doi: 10.3389/frbis.2023.1208763

Grand challenges in biomolecular condensates: structure, function, and formation

Danny M. Hatters*

Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC, Australia

Biomolecular condensates describe concentrated nonstoichiometric assemblies of biomolecules that can form by a range of different mechanisms 1). Biomolecular condensates can arise by phase separation, which in biology involves the demixing of a water-soluble polymer into two co-existing phases: a polymer-dilute phase and a polymer-dense phase. Coacervates describe phase separation mediated by a third element, which may typically be a ligand (such as RNA) to the polymer (such as a protein) that undergoes phase separation. Protein aggregation into amyloids and amorphous aggregates, and the formation of RNA granules, represent other forms of biomolecular condensates. The assembly of proteins and other biomolecules into complexes is a fundamental feature for the execution of biological functions. Biomolecular condensates are a natural variation of the assembly theme. There is an incredible complexity and diversity to how condensates form, are regulated and are structured (reviewed recently in 2)). And there is incredible diversity to how condensates are used by nature to drive biological functions and how when their assemblies go wrong, they can drive disease mechanisms, such as amyloids in neurodegeneration.

KEYWORDS

coacervates, biomolecular condensates, membraneless organelles, protein aggregation, amyloid aggregation, liquid-liquid phase separation

1 Introduction

Biomolecular condensates describe concentrated nonstoichiometric assemblies of biomolecules that can form by a range of different mechanisms (Choi et al., 2020). Biomolecular condensates can arise by phase separation, which in biology involves the demixing of a water-soluble polymer into two co-existing phases: a polymer-dilute phase and a polymer-dense phase. Complex coacervates describe phase separation mediated by a second element, which may typically be a ligand (such as RNA) to the polymer (such as a protein) that undergoes phase separation. Protein aggregation into amyloids and amorphous aggregates, and the formation of RNA granules, represent other forms of biomolecular condensates.

The assembly of proteins and other biomolecules into complexes is a fundamental feature for the execution of biological functions. Biomolecular condensates are a natural variation of the assembly theme. There is an incredible complexity and diversity to how condensates form, are regulated and are structured (reviewed recently in (Hirose et al., 2022)). And there is incredible diversity to how condensates are used by nature to drive biological functions and how when their assemblies go wrong, they can drive disease mechanisms, such as amyloids in neurodegeneration.

With this incredible diversity in mind, what are some of the grand challenges that exist in the research field of biomolecular condensates? Below are some of my picks.

2 Understanding principles of functioning of cellular condensates: role of molecular grammar and cellular processes

The concept of phase-separation of proteins into liquid droplets has become a hot-topic mechanism in protein biochemistry and cell biology over the last decade stimulated by the remarkable work of Brangwynne, Hyman and colleagues (Brangwynne et al., 2009). The work showed P-granules in *C. elegans* germ cells displayed properties consistent with liquid condensates. Under shear force, P granules displayed classic liquid properties of flowing, dripping and fusing into larger droplets (Brangwynne et al., 2009). The study sparked the popularization of the mechanism of phase separation for directing the compartmentalization of biomolecules. Such a mechanism was indeed a paradigm shift in that compartmentalization was previously thought to be mostly mediated by membrane-bound organelles or macromolecular scaffolds. Phase separation is now known to be involved in the formation of at least 24 membraneless organelles: P-body, U-body, Balbiani body, germ granules, RNA transport granules, synaptic densities, stress granules, nuclear pore complex, Cajal body, Cleavage body, Gem, nuclear speckles, nucleolus, OPT domain, PcG body, perinucleolar compartment, PML bodies, histone locus body, paraspeckles, focal adhesions, nephrin clusters, TCR clusters, podosomes, and actin patches (Banani et al., 2017). The number of publications in Pubmed using the search phrase, "liquid-liquid phase separation" has grown exponentially from 18 in 2009 to 572 papers in 2022 at the time of writing (May 2023).

