

Editorial

Orchestration of cell plasticity by phase separation

Liquid–liquid phase separation (LLPS) of biomolecules drives the formation of cellular compartments, which determines cell plasticity and fate decision. Emerging evidence indicates that a large number of membraneless organelles (MLOs), including nucleoli, Cajal bodies, P-bodies, and stress granules, exist as liquid droplets within the cell and orchestrate the cell plasticity control in response to the dynamics of extracellular cues. The MLOs provide not only organizers for signaling cascade but also nano-reactors for signaling catalysis (Liu et al., 2020). However, the molecular mechanisms underlying MLO dynamics and its relation to the cell plasticity control have remained elusive (Alberti and Hyman, 2021). MLO assembly is tightly regulated in the intracellular environment, and failure to control its dynamic properties often leads to protein misfolding and aggregation, which perturbs cell plasticity and elicits pathogenic reaction. In this special issue, we describe the mechanisms and regulation of condensate assembly and dissolution, highlight recent advances in understanding the role of biomolecular condensates in aging and disease, and discuss how cellular stress, aging-related loss of homeostasis, and a decline in protein quality control may contribute to the formation of aberrant, disease-causing condensates.

Emerging evidence revealed that aberrant biomolecular condensation results in perturbation of cell plasticity and neurodegenerative disorders. Ismail et al. (2021) highlight the current understanding of phase separation, unifying structural and mechanistic principles underlying LLPS, underlying mechanisms of LLPS regulation *in situ*, and emerging interrogation strategies centered on aberrant LLPS.

Coronavirus disease 2019 (COVID-19) is a pandemic that has caused significant morbidity and mortality worldwide. Viruses hijack host cell functions to interrogate host cellular hierarchy and host cell plasticity. Scoca and Di Nunzio (2021)

examined the possibility of SARS-CoV-2

nucleocapsid protein condensation in viral genome packaging of intact viral genomes and speculated that LLPS serves as a noise-filtering mechanism to separate the function of host cell RNAs from viral genomes. Although major breakthrough and excitements have been made toward the atomic resolution of viral replication machinery, the greater excitement and challenge ahead are to understand host cell factors, in particular pathogen-induced MLOs in the COVID-19 disparity (Lythgoe et al., 2021).

Precise regulation of gene transcription is of great importance to development and diseases. The pandemic had prompted the studies of viral regulation of host gene transcription. Promoter-proximal transcriptional pause is a key feature of metazoans for precise control of transcription. Guo et al. (2021) have reviewed the recent progresses toward a better understanding of fine regulation of Pol II pausing and release with regard to the super elongation complex. However, the current challenge is the limitation of precise measurement of phase-separated properties of condensates and the functional relevance to *in situ* transcriptional regulation. Molecular delineation of physiochemical properties of Pol II condensates in space and time would not only improve our understanding of transcriptional regulation but also provide targets for interrogation of disease processes.

Super-enhancers comprise large clusters of enhancers, which are co-occupied by multiple lineage-specific and master transcription factors, and play crucial roles in regulating cell plasticity. However, it has been elusive whether and how super-enhancers are regulated by the noncoding sequences of the genome. Yan et al. (2021) found that long noncoding RNA (lncRNA) genes preferentially lie next to super-enhancers. They then focused on the lncRNA gene *Platr22* and revealed its critical regulatory role in modulating the activity of a nearby super-enhancer and the expression of the nearby pluripotency regulator *ZFP281*.

Mechanistically, *Platr22* transcripts coat

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chromatin near the super-enhancer region and interact with DDX5 and hnRNP-L, both possessing strong LLPS activity. DDX5 further recruits p300 and other factors related to active transcription. This study highlights an unanticipated role for a class of lncRNAs in epigenetically controlling cell plasticity. The excitement ahead is to delineate what transcriptional factors are compartmentalized and how this LLPS property is regulated during cell division cycle.

Steady-state RNA levels are controlled by the balance between RNA synthesis and RNA metabolism. Lee et al. (2021) have postulated that neurons possess a selective RNA turnover mechanism via nonsense-mediated RNA decay (NMD). NMD has been shown to influence neural development, axon guidance, and synaptic plasticity. In humans, NMD factor gene mutations cause some forms of intellectual disability and are associated with neurodevelopmental disorders, including schizophrenia, autism, and amyotrophic lateral sclerosis (ALS). Approximately 15% of ALS cases are familial, and mutations in the *fused in sarcoma (FUS)* gene contribute to a subset of familial ALS cases. FUS is a multifunctional protein participating in many RNA metabolism pathways. ALS-linked mutations elicit a phase-separated property of FUS protein *in vitro*, inducing the formation of cytoplasmic granules and inclusions. Recent studies show that the ALS mutations in FUS indeed suppressed protein translation in fibroblast cells derived from FUS ALS cases. In addition, the NMD pathway, which is closely related to protein translation, was altered by mutant FUS. Alterations in NMD factors and elevated activity were also observed in the fibroblast cells of FUS ALS cases, which suggests the importance of homeostatic control of protein activity in space and time. Indeed, misfolded α -synuclein is a major component of Lewy bodies, which are a hallmark of Parkinson's disease. A large body of evidence shows that α -synuclein can aggregate into amyloid fibrils, but the relationship between α -synuclein self-assembly and Lewy body formation remains unclear. Hardenberg et al. (2021) show, both *in vitro* and in a *Caenorhabditis elegans* model of Parkinson's disease, that α -synuclein undergoes LLPS by forming a liquid droplet, which then converts into an amyloid-rich hydrogel with Lewy body-like properties. This maturation process toward the amyloid state is delayed in the presence of model synaptic vesicles *in vitro*. These results suggest that the formation of Lewy bodies may perturb maturation of α -synuclein condensates in the presence of lipids and other cellular components. It would be of great interest to ascertain the precise involvement of LLPS in the NMD pathway and protein homeostasis using brain organoid models.

LLPS also occurs at membranes, where both lipids and membrane-associated proteins can de-mix to form phase-separated compartments. Investigation of these membrane-associated condensates using *in vitro* biochemical reconstitution and cell biology has provided key insights into the role of phase separation in membrane domain formation and function. However, these studies have generally been limited by available technologies to study LLPS on model membranes and the complex

cellular environment that regulates condensate formation, composition, and function. Thus, Ditlev (2021) highlighted the perspectives and new technologies for studying membrane-associated condensates in cell plasticity control.

The molecular delineation of organoid formation thus provides valuable information about the mechanisms underlying human organogenesis, tissue regeneration, and tumorigenesis, highlighting their value for mechanistic study of fundamental biology in addition to their potential application in precision medicine. The ability of 3D organoids to recapitulate the stem cell lineage and mimic the context-dependent biology has expedited the modeling LLPS in organoids (Liu et al., 2020). This provides a unique stepping stone to pinpoint and rewire context-dependent LLPS for precision drug discovery.

Visualization of specific molecules and their interactions in real space and time is essential to delineate how cellular plasticity is achieved and orchestrated, as perturbation of cellular dynamics is detrimental to health. Given the prevalence of LLPS perturbation phenotype in neural diseases, Liu et al. (2020) developed a strategy to model context-dependent cell renewal in organoids using a light sheet microscope, which will illuminate the molecular dynamics in LLPS for cell plasticity control. There is no doubt that consolidation of protein-protein interaction in droplets in live cell combined with illumination of molecular dynamics in context-dependent 3D organoids will enable us to delineate the molecular mechanisms underlying LLPS-driven pathogen-host cell interaction and host cell plasticity in space and time.

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