Trends in Biochemical Sciences

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Feature Review

Organization and Function of Non-dynamic Biomolecular Condensates

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Cells compartmentalize biochemical reactions using organelles. Organelles can be either membrane-bound compartments or supramolecular assemblies of protein and ribonucleic acid known as 'biomolecular condensates'. Biomolecular condensates, such as nucleoli and germ granules, have been described as liquid like, as they have the ability to fuse, flow, and undergo fission. Recent experiments have revealed that some liquid-like condensates can mature over time to form stable gels. In other cases, biomolecular condensates solidify into amyloid-like fibers. Here we discuss the assembly, organization, and physiological roles of these more stable condensates in cells, focusing on Balbiani bodies, centrosomes, nuclear pores, and amyloid bodies. We discuss how the material properties of these condensates can be explained by the principles of liquid–liquid phase separation and maturation.

Highlights

Biomolecular condensates are membrane-less organelles that form through phase separation of scaffolding proteins.

Biomolecular condensates can exhibit a broad spectrum of material properties, from dynamic liquid-like droplets to non-dynamic gels and solid amyloids. *In vitro* reconstitution suggests that non-dynamic condensates can start as liquid droplets that rapidly solidify.

Bioreactive gels are condensates that promote biochemical reactions, while functional amyloids shut down biochemical reactions and promote cellular dormancy.

Introduction

Cells must orchestrate a multitude of complex biochemical reactions to grow and divide. To control the rate and direction of these reactions, cells compartmentalize reactants, enzymes, and end products. The canonical unit of compartmentalization in a cell is the organelle. Early cytologists defined organelles as lipid-membrane-bound vesicles (e.g., the nucleus, mitochondria, the Golgi apparatus), but the advent of high-resolution electron microscopy and tools to label cellular structures *in situ* (e.g., immunofluorescence, GFP labeling) revealed numerous subcellular compartments that lack membranes. These non-membrane-bound organelles can be further subdivided based on structural features. One class includes macromolecular protein assemblies with a defined structure, such as ribosomes or the proteasome. Another class, recently termed 'biomolecular condensates' [1], includes supramolecular assemblies of protein and RNA that tend to lack long-range structure and can grow up to several microns in diameter. Nucleoli, germ granules, Cajal bodies, and P bodies are typical examples.

Biomolecular condensates have stepped into the limelight recently because, for many cases, their assembly and composition control is well described by liquid–liquid phase separation and other concepts borrowed from condensed matter physics. For example, germ granules from the nematode *Caenorhabditis elegans* and nucleoli from the frog *Xenopus laevis* exhibit liquid-like properties, such as the ability to coalesce and drip under shear force [2,3]. Since then, liquid-like properties have been observed for a number of protein compartments, suggesting that liquidity and dynamicity are common properties of biomolecular condensates. However, this view has been refined by recent studies that used *in vitro* reconstitution to study less dynamic biomolecular condensates such as centrosomes, the nuclear pore, and Balbiani bodies. Thus, it is now becoming clear that biomolecular condensates can exhibit a range of material states, from viscous liquids to gels to solid-like, functional amyloids. In this review we highlight the diversity of biomolecular condensates and speculate on how their material states

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relate to their diverse physiological roles inside cells. We place primary emphasis on the less dynamic class of biomolecular condensates, as several recent reviews nicely overview the more dynamic liquid-like class [1,4].

Bioreactive Gels: Amorphous Assemblies That Promote Selectivity and Reactivity

The main function of organelles is to compartmentalize reactants to facilitate biochemical reactions. A classic example is the promotion of ATP synthesis by establishing a proton gradient across the inner and outer mitochondrial lumen. Stable biomolecular condensates, such as centrosomes and nuclear pores, can also selectively concentrate and exclude reactants to promote biochemistry. Since these porous condensates are made from non-covalently crosslinked proteins and allow diffusion of desired macromolecules (permselectivity), we refer to them as 'bioreactive gels'.

Centrosomes

Centrosomes are micron-scale organelles that organize hundreds to thousands of microtubules that build and position the mitotic spindle in metazoans (Figure 1A). A centrosome is a composite material comprising a pair of 200-nm-long barrel-shaped centrioles surrounded by a dynamic protein mass called the pericentriolar material (PCM). The PCM comprises a small, patterned interphase layer [5–8] around which a much larger, amorphous mitotic PCM assembles (Figure 1B). The seemingly unstructured mitotic PCM provides most of the mass and microtubule nucleation capacity of the centrosome; thus, we focus on the mitotic PCM as a proxy for centrosome properties.