The range of biological processes linked with phase separation is diverse, and includes RNA metabolism, ribosome biogenesis, the DNA damage response and signal transduction (Banani et al., 2017). Studies have proposed that dense phases can provide crucibles for reactions by concentrating reactants, for funnelling linked metabolic reactions, as well as others (comprehensively reviewed in (Lyon et al., 2021)). But the field has likely only scratched the surface for how these functions operate at the molecular level and why they require the condensate state. There is clearly a lot more to learn to gain a full understanding of all the mechanisms, and in particular, how the condensation of biomolecules drives functional outputs.

3 Understanding the origins and functional implications of heterogeneity in cellular condensates

While we still are unearthing the functional role of membraneless organelles, another aspect is how such organelles interact with each other and evolve in function and composition temporally and under different cellular settings. For example, stress granules and processing bodies are spatially juxtaposed and appear to be functionally connected (Riggs et al., 2020). But the mechanisms for how and why they are connected remain incompletely understood. Both membraneless organelles contain mRNAs, suggesting a role in the regulation of RNA metabolism. Processing bodies may play a role as a reservoir of RNA and stress granules may also mediate signalling pathways under stress. We also understand little about how the compositional heterogeneity changes over time within individual condensate entities. For example, stress granules appear to contain distinct subproteomes under different stresses (Aulas et al., 2017; Markmiller et al., 2018) and can mature into solid- or gel-like inclusions that persist within the cell in neurodegenerative diseases (Rhine et al., 2022). At least 238 proteins have been curated to reside in stress granules (Markmiller et al., 2018). Hence, a grand challenge remains in understanding what the heterogeneity is of biomolecular condensate structures and how the heterogeneity relates to function and changes under different conditions and in disease. Other questions include what drives the selectivity of composition, and what the functional reasons are for different compositions and structures. Linked to these questions are other open questions as to how the cellular location and juxtaposition with other condensates relates to function.

The most well-known function of biomolecular condensates is the sheer process of assembling a membraneless organelle entity. Yet very distinct functions are emerging. For example, PopZ forms condensates at the poles of the bacterium *Caulobacter crescentus* that direct signalling pathways for cell-cycle regulation (Lasker et al., 2022). The condensation of proteins in a T cell signalling pathway into microclusters directs the signalling responses (Su et al., 2016). As part of this effect, the phase separation of signalling complex proteins at a membrane surface invokes membrane phase separation and in turn an altered co-mixing of other proteins into the new phases (Chung et al., 2021). Other work has shown that protein condensates formed at the surface of membranes can drive membrane curvature by applying compressive stress to the membrane surface (Yuan et al., 2021). Given the incredible density and crowdedness of intracellular environments, are there other functions yet to be discovered? Hence a grand challenge remains in uncovering the full diversity by which condensates direct cellular functions. Furthermore, are there novel modes of substructural organization within the cytosol driven by phase separation?

4 Deciphering the structural organization of cellular condensates

Phase separation may imply biomolecular condensates adopt a rather amorphous structure. However, membraneless organelles at least in some instances display substructural organization that indicate cellular mechanisms have been deployed to mould the organization for functional purposes. The nucleolus, Cajal bodies, nuclear speckles, PML bodies, and paraspeckles contain distinct internal domains, such as cores, that confer distinct functional features (Hirose et al., 2022). Some of the substructure may be direct functions of multi-phasic behaviour such as in nucleoli where immiscible liquid phases form that can be modelled *in vitro* with purified

