RNA phase separation–mediated direction of molecular trafficking under conditions of molecular crowding

Kazuki Kohata¹ · Daisuke Miyoshi¹

Received: 28 January 2020 / Accepted: 28 April 2020 / Published online: 16 May 2020

© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Living cells are highly crowded with large and small biomolecules. The total concentration of biomolecules can reach 400 mg/ml, and 40% of the cell volume is occupied by biomolecules. Droplet formation in cells via liquid-liquid phase separation may play a role in controlling biochemical reactions in this complex molecular environment. Liquid-liquid phase separation generally involves nucleic acids and proteins as anionic and cationic components, respectively. Significant characteristics of droplets, which make them different from protein aggregation or fibril formation, are reversibility of formation and responsiveness to the molecular environment. In this review, we quantitatively describe the molecular environment inside cells and droplets that participate in controlling central dogma reactions. Finally, we discuss the importance of droplets under conditions of molecular crowding within living cells.

Keywords Molecular crowding · Phase separation · Nucleic acid · Droplet · Central dogma

Molecular environment inside cells

The molecular environment within living cells is surprisingly crowded with a high number and variety of biomolecules and differs dramatically from that in a test tube (Fig. 1a) (Minton 2001; Goodsell 2011), where most biochemical and biophysical studies have been carried out (Ellis 2001). Approximately 40% of the cell volume is occupied by macromolecular complexes such as the cytoskeleton and ribosomes and biomolecules such as proteins and nucleic acids (Zimmerman and Trach 1991; Minton 2001; Rivas et al. 2004; Goodsell 2011). Moreover, the total concentration of small compounds, including metabolites and osmolytes, can reach the molar level (Zimmerman and Trach 1991; Thirumalai et al. 2003). These large and small biomolecules render the inside of a cell highly crowded. As an example, there are 4288 different proteins in an *E. coli* cell (Blattner et al. 1997), occupying approximately 17 wt% (~56% of 30%) of cell (Fig. 1b). The weight percentages of nucleic acids, RNA, and DNA in *E. coli*

are approximately 6 and 3%, respectively (Neidhardt et al. 1990; Milo and Phillips 2015). There are also other large biomolecules in cells, such as lipids and saccharides. In addition to these large biomolecules, the metabolite concentration in *E. coli* is near 200 mM (Bennett et al. 2009), as shown in Fig. 1c. Interestingly, most intracellular cations are inorganic potassium ions, whereas most anions are amino acids and nucleic acids (Blattner et al. 1997; Bennett et al. 2009; Milo and Phillips 2015). The total concentration of biomolecules in *E. coli* is 300–400 mg/ml (Zimmerman and Trach 1991). Such an intracellular environment is characterized by the term ‘molecular crowding’ (MC).

Liquid-liquid phase separation for biological processes

To maintain cellular homeostasis, biochemical reactions such as those associated with the central dogma are subject to temporal, spatial, and specific control. From a biochemistry and biophysics perspective, it is surprising that selective interactions and specific functions of biomolecules are controlled in a temporal- and spatial-specific manner in complex MC environments involving numerous other biomolecules. A key feature that enables specific interactions between biomolecules is the collective and congregative effect of

✉ Daisuke Miyoshi
miyoshi@konan-u.ac.jp

¹ Faculty of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, 7-1-20 Minatogima-minamimachi, Chuo-ku, Kobe 650-0047, Japan

Fig. 1 **a** Schematic illustration of the difference between the molecular environment in a cell and in a test tube. **b** Molecular components of a cell (*E. coli*). **c** Contents of small molecules in a cell (*E. coli*)

biomolecular properties and behaviors, which has attracted considerable research attention in recent years (Banani 2017; Alberti 2017). The biomolecular localization and compartmentalization system using droplets formed via liquid-liquid phase separation (LLPS) as observed in the cytoplasm and nucleus is becoming one of the most hot research topics in cellular, chemical, and physical biology. Droplets, often called membrane-less organelles, concentrates, coacervates, or granules, lack a membrane and are thus distinct from typical organelles having membranes (Fox et al. 2002; Lamond and Sleeman 2003; Cioce and Lamond 2005; Pederson 2011; Anderson et al. 2015). Droplets are temporary, and their formation is reversible and responsive to various external signals associated with cellular stressors (Brangwynne et al. 2009; Banani et al. 2016). The reversibility and signal responsiveness of droplets contrast with biomolecule aggregation, which is generally irreversible (Yoshimura et al. 2012). LLPS is a phenomenon in which a homogeneous solution with solutes spontaneously separates into two or more liquid phases that can stably coexist (Alberti 2017). Thus, the entropically less-stable heterogeneous phase-separated state should become more stable than the entropically more-favorable homogeneous single phase. This indicates that there is an enthalpic contribution by factors such as solute-solute interactions that maintain the stability of the phase-separated state. The interface between phases has no partition barrier such as a lipid, enabling the free passage of water molecules and solutes through the interface. Thus, droplets can dynamically exchange contents with the external environment (Elbaum-Garfinkle et al. 2015; Schuster et al. 2018). These features of droplets could be crucial for facilitating the specific functions and interactions between biomolecules under conditions of MC.