A striking feature of mitotic PCM is that, in most cells studied, it is a spherical compartment comprising self-organizing proteins (Figure 1C), like many liquid-like biomolecular condensates. This would suggest that mitotic PCM could also be liquid like. However, the main proteins needed to build mitotic PCM – long coiled-coil proteins such as centrosomin (*Drosophila*), CDK5Rap2 (humans), and SPD-5 (*C. elegans*) – are quite stable in the PCM and can even resist harsh dilution and salt extraction [9–11]. Although mitotic PCM is dynamic on cell-cycle timescales, these observations suggested that mitotic PCM, once assembled, is rather a stable scaffold to which client proteins bind. How mitotic PCM can act as a stable scaffold while appearing amorphous and spherical remains a riddle.

In vitro reconstitution of mitotic PCM with defined components has shown promise in solving this riddle. Using recombinant *C. elegans* proteins, it has been possible to assemble minimal mitotic PCM capable of nucleating microtubule asters *in vitro* [12]; these assemblies are called 'SPD-5 condensates', referencing the *C. elegans* scaffold protein needed to build them. SPD-5 condensates have been useful to study because they exhibit many features of *in vivo* mitotic PCM,: they are spherical and amorphous, selectively recruit PCM client proteins (e.g., homologs of TPX2, XMAP215, and Polo kinase), and nucleate microtubule asters. Unexpectedly, SPD-5 condensates began as dynamic, liquid-like droplets that quickly matured into solid-like structures. For instance, young (<2 min after formation) SPD-5 condensates could fuse after contact, whereas aged condensates (>10 min) could not fuse and instead clumped together. The aged SPD-5 condensates also did not show recovery of fluorescence after photobleaching (Figure 1D). Likewise, in mitotically arrested *C. elegans* embryos, PCM labeled with GFP::SPD-5 did not recover after photobleaching [10] (Figure 1D). Earlier in the cell cycle, *in vivo* PCM enlarges rapidly through accumulation of SPD-5 and PCM client proteins, making it difficult to interpret photobleaching experiments. However, newly incorporated SPD-5 can be seen throughout the PCM and overall PCM expansion is isotropic [10] (Figure 1D). This behavior