proteins (Feric et al., 2016). Other substructures appear to involve other factors. For example, paraspeckles require an RNA scaffold, NEAT1, to anchor the protein condensates (Yamazaki et al., 2018). Indeed, a class of long non-coding RNAs have been termed architectural RNAs (arcRNAs) for such scaffolds, with 5 identified in mammals, insects, and yeast (Chujo et al., 2016). Stress granules contain a stable core structure surrounded by a dynamic shell with assembly, disassembly, and transitions between the core and shell modulated by numerous protein and RNA remodelling complexes (Jain et al., 2016). Recently it was shown that TDP-43 formed "anisosomes" which have spherical shells of TDP-43 in a liquid crystal state around a core of a HSP70 chaperone that was required to maintain the condensate in a liquid state (Yu et al., 2021). Addition of RNA can also modulate the miscibility of proteins in the dense phases. Many proteins that display disordered domains, which can be the key lever for driving condensation, also contain other globular and structured domains that may not. Hence, how do different domain structures in proteins that form condensates dictate structural and functional properties of the condensates? There is still much to learn about how different domains work together to shape the substructure of the condensates, as well as how ligand binding and post-translational modifications play roles in that.

5 Deciphering molecular grammar encoding material properties of cellular condensates

Motifs that drive the interactions of amino acids in intrinsically disordered motifs are beginning to emerge -such as the cation-pi interactions (e.g., Arg and Tyr) in DDX4, FUS and other proteins (Nott et al., 2015; Wang et al., 2018). In functional amyloids, an "imperfect" amyloid motif, called low-complexity amyloid-like reversible kinked segments (LARKS), has been proposed to mediate the reversibility of functional amyloid assembly (Hughes et al., 2018). LARKS form kinked β-sheets that weakly interact by polar atoms and aromatic sidechains and differ from conventional amyloid fibrils that form far more stably associated steric zippers (Hughes et al., 2018). The interactions that stabilize condensates of a wide range of condensate-forming proteins are known to be modulated by post-translational modifications, suggestive of intricate regulatory mechanisms to shift the phase boundaries (Owen and Shewmaker, 2019). Key questions remain as to how such structures are regulated more generally for functional processes, and how these intersect with the events leading to inappropriate aggregation in disease.

Other areas of discovery include understanding the mechanisms of condensate dynamics and flow (rheology), and the manner by which cells can tune this behaviour for biological functions. Various studies point to complexity in the rheological behaviour of condensates and how this can be important for influencing biological function. In one, optically trapped polystyrene beads were used to measure the viscous and elastic moduli and the interfacial tensions of four types of biomolecular condensates (Ghosh et al., 2021). The study found that condensates could not be reliably modelled just as viscous liquids and instead required different effects of viscoelasticity to be accounted for, shear thickening or thinning, depending on the type of condensate (Ghosh et al., 2021). In another study, the viscous and elastic regimes of condensates formed by Arg/Gly-rich sticker-spacer-based polypeptides, which mimic motifs found in many RNA binding proteins, and RNA, was found to be tunable depending on the sequence properties of the sticker and spacers (Alshareedah et al., 2021). In another study, the material properties of PopZ condensates were found to be regulated by two internal domains (Lasker et al., 2022). A C-terminal helical domain is required to form very dense condensates, while an IDR domain confers greater fluidity. It was found that the IDR length, as well as the charge distribution of the amino acids in the IDR, were critical in specifying PopZ material state properties and that these properties in turn were important for the correct cellular localization of the condensates as well as how they functioned.

6 The impact of a delicately poised solubility threshold of cellular proteomes

The intracellular environment of cells is extremely crowded. A wide range of proteins are believed to be close to their threshold concentration for phase separation, which is exacerbated under conditions of macromolecular crowding (Walter and Brooks, 1995). The large numbers of protein species could lead to multiple co-existing phases. Each phase will be localized by interfacial tension and/or nearby solid bounding surfaces with which the phase is in contact with (Walter and Brooks, 1995). Hence how non-homogenous is the cytosol and does it comprise a sea of microphases? Do these phases have fuzzy boundaries? One interesting finding was that under osmotic stress that concentrates the cytosol of cells, multimeric proteins appear to readily phase separate into droplets (Jalihal et al., 2020). Hence, can cells use changes in the phase boundary as a sensor to measure dehydration stress? Do cells exploit a delicately poised phase boundary for functional purposes?