Previous studies have shown that droplets can be formed by cationic peptides and mononucleotides in a test tube (Koga et al. 2011). Moreover, droplets formed in cells often contain polyions such as cationic proteins and RNAs (Buchan 2014) (Fig. 2). Droplets are stabilized by electrostatic interactions, cation- π interactions (Nott et al. 2015), and π - π interactions (Frey et al. 2006; Kato et al. 2012) between these molecules. Droplets inside a test tube are fluid assemblies because they are formed by a number of weak interactions, and they are dynamic assemblies that repeatedly form and dissociate in response to changes in temperature and pH and the presence of small molecules (Brangwynne et al. 2011). Droplets formed in cells fuse and separate on the order of several tens to hundreds of seconds (Brangwynne et al. 2011; Shin et al. 2017). Such responsiveness of droplets to external stimuli provides a mechanism for controlling biological reactions temporally and spatially even under complex MC conditions.

Droplets controlling central dogma-associated reactions

To date, various droplets have been identified in cells, and it has been determined that each droplet plays a biological role. Figure 3 shows a schematic illustration of droplets participating in control of reactions associated with the central dogma along with their components. The nucleolus (Hernandez-Verdun 2011), paraspeckles (Fox and Lamond 2010), nuclear speckles (Lamond and Spector 2003), promyelocytic leukemia (PML) bodies (Lallemant-Breitenbach 2010), and Cajal bodies (Cioce and Lamond 2005) can be observed in the nucleus. These droplets play a role in supplying or storing RNA and transcriptional factors through droplet formation and

Fig. 2 Formation of droplets via interactions between RNA-binding proteins (RBPs) and mRNAs observed in the cell nucleus and cytoplasm

dissolution in the nucleus, and they regulate gene expression and control reactions related to RNA metabolism. P-bodies, stress granules, and reproductive granules have also been identified in the cytoplasm, where they control reactions related to RNA metabolism and cell homeostasis.

Nucleolus The nucleolus, the largest droplet in the cell, is involved in ribosome biosynthesis (Hernandez-Verdun 2011). Transmission electron microscopy analyses revealed that nucleoli are formed and degraded depending on the ribosome synthesis stage (López et al. 2005). Ribosome

biosynthesis begins when the transcription factor UBF binds to ribosomal DNA (rDNA), and transcription is initiated by RNA polymerase I (RNA Pol I). At this stage, nucleoli are formed. The transcribed ribosomal RNA (rRNA) is then spliced to assemble the small and then large ribosomal subunits. With the formation of the large ribosomal subunit, the nucleolus is degraded, and the biosynthesized ribosome units are transferred to the cytoplasm. The nucleolus is responsive to cellular stress. For example, in cancer cells, depending on the expression level of p53, ribosome biosynthesis stops, and the nucleolus retains the ribosomes in the nucleus (Pederson

Fig. 3 **a** Droplets play a role in the reactions associated with the central dogma. **b** Biological roles and components of the droplets shown in panel **a**

2011). Thus, the nucleolus suppresses the activity of ribosomes under conditions that are not suitable for cell growth and proliferation and contributes to efficient energy consumption by cells.

Paraspeckles Paraspeckles control gene expression by retaining RNA targeted by adenosine-inosine editing in the nucleus (Fox and Lamond 2010). Proteins involved in transcription and RNA processing have been identified as components of paraspeckles (Fox et al. 2002; Cioce and Lamond 2005; Passon et al. 2012). RNA polymerase II is also involved in transcription, and DBHS family proteins known as splicing proteins, as well as a long untranslated RNA, NEAT 1, have been identified. The paraspeckle, like the nucleolus, is responsive to cellular stress. In addition, paraspeckles and nucleoli can exchange their constituents depending on the type of stress and the environment surrounding the cells (Fox et al. 2002; Cioce and Lamond 2005). Furthermore, it was reported that RNA Pol II activates transcription by colocalizing with paraspeckles and suppresses transcription by colocalizing with the nucleolus (Fox et al. 2002). These results suggest that paraspeckles and nucleoli can respond to a variety of signals and limit the dynamic behavior of proteins in the nucleus to control protein activity in a cooperative manner.

Nuclear speckles Nuclear speckles have a structure similar to those of the nucleolus and paraspeckle, and they repeatedly form and decompose depending on the intracellular environment (Lamond and Spector 2003; Handwerger et al. 2005). A number of components of nuclear speckles have been identified, including serine- and arginine-rich proteins, adenine-rich RNAs, and proteins involved in pre-mRNA splicing (Visa et al. 1993). Nuclear speckles also suppress pre-mRNA splicing by encapsulating these components in droplets, resulting in inactivation of gene expression (Zong et al. 2011).