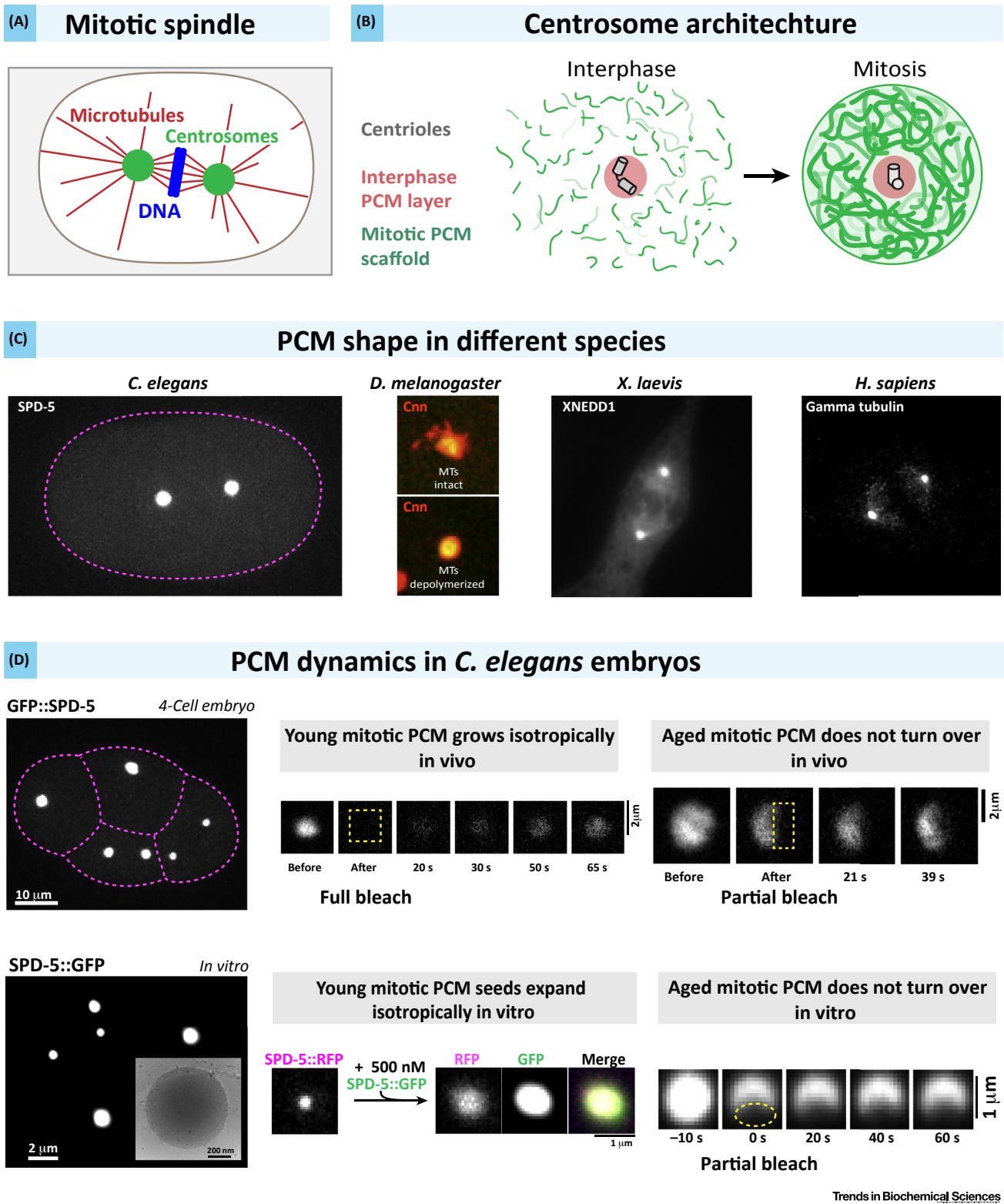

Figure 1. Centrosomes Are Spherical Organelles Comprising a Scaffold Phase That Becomes Less Dynamic over Time. (A) Schematic of the mitotic spindle in a dividing somatic cell. (B) Centrosome architecture at different cell-cycle stages. (C) Pericentriolar material (PCM) shape is spherical in various species.

(See figure legend on the bottom of the next page.)

is predicted for a growing liquid droplet: molecules are incorporated first at the surface and then evenly distribute throughout the droplet via diffusion. Droplet growth appears isotropic due to this redistribution of molecules in combination with surface tension, which maintains the droplet's spherical shape. Thus, the *in vitro* and *in vivo* findings together suggest that the mitotic PCM first assembles through phase separation of SPD-5 molecules around the centrioles. Then, the SPD-5 molecules rapidly form stable connections with each other and mature into a porous, gel-like material. Liquid–liquid phase separation of the SPD-5 scaffold followed by maturation into a gel could explain why mitotic PCM is both spherical and stable. Proper assessment of this idea would require nanoscale rheology to assess the material properties of the PCM scaffold both *in vivo* and *in vitro*.

In *C. elegans* the available evidence suggests that mitotic PCM comprises a gelating scaffold that concentrates client proteins, such as tubulin and microtubule-stabilizing proteins. These client proteins are mobile and diffuse through the scaffold phase regardless of scaffold age. Such organization makes sense with regard to PCM function, as it would promote biochemical reactions, like microtubule polymerization, while allowing structural stability to resist microtubule-mediated pulling forces.

How applicable are these organizational principles in other organisms? Mitotic PCM from other organisms is also spherical and fits the scaffold–client organizational model (Figure 1C). However, the material state of PCM has not been carefully assessed outside nematodes. One major limitation is the lack of *in vitro* reconstitution systems. Major steps forward are being made in reconstituting centrosomin from *Drosophila* and similarities to *C. elegans* SPD-5 are becoming apparent. Both proteins are enriched in coiled-coil domains and their self-assembly is accelerated by Polo kinase phosphorylation. Feng *et al*. demonstrated that two Centrosomin domains, termed PReM and CM2, together are sufficient to form micron-scale assemblies. Using X-ray crystallography, they showed that the PReM leucine zipper domain and the CM2 domain form helical dimers that bundle into an unusual tetramer. Thus, coiled-coil domain interactions drive PCM scaffold formation in *Drosophila*. Similar structural studies have not yet been performed with SPD-5. However, it is likely that such a mechanism drives PCM formation in *C. elegans*, considering that SPD-5 contains nine predicted coil-coil domains that constitute ~40% of the protein [13,14]. Structural investigation of *C. elegans* SPD-5 and reconstitution of full-length *Drosophila* centrosomin are major challenges that, once overcome, will allow proper cross-species comparisons.