The other relevant point is that proteins when purified are rarely soluble at the concentration seen in cells (200–300 mg/mL) (Brown, 1991). Hence, how do cells keep the proteome soluble? We know that chaperones are critical to the folding and assembly of many proteins, as well as for dissolving particular protein aggregates (Rosenzweig et al., 2019). We know chaperones can bind to unfolded-like states of globular proteins to aid in their solubility in cells (Wood et al., 2018; Ruff et al., 2022). Hence to what extent do proteins such as chaperones play in regulating the phase boundaries of the proteome?

Another emerging question is whether cells can functionally exploit the changes to delicately poised phase boundaries induced by external forces such as temperature and mechanical compression. It seems there is evidence that they can. For example, paraspeckles can form under conditions of cellular mechanical stress and confinement and thus this behavior may play a regulatory role in confined migration and invasion in cancer cells (Todorovski et al., 2023). In another example, plant circadian clock signalling involves a condensate forming protein ELF3 that rapidly alternates between active and inactive states *via* a thermosensory-regulated phase transition (Jung et al., 2020).

7 Why inappropriate protein aggregation occurs in diseases

Mutations in proteins that reside in membraneless organelles are highly enriched in neurodegenerative diseases and form deposits (Nedelsky and Taylor, 2019; Ryan and Fawzi, 2019). The reasons for why these mutations are enriched and form deposits remains incompletely understood. However, key questions arise from this observation; namely, do they perturb natural-regulated mechanisms of phase separation and if so how does that relate to pathogenesis? In addition, is there a broader link between the aberrant phase separation mechanisms in membraneless organelles with other amyloid and amorphous forms of protein aggregation also linked to diseases? More generally, while it is well established that protein aggregates are generally the source of cellular dysfunction, especially smaller-sized oligomeric forms, the mechanisms involved remain unclear (Murakami and Ono, 2022). Furthermore, the mechanisms underpinning why some protein aggregates are toxic and others are not or less so remains unclear.

8 How inappropriate protein aggregation relates to pathogenesis in diseases

It has been well established for over 2 decades that misfolded proteins aggregate and are toxic to cell biology (Bucciantini et al., 2002). Yet we still don't have clear ideas as to why that is the case. Likely the mechanisms are multipronged meaning single mechanisms are hard to unambiguously identify. An additional challenge comes in properly understanding the compositional properties of the proteinaceous deposits in disease (Cox et al., 2020) and biases arising from model systems that don't accurately recreate the same aggregate structure as in disease (Lashuel, 2021). But more fundamental questions as to why aggregation is so pernicious and problematic to cellular health remains an important question to address. In addition, the basis for why some types of protein aggregates are more toxic than others is not clearly understood.

One hypothesis is that the proteome solubility is poised close to the threshold of supersaturation, the point at which proteins form aggregates. Work from Vendruscolo and colleagues showed that proteins prone to supersaturation are over-represented in biochemical pathways linked to neurodegenerative diseases (Ciryam et al., 2013). These supersaturated proteins are likely to require rigorous management by protein quality control mechanisms. Indeed genes corresponding to the metastable subproteome associated with Alzheimer's Disease are tightly co-expressed with specific components of protein quality control, namely, the ubiquitin–proteasome and the endosomal–lysosomal pathways (Kundra et al., 2017). Also related to this are the chaperone and co-chaperone networks (dubbed the chaperome) that change during ageing and in neurodegenerative diseases (Brehme et al., 2014). Nearly a third of the chaperome, corresponding to ATP-dependent chaperone machines, was repressed in normal human brain aging whereas nearly 20% of the chaperome, corresponding to ATP-independent chaperones and co-chaperones, was induced. There were enhancements of these chaperome subnetworks in Alzheimer's, Huntington's, or Parkinson's diseases. Hence, a grand challenge is in defining the intersectionality of mechanisms governing protein solubility and disease pathology. In turn, how do these mechanisms link to those raised above in my other points with respect to mechanisms of condensate formation, structure and function.