Cajal bodies Cajal bodies possess a dynamic structure that is dependent on stress and the cell cycle (Lamond and Sleeman 2003). Cajal bodies are involved in regulating the production of small nucleolar ribonucleoprotein particles (Cioce and Lamond 2005). Under conditions of stress, Cajal bodies incorporate and protect p53 (Young et al. 2002). Cajal bodies also incorporate and protect transcription factors such as RNA Pol I (Shav-Tal et al. 2005).

PML bodies PML bodies include PML protein as a primary constituent (Lallemand-Breitenbach 2010). PML, a member of the TRIM family of proteins, contains a RING domain, two B-box domains, and a coiled coil domain. All of these domains have been shown to be necessary for the formation of PML bodies (Lallemand-Breitenbach 2010). PML bodies regulate tumor pathways, defense responses to viruses, and also regulate pathways associated with apoptosis by incorporating

p53 and transcriptional regulatory factors (Eskiw et al. 2003; Condemine et al. 2007; Batty et al. 2012). PML bodies can also regulate gene expression via the inclusion and release of mRNA (Lallemand-Breitenbach 2010). Although these properties and functions are similar to other droplets, PML bodies do not require RNA for their formation (Boisvert et al. 2000), in contrast to other droplets found in the nucleus. PML bodies regulate gene expression through temporal control of mRNA.

P-bodies P-bodies contain proteins involved in mRNA transport, modification, and translation and function in the regulation of RNA metabolism. mRNA plays a central role in the formation of P-bodies (Jain and Parker 2013). Increases in untranslated mRNA molecules resulting from translation initiation or stress inhibition are correlated with increases in P-body size and number (Teixeira et al. 2005). In contrast, decreases in untranslated mRNA resulting from inhibition of the elongation step or enzymatic degradation of mRNAs are correlated with P-body degradation (Teixeira et al. 2005; Jain and Parker 2013).

Stress granules Stress granules assemble in response to stress signals and segregate transcriptionally silent mRNA molecules and transcription factors (Anderson et al. 2015). Translation initiation factors and small ribosomal subunit components were identified as proteins concentrated in stress granules (Buchan 2014). Stress granules are degraded by removal of the stress signal, resulting in resumption of mRNA translation. Similar to P-bodies, sequestration of untranslated mRNAs in the polysome suppresses the formation of stress granules. These results show that mRNA is required for their assembly (Kedersha et al. 2000). Furthermore, it was reported that yeast strains deficient in the formation of P-bodies cannot efficiently form stress granules (Kedersha et al. 2000). These observations indicate that the pre-assembly of a complex composed of mRNA and protein in the P-body is a prerequisite for the formation of stress granules (Patel et al. 2015).

Germ granules Germ granules are droplets found in special germ cells that generate sexual cells during meiosis of the developing embryo and regulate gene expression through post-transcriptional regulation of mRNA in primordial germ cells (Voronina et al. 2011). Germ granules are rich in mRNA, RNA helicase, and RNA-modifying enzymes. Germ granules are also involved in the localization of biomolecules in primordial germ cells (Wang et al. 2014a). For example, the nos-2 RNA is asymmetrically separated in the cytoplasm by reproductive granules during the development of *C. elegans* larvae. It was also shown that reproductive granules are required for the formation of mitotic structures in primordial germ granules and for mitosis (Wang et al. 2014b).

Generally, the droplets described above control biological reactions by locally adjusting the concentrations of specific biomolecules. When an enzyme is co-localized with a substrate in a droplet, the enzymatic reaction would be promoted. In contrast, an enzyme reaction would be suppressed by encapsulation of the enzyme in a droplet in the absence of the substrate. In the same manner, a reduction in an enzyme's concentration outside a droplet results in suppression of its activity outside the droplet. Such reaction control via molecular localization could be an important mechanism for controlling gene expression in cells and organelles under MC conditions. Moreover, some of the proteins in a droplet might have nuclear localization or nuclear export signals. Thus, droplets could play a role in the transportation of biomolecules across organelles.

Other biological roles of droplets

Biomolecule localization and reaction control via droplets are also involved in the enzyme reaction for repairing DNA damage. A compartment that promotes DNA repair reactions is formed at the site of DNA damage (Eskiw et al. 2003; Cioce and Lamond 2005; Louvet et al. 2006; Moore et al. 2011; Louvet et al. 2014). This compartment is a dynamic droplet with liquid properties and encapsulates a variety of enzymes involved in DNA damage repair by forming in proximity to DNA lesions. These droplets accelerate the repair process by concentrating DNA repair enzymes close to DNA lesions.