The Nuclear Pore

Nuclear pore complexes (NPCs) are proteinaceous assemblies that fenestrate the nuclear envelope and provide a selectively permeable barrier that separates the inside of the nucleus from the cytoplasm. NPCs comprise multiple copies of ~32 proteins called nucleoporins (Nups) that are circularly arranged. Forming the outside of the circle are anchoring domains that connect the Nups to the nuclear envelope; facing the inside are disordered domains that are rich in phenylalanine and glycine dipeptide motifs (FG domains). This arrangement creates a central channel ~120 nm wide and ~85 nm deep called the nuclear pore, which serves as a

Examples are shown from a *Caenorhabditis elegans* embryo (cell membrane outlined in magenta), a *Drosophila melanogaster* embryo (lower panel shows a centrosome treated with nocodazole), *Xenopus laevis* tissue culture, and U2OS human cells. Images reproduced from [56–58]. (D) PCM dynamics change over time. Top: In *C. elegans* embryos, the PCM grows isotropically before mitosis, indicating the ability to rearrange; during mitosis, the PCM does not recover after photobleaching, indicating stability. The cell membrane is outlined in magenta. Bottom: Reconstituted PCM *in vitro* also expands isotropically before settling into a stable configuration that does not recover after photobleaching. Images reproduced from [10,12].

protein-dense passageway for transport of cargo between the nucleus and the cytoplasm (Figure 2A). Small molecules can freely diffuse through the pore but larger molecules (>5 nm in diameter or >30 kDa in mass) must bind first to nuclear transport receptors (NTRs) (Figure 2B).

How do the FG domains in the nuclear pore create a selective barrier? Over the past several decades, several models have been proposed, and their history is covered elsewhere [15]. The most compelling explanation is that the nuclear pore acts as a selective phase, suggesting that it can be described as a biomolecular condensate. Reconstituted FG domains from Nups from various species readily phase separate and form spherical condensates that reach >10 μm in