In conclusion, the field of biomolecular condensates is at an exciting point of discovery. *The Coacervates and Biological Condensates* specialty section of *Frontiers in Biophysics* offers authors the opportunity to disseminate findings in this journey of discovery. This includes manuscripts related to any of these questions and grand challenges raised above, as well as others not covered here.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

Funding

DH is supported by a National Health and Medical Research Council Senior Research fellowship (APP1154352) and grants from the Australian Research Council (DP230101050) and FightMND (02_Discovery_2022).

Conflict of interest

The author(s) [DMH] declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

Alshareedah, I., Moosa, M. M., Pham, M., Potoyan, D. A., and Banerjee, P. R. (2021). Programmable viscoelasticity in protein-RNA condensates with disordered sticker-spacer polypeptides. *Nat. Commun.* 12 (1), 6620. doi:10.1038/s41467-021-26733-7

Aulas, A., Fay, M. M., Lyons, S. M., Achorn, C. A., Kedersha, N., Anderson, P., et al. (2017). Stress-specific differences in assembly and composition of stress granules and related foci. *J. Cell Sci.* 130 (5), 927–937. doi:10.1242/jcs.199240

Banani, S. F., Lee, H. O., Hyman, A. A., and Rosen, M. K. (2017). Biomolecular condensates: Organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* 18 (5), 285–298. doi:10.1038/nrm.2017.7

Brangwynne, C. P., Eckmann, C. R., Courson, D. S., Rybarska, A., Hoege, C., Gharakhani, J., et al. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* 324 (5935), 1729–1732. doi:10.1126/science.1172046

Brehme, M., Voisine, C., Rolland, T., Wachi, S., Soper, J. H., Zhu, Y., et al. (2014). A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep.* 9 (3), 1135–1150. doi:10.1016/j.celrep.2014.09.042

Brown, G. C. (1991). Total cell protein concentration as an evolutionary constraint on the metabolic control distribution in cells. *J. Theor. Biol.* 153 (2), 195–203. doi:10.1016/s0022-5193(05)80422-9

Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., et al. (2002). Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* 416 (6880), 507–511. doi:10.1038/416507a

Choi, J.-M., Holehouse, A. S., and Pappu, R. V. (2020). Physical principles underlying the complex biology of intracellular phase transitions. *Annu. Rev. Biophysics* 49 (1), 107–133. doi:10.1146/annurev-biophys-121219-081629

Chujo, T., Yamazaki, T., and Hirose, T. (2016). Architectural RNAs (arcRNAs): A class of long noncoding RNAs that function as the scaffold of nuclear bodies. *Biochimica Biophysica Acta (BBA) - Gene Regul. Mech.* 1859 (1), 139–146. doi:10.1016/j.bbagrm.2015.05.007

Chung, J. K., Huang, W. Y. C., Carbone, C. B., Nocka, L. M., Parikh, A. N., Vale, R. D., et al. (2021). Coupled membrane lipid miscibility and phosphotyrosine-driven protein condensation phase transitions. *Biophysical J.* 120 (7), 1257–1265. doi:10.1016/j.bpj.2020.09.017

Ciryam, P., Tartaglia, G. G., Morimoto, R. I., Dobson, C. M., and Vendruscolo, M. (2013). Widespread aggregation and neurodegenerative diseases are associated with supersaturated proteins. *Cell Rep.* 5 (3), 781–790. doi:10.1016/j.celrep.2013.09.043

Cox, D., Raeburn, C., Sui, X., and Hatters, D. M. (2020). Protein aggregation in cell biology: An aggregomics perspective of health and disease. *Seminars Cell and Dev. Biol.* 99, 40–54. doi:10.1016/j.semcdb.2018.05.003

Feric, M., Vaidya, N., Harmon, T. S., Mitrea, D. M., Zhu, L., Richardson, T. M., et al. (2016). Coexisting liquid phases underlie nucleolar subcompartments. *Cell* 165 (7), 1686–1697. doi:10.1016/j.cell.2016.04.047