Enrichment of enzymes and proteins in droplets plays a role in various biological processes and reactions in cells. Droplets that promote phosphorylation and methylation via encapsulation of kinases and methylation enzymes, respectively, have been reported (Wang et al. 2014a; Hofweber et al. 2018). An increasing number of biological processes utilizing droplets have been identified, such as signal transduction of T cell receptors (Su et al. 2016), assembly of membrane-bound proteins to initiate endocytosis (Bergeron-Sandoval et al. 2017), formation of nuclear pore complex (Vovk et al. 2016), extracellular matrix protein networks (Rauscher and Pomès 2017), and silencing chromatin (Larson et al. 2017; Strom et al. 2017). Droplets are further involved in formation of microtubules and actin fibers. Tubulin is concentrated to droplets in the centrosome to promote the microtubule positive reaction (Woodruff et al. 2017). Similarly, around the vicinity of the cell membrane, actin formation is promoted via the concentration of actin fiber-forming biomolecules in droplets (Feric and Brangwynne 2013). From these reports, it is possible to conclude that droplets are essential components of cells and facilitate complex and multiplex biological reactions in biosystems under MC conditions.

Droplets and diseases

Droplets are not only involved in the control of normal biological cellular responses, they also play roles in the onset of various diseases (Ramaswami et al. 2013; Kim et al. 2013; Molliex et al. 2015; Patel et al. 2015). It is increasingly evident that inhibition of RNA metabolism due to the formation or accumulation of pathologic droplets in cells is involved in the onset of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTD), and fragile X syndrome (FXTAS) (Ramaswami et al. 2013; Kim et al. 2013; Alberti 2017). It was also suggested that pathologic droplets are involved in the onset of cancer (Anderson et al. 2015). These pathologic droplets are composed of proteins and RNAs in the same manner as normal droplets (Lin et al. 2015; Molliex et al. 2015; Patel et al. 2015). Interestingly, many proteins associated with pathologic droplets contain prion-like domains and RNA-binding domains such as RRM and RGG domains (Ramaswami et al. 2013; Kim et al. 2013; Lin et al. 2015; Molliex et al. 2015; Patel et al. 2015). Proteins found in stress granules, such as FUS (Lin et al. 2015; Molliex et al. 2015), hnRNPA1 (Kim et al. 2013; Lin et al. 2015; Molliex et al. 2015), and hnRNPA2 (Kim et al. 2013), have also been observed in ALS-related pathologic droplets. It is becoming clear that protein-RNA interactions via the above-mentioned domains in proteins are essential for droplet formation. Mutations in RRM and RGG domains suppress the formation of pathologic droplets both in vivo and in vitro (Burke et al. 2015; Tsang et al. 2019). RNAs found in pathologic droplets often have expanded repetitive sequences, such as GGC and GGGGCC, which are related to neurodegenerative diseases (Green et al. 2017; Cid-Samper et al. 2018). As with the case of proteins, mutations in such repetitive RNA sequences suppress the formation of pathologic droplets in vivo and in vitro (Lee et al. 2013; Jain and Vale 2017). The loss of dynamic and reversible properties of droplets induced by mutations in proteins and RNAs may cause biomolecular aggregation, resulting in neurodegenerative and other diseases. Notably, not only the mutations but also other factors such as chemical modifications of biomolecules and the molecular environment itself can alter droplet properties and lead to the irreversible aggregation of droplets.

Droplets and chemical modifications

Chemical modifications of proteins control droplet formation. For example, ADP ribosylation promotes droplet formation. The ribosylation of ADP in *Drosophila* is activated by p53-independent stress responses. Ribosylation of ADP promotes the formation of droplets composed of specific polymerases and controls the activity of these polymerases (Liu and Fang 2019). It has also been

reported that phosphorylation of tyrosine residues of certain proteins by kinases promotes the formation of droplets in mammalian cells (Louvet et al. 2006; Li et al. 2012; Wippich et al. 2013; Wang et al. 2014a). Phosphorylation/dephosphorylation cycles also regulate the formation and dissolution of nucleoli and germ granules (Louvet et al. 2006; Wang et al. 2014b). Furthermore, methylation of arginine residues of certain proteins, such as the RNA helicase Ddx4, has been shown to regulate the activity enzymes by promoting droplet formation (Nott et al. 2015). Ubiquitination of certain proteins has been shown to inhibit droplet formation (Dao et al. 2018). As these chemical modifications alter the net charge, charge density, water solubility, hydrophobicity, and ability to form non-covalent interactions (e.g., electrostatic, H-bonding, π - π , cation- π , and CH- π interactions), chemical modifications have a marked affect of droplet formation and properties.