Ghosh, A., Kota, D., and Zhou, H. X. (2021). Shear relaxation governs fusion dynamics of biomolecular condensates. *Nat. Commun.* 12 (1), 5995. doi:10.1038/s41467-021-26274-z

Hirose, T., Ninomiya, K., Nakagawa, S., and Yamazaki, T. (2022). A guide to membraneless organelles and their various roles in gene regulation. *Nat. Rev. Mol. Cell Biol.* 24, 288–304. doi:10.1038/s41580-022-00558-8

Hughes, M. P., Sawaya, M. R., Boyer, D. R., Goldschmidt, L., Rodriguez, J. A., Cascio, D., et al. (2018). Atomic structures of low-complexity protein segments reveal kinked β sheets that assemble networks. *Science* 359 (6376), 698–701. doi:10.1126/science.aan6398

Jain, S., Wheeler, Joshua R., Walters, Robert W., Agrawal, A., Barsic, A., and Parker, R. (2016). ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* 164 (3), 487–498. doi:10.1016/j.cell.2015.12.038

Jalihal, A. P., Pitchiaya, S., Xiao, L., Bawa, P., Jiang, X., Bedi, K., et al. (2020). Multivalent proteins rapidly and reversibly phase-separate upon osmotic cell volume change. *Mol. Cell* 79 (6), 978–990.e5. doi:10.1016/j.molcel.2020.08.004

Jung, J.-H., Barbosa, A. D., Hutin, S., Kumita, J. R., Gao, M., Derwort, D., et al. (2020). A prion-like domain in ELF3 functions as a thermosensor in Arabidopsis. *Nature* 585 (7824), 256–260. doi:10.1038/s41586-020-2644-7

Kundra, R., Ciryam, P., Morimoto, R. I., Dobson, C. M., and Vendruscolo, M. (2017). Protein homeostasis of a metastable subproteome associated with Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 114 (28), E5703–E5711. doi:10.1073/pnas.1618417114

Lashuel, H. A. (2021). Rethinking protein aggregation and drug discovery in neurodegenerative diseases: Why we need to embrace complexity? *Curr. Opin. Chem. Biol.* 64, 67–75. doi:10.1016/j.cbpa.2021.05.006

Lasker, K., Boeynaems, S., Lam, V., Scholl, D., Stainton, E., Briner, A., et al. (2022). The material properties of a bacterial-derived biomolecular condensate tune biological function in natural and synthetic systems. *Nat. Commun.* 13 (1), 5643. doi:10.1038/s41467-022-33221-z

Lyon, A. S., Peeples, W. B., and Rosen, M. K. (2021). A framework for understanding the functions of biomolecular condensates across scales. *Nat. Rev. Mol. Cell Biol.* 22 (3), 215–235. doi:10.1038/s41580-020-00303-z

Markmiller, S., Soltanieh, S., Server, K. L., Mak, R., Jin, W., Fang, M. Y., et al. (2018). Context-dependent and disease-specific diversity in protein interactions within stress granules. *Cell* 172 (3), 590–604 e13. doi:10.1016/j.cell.2017.12.032

Murakami, K., and Ono, K. (2022). Interactions of amyloid coaggregates with biomolecules and its relevance to neurodegeneration. *FASEB J. official Publ. Fed. Am. Soc. Exp. Biol.* 36 (9), e22493. doi:10.1096/fj.202200235R

Nedelsky, N. B., and Taylor, J. P. (2019). Bridging biophysics and neurology: Aberrant phase transitions in neurodegenerative disease. *Nat. Rev. Neurol.* 15 (5), 272–286. doi:10.1038/s41582-019-0157-5

Nott, T. J., Petsalaki, E., Farber, P., Jervis, D., Fussner, E., Plochowietz, A., et al. (2015). Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. *Mol. Cell* 57 (5), 936–947. doi:10.1016/j.molcel.2015.01.013