Droplets and the molecular environment

The salt concentration and presence of organic solvents such as hexane diol affect droplet formation and are thus used to show the dynamic and reversible behavior of droplets (Strom et al. 2017; Lu et al. 2018). As discussed above, it is also important to study the effects of MC on droplet properties. Droplet formation in the presence of nonionic and ionic crowding reagents has been reported. It was shown that crowding reagents can be used to control droplet formation both *in test a tube* and *in a cell*. For example, droplet formation is promoted by the high density of macromolecules in a cell (Kim et al. 2013; Burke et al. 2015; Molliex et al. 2015). In a test tube, droplet formation is promoted by crowding reagents such as polyethylene glycol (PEG) (Kim and Lee 1998; Wang et al. 2014a). Interestingly, it has also been reported that when cells are stressed, the cell volume decreases, resulting in an increase in intracellular congestion (Burke et al. 2015; Molliex et al. 2015). An increase in intracellular congestion has been shown to promote droplet formation. Certain proteins associated with signal transduction, such as mTORC1, regulate intracellular levels of ribosomes by controlling ribosome biogenesis and autophagy (Sabatini 2017). An increase in intracellular ribosome congestion also has been shown to promote droplet formation (Delarue 2018). In this manner, cells can adjust the intracellular congestion and promote droplet formation during periods of stress (Burke et al. 2015; Molliex et al. 2015; Delarue 2018). This hypothesis is consistent with stress granules and other droplets that control protein synthesis at different steps in the reactions associated with the central dogma.

Conclusions and future perspectives

Cells are highly crowded with various macromolecules as well as small molecules. MC is the fundamental difference between the intracellular molecular environment and that in a test tube. Gene expression associated with reactions of the central dogma in such a complex molecular environment is critically dependent on droplets as molecular sorters, molecular transporters, and probably signal amplifiers.

MC affects the structure, stability, interactions, and function of biomolecules (Zhou et al. 2008). Therefore, MC itself affects the properties of droplets. The effects of MC on droplets have been studied primarily using nonionic and inert synthetic polymers *in vitro*, while most biomolecules have functional groups that facilitate non-covalent interactions with other biomolecules. In addition, many osmolytes, which are abundant inside cells, are zwitterionic, and most of the anions inside a cell are small organic molecules. Therefore, the MC effects on droplets should be studied more systematically with such molecules. These studies may enhance our understanding of how droplets respond to the surrounding molecular environment and by extending our understanding of cellular stress, cell status, and the cell cycle.

Funding information This work was supported by JSPS KAKENHI (grant numbers 18K19153 and 17H06351 [Grant-in-Aid for Scientific Research on Innovative Areas “Chemistry for Multimolecular Crowding Biosystems”]) and a research grant from the Asahi Glass Foundation, Japan.

References

- Anderson P, Kedersha N, Ivanov P (2015) Stress granules, P-bodies and cancer. *Biochim Biophys Acta* 1849:861–870
- Alberti S (2017) The wisdom of crowds: regulating cell function through condensed states of living matter. *J Cell Sci* 130:2789–2796
- Banani SF, Lee HO, Hyman AA, Rosen MK (2017) Biomolecular condensates: organizers of cellular biochemistry. *Nat Rev Mol Cell Biol* 18:285–298
- Banani SF, Rice AM, Peeples WB, Lin Y, Jain S, Parker R, Rosen KM (2016) Compositional control of phase-separated cellular bodies. *Cell* 166:651–663
- Batty EC, Jensen K, Freemont PS (2012) PML nuclear bodies and other trim-defined subcellular compartments. *Adv Exp Med Biol* 770:39–58
- Bennett BD, Kimball EH, Gao M, Osterhout R, Van Dien SJ, Rabinowitz JD (2009) Absolute metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*. *Nat Chem Biol* 5:593–599
- Bergeron-Sandoval LP, Heris HK, Hendricks AG, Ehrlicher AJ, François P, Pappu RV, Michnick SW (2017) Endocytosis caused by liquid-liquid phase separation of proteins. *BioRxiv* 10:145664–145688
- Blattner FR, Plunkett G III, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* 277:1453–1462