Owen, I., and Shewmaker, F. (2019). The role of post-translational modifications in the phase transitions of intrinsically disordered proteins. *Int. J. Mol. Sci.* 20 (21), 5501. doi:10.3390/ijms20215501

Rhine, K., Al-Azzam, N., Yu, T., and Yeo, G. W. (2022). Aging RNA granule dynamics in neurodegeneration. *Front. Mol. Biosci.* 9, 991641. doi:10.3389/fmolb.2022.991641

Riggs, C. L., Kedersha, N., Ivanov, P., and Anderson, P. (2020). Mammalian stress granules and P bodies at a glance. *J. Cell Sci.* 133 (16), jcs242487. doi:10.1242/jcs.242487

Rosenzweig, R., Nillegoda, N. B., Mayer, M. P., and Bukau, B. (2019). The Hsp70 chaperone network. *Nat. Rev. Mol. Cell Biol.* 20 (11), 665–680. doi:10.1038/s41580-019-0133-3

Ruff, K. M., Choi, Y. H., Cox, D., Ormsby, A. R., Myung, Y., Ascher, D. B., et al. (2022). Sequence grammar underlying the unfolding and phase separation of globular proteins. *Mol. Cell* 82 (17), 3193–3208 e8. doi:10.1016/j.molcel.2022.06.024

Ryan, V. H., and Fawzi, N. L. (2019). Physiological, pathological, and targetable membraneless organelles in neurons. *Trends Neurosci.* 42 (10), 693–708. doi:10.1016/j.tins.2019.08.005

Su, X., Ditlev, J. A., Hui, E., Xing, W., Banjade, S., Okrut, J., et al. (2016). Phase separation of signaling molecules promotes T cell receptor signal transduction. *Science* 352 (6285), 595–599. doi:10.1126/science.aad9964

Todorovski, V., McCluggage, F., Li, Y., Meid, A., Spatz, J. P., Holle, A. W., et al. (2023). Confined environments induce polarized paraspeckle condensates. *Commun. Biol.* 6 (1), 145. doi:10.1038/s42003-023-04528-4

Walter, H., and Brooks, D. E. (1995). Phase separation in cytoplasm, due to macromolecular crowding, is the basis for microcompartmentation. *FEBS Lett.* 361 (2-3), 135–139. doi:10.1016/0014-5793(95)00159-7

Wang, J., Choi, J. M., Holehouse, A. S., Lee, H. O., Zhang, X., Jahnel, M., et al. (2018). A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. *Cell* 174 (3), 688–699.e16. doi:10.1016/j.cell.2018.06.006

Wood, R. J., Ormsby, A. R., Radwan, M., Cox, D., Sharma, A., Vopel, T., et al. (2018). A biosensor-based framework to measure latent proteostasis capacity. *Nat. Commun.* 9 (1), 287. doi:10.1038/s41467-017-02562-5

Yamazaki, T., Souquere, S., Chujo, T., Kobelke, S., Chong, Y. S., Fox, A. H., et al. (2018). Functional domains of NEAT1 architectural lncRNA induce paraspeckle assembly through phase separation. *Mol. Cell* 70 (6), 1038–1053.e7. doi:10.1016/j.molcel.2018.05.019

Yu, H., Lu, S., Gasior, K., Singh, D., Vazquez-Sanchez, S., Tapia, O., et al. (2021). HSP70 chaperones RNA-free TDP-43 into anisotropic intranuclear liquid spherical shells. *Science* 371 (6529), eabb4309. doi:10.1126/science.abb4309

Yuan, F., Alimohamadi, H., Bakka, B., Trementozzi, A. N., Day, K. J., Fawzi, N. L., et al. (2021). Membrane bending by protein phase separation. *Proc. Natl. Acad. Sci. U. S. A.* 118 (11), e2017435118. doi:10.1073/pnas.2017435118