- Boisvert FM, Hendzel MJ, Bazett-Jones DP (2000) Promyelocytic leukemia (PML) nuclear bodies are protein structures that do not accumulate RNA. *J Cell Biol* 24:283–292
- Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, Jülicher F, Hyman AA (2009) Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* 324:1729–1732
- Brangwynne CP, Mitchison TJ, Hyman AA (2011) Active liquid-like behavior of nucleoli determines their size and shape in *Xenopus laevis* oocytes. *Proc Natl Acad Sci U S A* 108:4334–4339
- Buchan JR (2014) mRNP granules. Assembly, function, and connections with disease. *RNA Biol* 11:1019–1030
- Burke KA, Janke AM, Rhine CL, Fawzi NL (2015) Residue-by-residue view of *in vitro* FUS granules that bind the C-terminal domain of RNA polymerase II. *Mol Cell* 60:231–241
- Cid-Samper F, Gelabert-Baldrich M, Lang B, Lorenzo-Gotor N, Ponti RD, Severijnen AWF, Bolognesi B, Gelpi E, Hukema RK, Botta-Orfila T, Tartaglia GG (2018) An integrative study of protein-RNA condensates identifies scaffolding RNAs and reveals players in fragile X-associated tremor/ataxia syndrome. *Cell Rep* 25:3422–3434.e7
- Cioce M, Lamond AI (2005) Cajal bodies: a long history of discovery. *Annu Rev Cell Dev Biol* 21:105–131
- Condemine W, Takahashi Y, Bras ML, de Thé H (2007) A nucleolar targeting signal in PML-I addresses PML to nucleolar caps in stressed or senescent cells. *J Cell Sci* 120:3219–3227
- Dao TP, Kolaitis R-M, Kim HJ, O'Donovan K, Martyniak B, Colicino E, Hehnly H, Taylor JP, Castañeda CA (2018) Ubiquitin modulates liquid-liquid phase separation of UBQLN2 via disruption of multivalent interactions. *Mol Cell* 69:965–978
- Delarue M, Brittingham GP, Pfeffer S, Surovtsev IV, Pinglay S, Kennedy KJ, Schaffer M, Gutierrez JL, Sang D, Poterewicz G, Chung JK, Plitzko JM, Groves JT, Jacobs-Wagner C, Engel BD, Holt LJ (2018) mTORC1 Controls Phase Separation and the Biophysical Properties of the Cytoplasm by Tuning Crowding. *Cell* 174 (2):338–349.e20
- Ellis RJ (2001) Macromolecular crowding: obvious but underappreciated. *Trends Biochem Sci* 26:597–604
- Eskiw CH, Dellaire G, Mymryk JS, Bazett-Jones DP (2003) Size, position and dynamic behavior of PML nuclear bodies following cell stress as a paradigm for supramolecular trafficking and assembly. *J Cell Sci* 116:4455–4466
- Elbaum-Garfinkle S, Kim Y, Szczepaniak K, Chih-Hsiung Chen C, Eckmann CR, Myong S, Brangwynne PB (2015) The disordered P granule protein LAF-1 drives phase separation into droplets with tunable viscosity and dynamics. *Proc Natl Acad Sci U S A* 112:7189–7194
- Feric M, Brangwynne CP (2013) A nuclear F-actin scaffold stabilizes ribonucleoprotein droplets against gravity in large cells. *Nat Cell Biol* 15:1253–1259
- Fox AH, Lamond AI (2010) Paraspeckles. *Cold Spring Harb Perspect Biol*, New York
- Fox AH, Lam YW, Leung AKL, Lyon CE, Andersen J, Mann M, Lamond AI (2002) Paraspeckles: a novel nuclear domain. *Curr Biol* 12:13–25
- Frey S, Richter RP, Gorlich D (2006) FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties. *Science* 314:815–817
- Goodsell DS (2011) Eukaryotic cell panorama. *BAMBED* 39:91–101
- Green KM, Glineburg MR, Kears MG, Flores BN, Linsalata AE, Fedak SJ, Goldstrohm AC, Barmada SJ, Todd PK (2017) RAN translation at C9orf72-associated repeat expansions is selectively enhanced by the integrated stress response. *Nat Commun* 8:2005
- Handwerker KE, Cordero JA, Gall JG (2005) Cajal bodies, nucleoli, and speckles in the *Xenopus* oocyte nucleus have a low-density, sponge-like structure. *Mol Biol Cell* 16:202–211
- Hernandez-Verdun D (2011) Assembly and disassembly of the nucleolus during the cell cycle. *Nucleus* 2:189–194
- Hofweber M, Hutten S, Bourgeois B, Spreitzer E, Niedner-Boblentz A, Schifferer M, Ruepp MD, Simons M, Niessing D, Madl T, Dormann D (2018) Phase separation of FUS is suppressed by its nuclear import receptor and arginine methylation. *Cell* 173:706–719
- Jain A, Vale RD (2017) RNA phase transitions in repeat expansion disorders. *Nature* 546:243–247
- Jain S, Parker R (2013) The discovery and analysis of P bodies. *Adv Exp Med Biol* 768:23–43
- Kato M, Han TW, Xie S, Shi K, Du X, Wu LC, Mirzaei H, Goldsmith EJ, Longgood J, Pei J, Grishin NV, Frantz DE, Schneider JW, Chen S, Li L, Sawaya MR, Eisenberg D, Tycko R, McKnight SL (2012) Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell* 149:753–767
- Kedersha N, Cho MR, Li W, Yacono PW, Chen S, Gilks N, Golan DE, Anderson P (2000) Dynamic shuttling of Tia-1 accompanies the recruitment of mRNA to mammalian stress granules. *J Cell Biol* 151:1257–1268
- Kim HJ, Kim NC, Wang YD, Scarborough EA, Moore J, Diaz Z, MacLea KS, Freibaum B, Li S, Molliex A, Kanagaraj AP, Carter R, Boylan KB, Wojtas AM, Rademakers R, Pinkus JL, Greenberg SA, Trojanowski JQ, Traynor BJ, Smith BN, Topp S, Gkazi AS, Miller J, Shaw CE, Kottlors M, Kirschner J, Pestronk A, Li Y, Ford AF, Gitler AD, Benatar M, King OD, Kimonis VE, Ross ED, Weihl CC, Shorter J, Taylor JP (2013) Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature* 495:467–473
- Kim JH, Lee KH (1998) Effect of PEG additive on membrane formation by phase inversion. *J memb sci* 138:153–163
- Koga S, Williams DS, Perriman AW, Mann S (2011) Peptide-nucleotide microdroplets as a step towards a membrane-free protocell model. *Nat Chem* 3:720–724
- Lallemant-Breitenbach V (2010) The PML nuclear bodies. *Cold Spring Harb Perspect Biol*, New York
- Lamond AI, Sleeman JE (2003) Nuclear substructure and dynamics. *Curr Biol* 13:R825–R828
- Lamond AI, Spector DL (2003) Nuclear speckles: a model for nuclear organelles. *Nat Rev Mol Cell Biol* 4:605–612
- Larson A, Elnatan D, Keenen M, Trmka M, Johnston J, Burlingame A, Agard D, Redding S, Narlikar G (2017) Liquid droplet formation by HP1 α suggests a role for phase separation in heterochromatin. *Nature* 547:236–240
- Lee YB, Chen HJ, Peres JN, Gomez-Deza J, Attig J, Štalekar M, Troakes C, Nishimura AL, Scotter EL, Vance C, Adachi Y, Sardone V, Miller JW, Smith BN, Gallo JM, Ule J, Hirth F, Rogelj B, Shaw CE (2013) Hexanucleotide repeats in ALS / FTD form length-dependent RNA foci, sequester RNA binding proteins, and are neurotoxic. *Cell Rep* 5:1178–1186
- Li P, Banjade S, Cheng HC, Kim S, Chen B, Guo L, Llaguno M, Hollingsworth JV, King DS, Banani SF, Russo PS, Jiang QX, Nixon BT, Rosen MK (2012) Phase transitions in the assembly of multivalent signalling proteins. *Nature* 483:336–340
- Lin Y, Protter DS, Rosen MK, Parker R (2015) Formation and maturation of phase-separated liquid droplets by RNA-binding proteins. *Mol Cell* 60:208–219
- Liu C, Fang Y (2019) New insights of poly(ADP-ribosylation) in neurodegenerative diseases: a focus on protein phase separation and pathologic aggregation. *Biochem Pharmacol* 167:58–63
- López CG, Hernández R, López-Villaseñor I, Reyes-Vivas H, de L Segura-Valdez M, Jiménez-García LF (2005) Electron microscopy analysis of the nucleolus of Trypanosoma. *Microsc Microanal* 11:293–299
- Louvet E, Junera HR, Berthuy I, Hernandez-Verdun D (2006) Compartmentation of the nucleolar processing proteins in the

- granular component is a CK2-driven process. *Mol Biol Cell* 17: 2537–2546
- Louvet E, Yoshida A, Kumeta M, Takeyasu K (2014) Probing the stiffness of isolated nucleoli by atomic force microscopy. *Histochem Cell Biol* 141:365–381
- Lu H, Yu D, Hansen AS, Ganguly S, Liu R, Heckert A, Darzacq X, Zhou Q (2018) Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II. *Nature* 558:318–323
- Milo R, Phillips R (2015) *Cell biology by the numbers*. Garland Science, New York
- Minton AP (2001) The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. *J Biol Chem* 276:10577–10580
- Molliex A, Temirov J, Lee J, Coughlin M, Kanagaraj AP, Kim HJ, Mittag T, Taylor JP (2015) Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell* 163:123–133
- Moore MH, Bai B, Boisvert FM, Latonen L, Rantanen V, Simpson JC, Pepperkok R, Lamond AI, Laiho M (2011) Quantitative proteomics and dynamic imaging of the nucleolus reveal distinct responses to UV and ionizing radiation. *Mol Cell Proteomics* 10:10–24
- Neidhardt FC, Ingraham JL, Schaechter M (1990) *Physiology of the bacterial cell: a molecular approach*, Sinauer associates
- Nott TJ, Petsalaki E, Farber P, Jervis D, Fussner E, Plochowitz A, Craggs TD, Bazett-Jones DP, Pawson T, Forman-Kay JD, Baldwin AJ (2015) Phase transition of a disordered Nuage protein generates environmentally responsive membraneless organelles. *Mol Cell* 57:936–947
- Passon DM, Lee M, Rackham O, Stanley WA, Sadowska A, Filipovska A, Fox AH, Bond CS (2012) Structure of the heterodimer of human NONO and paraspeckle protein component 1 and analysis of its role in subnuclear body formation. *Proc Natl Acad Sci U S A* 109:4846–4850
- Patel A, Lee HO, Jawerth L, Maharana S, Jahnke M, Hein MY, Stoykov S, Mahamid J, Saha S, Franzmann TM, Poznaniakowski A, Poser I, Maghelli N, Royer LA, Weigert M, Myers EW, Grill S, Drechsel D, Alberti S (2015) A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162:1066–1077
- Pederson T (2011) *The nucleus introduced*. Cold Spring Harb Perspect Biol, New York
- Ramaswami M, Taylor JP, Parker R (2013) Altered ribostasis: RNA-protein granules in degenerative disorders. *Cell* 154:727–736
- Rauscher S, Pomès R (2017) The liquid structure of elastin. *Elife* 6: 26526–26547
- Shav-Tal Y, Blechman J, Darzacq X, Montagna C, Dye BT, Patton JG, Singer RH, Zipori D (2005) Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition. *Mol Biol Cell* 16:2395–2413
- Rivas G, Ferrone F, Herzfeld J (2004) Life in a crowded world. *EMBO Rep* 5:23–27
- Strom AR, Emelyanov AV, Mir M, Fyodorov DV, Darzacq X, Karpen GH (2017) Phase separation drives heterochromatin domain formation. *Nature* 547:241–245
- Sabatini DM (2017) Twenty-five years of mTOR: uncovering the link from nutrients to growth. *Proc Natl Acad Sci U S A* 114:11818–11825
- Schuster BS, Reed EH, Parthasarathy R, Jahnke CN, Caldwell RM, Bermudez JG, Ramage H, Good MC, Hammer DA (2018) Controllable protein phase separation and modular recruitment to form responsive membraneless organelles. *Nat Commun* 9:2985
- Shin Y, Berry J, Pannucci N, Haataja MP, Toettcher JE, Brangwynne CP (2017) Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. *Cell* 168:159–171
- Su X, Ditlev JA, Hui E, Xing W, Banjade S, Okrut J, King DS, Taunton J, Rosen MK, Vale RD (2016) Phase separation of signaling molecules promotes T cell receptor signal transduction. *Science* 352: 595–599
- Teixeira D, Sheth U, Sanchez MAV, Brengues M, Parker R (2005) Processing bodies require RNA for assembly and contain nontranslating mRNAs. *RNA* 11:371–382
- Thirumalai D, Klimov DK, Lorimer GH (2003) Caging helps proteins fold. *Proc Natl Acad Sci U S A* 100:11195–11197
- Tsang B, Arsenault J, Vernon RM, Lin H, Wang LY, Bah A, Forman-Kay D (2019) Phosphoregulated FMRP phase separation models activity-dependent translation through bidirectional control of mRNA granule formation. *Proc Natl Acad Sci U S A* 116:4218–4227
- Visa N, Puvion-Dutilleul F, Harper F, Bachellerie JP, Puvion E (1993) Intracellular distribution of poly (A) RNA determined by electron microscope *in situ* hybridization. *Cell Res* 208:19–34
- Vovk A, Gu C, Opferman MG, Kapinos LE, Lim RYH, Coalson RD, Jasnow D, Zilman A (2016) Simple biophysics underpins collective conformations of the intrinsically disordered proteins of the nuclear pore complex. *Elife* 5:10785–10814
- Wang Y, Latypov RF, Lomakin A, Meyer JA, Kerwin BA, Vunnum S, Benedek GB (2014a) Quantitative evaluation of colloidal stability of antibody solutions using PEG-induced liquid-liquid phase separation. *Mol Pharm* 11:1391–1402
- Wang JT, Smith J, Chen BC, Schmidt H, Rasoloson D, Paix A, Lambrus BG, Calidas D, Betzig E, Seydoux G (2014b) Regulation of RNA granule dynamics by phosphorylation of serine-rich, intrinsically disordered proteins in *C. elegans*. *Elife* 3:4591–4614
- Voronina E, Seydoux G, Sassone-Corsi P, Nagamori I (2011) RNA granules in germ cells. *Cold Spring Harb Perspect Biol*, New York
- Wippich F, Bodenmiller B, Trajkovska MG, Wanka S, Aebersold R, Pelkmans L (2013) Dual specificity kinase DYRK3 couples stress granule condensation / dissolution to mTORC1 signaling. *Cell* 152: 791–805
- Woodruff JB, Gomes BF, Widlund PO, Mahamid J, Honigsmann A, Hyman AA (2017) The centrosome is a selective condensate that nucleates microtubules by concentrating tubulin. *Cell* 169:1066–1077
- Yoshimura Y, Lin Y, Yagi H, Lee YH, Kitayama H, Sakurai K, So M, Ogi H, Naiki H, Goto Y (2012) Distinguishing crystal-like amyloid fibrils and glass-like amorphous aggregates from their kinetics of formation. *Proc Natl Acad Sci U S A* 109:14446–14451
- Young PJ, Day PM, Zhou J, Androphy EJ, Morris GE, Lorson CL (2002) A direct interaction between the survival motor neuron protein and p53 and its relationship to spinal muscular atrophy. *J Biol Chem* 277:2852–2859
- Zhou HX, Rivas G, Minton AP (2008) Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences. *Annu Rev Biophys* 37:375–397
- Zimmerman SB, Trach SO (1991) Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. *J Mol Biol* 222:599–620
- Zong X, Tripathi V, Prasanth KV (2011) RNA splicing control yet another gene regulatory role for long nuclear noncoding RNAs. *RNA Biol* 8:968–977