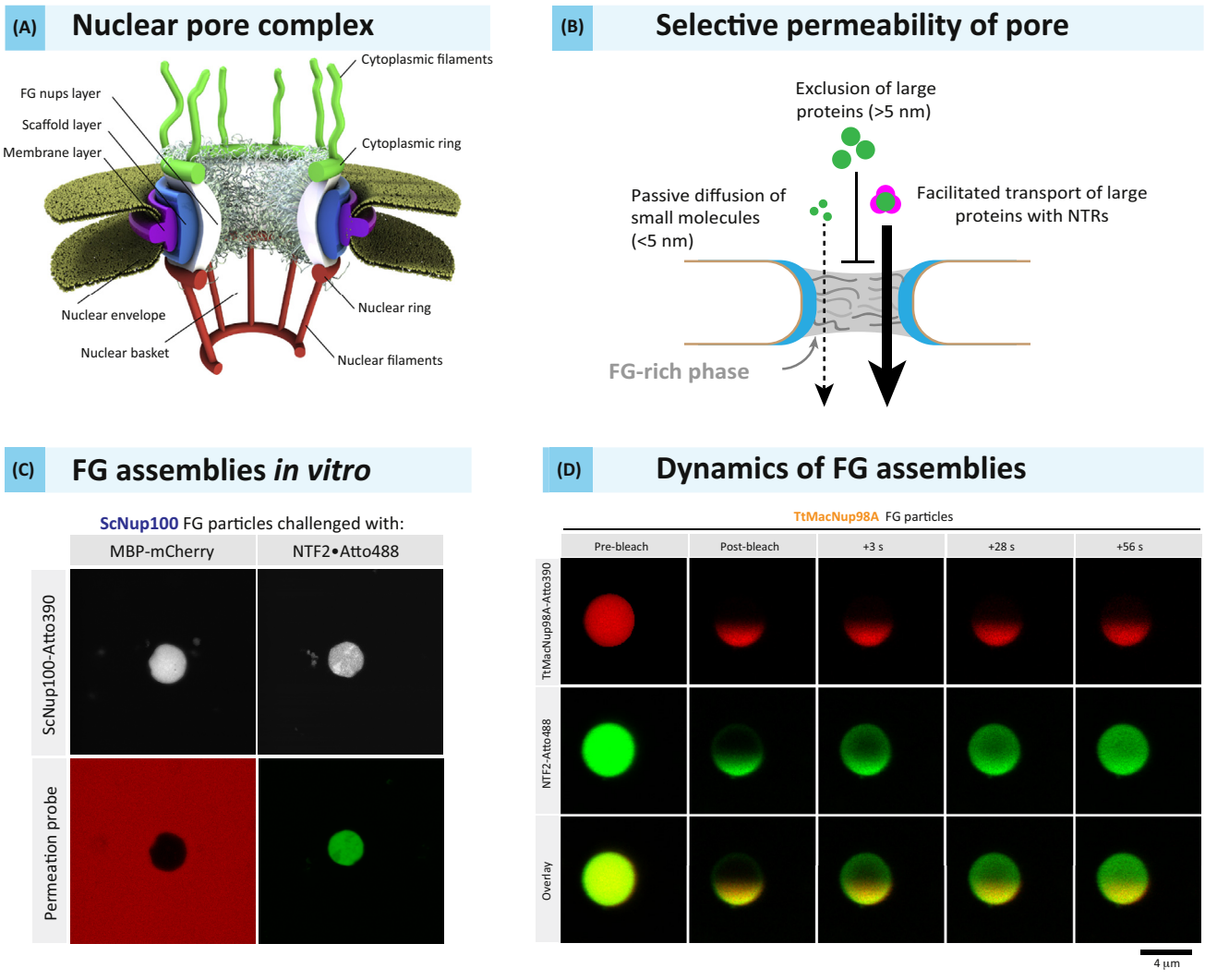

Figure 2. The Nuclear Pore Is a Dense Phase of Disordered Proteins That Forms a Selective Barrier. (A) Schematic of the nuclear pore complex. The nuclear pore is lined with disordered domains of nucleoporin (Nup) proteins containing phenylalanine and glycine (FG) dyad repeats. Image reproduced from [59]. (B) The nuclear pore permits the diffusion of small molecules but creates a selective barrier to large molecules. Cargo that is normally excluded from entering the FG-rich nuclear pore can enter if coupled to nuclear transport receptors (NTRs). (C) Purified Nup100 tails assemble into micron-scale spherical condensates *in vitro*. These condensates selectively concentrate nuclear transport receptors (NTRs) and exclude mCherry. Images reproduced from [16]. (D) Photobleaching of fluorescently labeled Nup98 condensates *in vitro*. The Nup proteins do not rearrange, whereas NTRs diffuse throughout the condensate. Images reproduced from [16].

diameter [16]. These FG condensates demonstrate the expected behavior of *in vivo* nuclear pores: small molecules (fluorescent dyes) passively diffuse through while large, inert tracer molecules are excluded (e.g., MBP-mCherry) and NTRs (e.g., importin β) are concentrated (Figure 2C). The spherical shape of the *in vitro* FG condensates might suggest that they behave as liquids. To the contrary, photobleaching experiments revealed that the FG proteins are stable within the condensate whereas the NTRs are dynamic and can transit through (Figure 2D). These results suggest that the nuclear pore comprises a gel-like phase of FG domains permeated by diffusing client proteins (NTRs coupled to cargo). Such organization is similar to that of centrosomes (discussed above) and other biomolecular condensates [12,17]. Given the similarities between centrosomes and nuclear pores, it is possible that FG domains in the nuclear pore initially assemble into a liquid-like phase that rapidly solidifies into a gel, but this idea remains to be tested.

Based on the selective phase model, selectivity of the pore is determined by the physicochemical properties of the FG-rich scaffold phase. Proteins that interact favorably with the FG-rich phase will enter the nuclear pore, whereas proteins that interact poorly will be effectively excluded. What chemical properties would allow a client protein to concentrate within the FG scaffold phase? Nup FG domains are highly hydrophobic and largely devoid of charged residues, and aliphatic alcohols, like 1,6-hexanediol, disrupt the formation of FG condensates *in vitro* and the selectivity of nuclear pores *in vivo*. Researchers have interpreted these data to mean that hydrophobic interactions dominate within the nuclear pore and determine which proteins enter. However, it has never been shown that 1,6-hexanediol exclusively disrupts hydrophobic interactions. Furthermore, the phenylalanine rings in the FG motif can participate in short-range Pi–Pi stacking, which can drive phase separation of disordered peptides [1]. Carefully delineation of the 'rules of attraction' would help us understand how large proteins and protein complexes without nuclear localization signals (which bind to NTRs) still enter the nucleus; a prominent example is alpha/beta tubulin dimers (8 nm long) [18].

Functional Amyloids: Protein Assemblies That Shut Down Biochemistry

So far we have focused on the formation of condensates that promote biochemical reactions. However, other condensates can slow reactions or stop them altogether. Important examples include Balbiani bodies and A-bodies, which are micron-scale condensates that exhibit solid-like behavior and are proposed to promote dormancy during oocyte storage and cellular stress, respectively. We classify these bodies as functional amyloids, since they contain prion-like domains but have physiological roles and their formation is reversible.

Balbiani Bodies

Balbiani bodies are amyloid-like cages that sequester RNA, proteins, and many membranous organelles, such as mitochondria, the endoplasmic reticulum, and the Golgi apparatus (Figure 3A). They are present in dormant, non-growing oocytes but disappear once the oocyte receives maturation signals [19,20]. Balbiani bodies are conserved in virtually all vertebrates and have been studied in mice [20], frogs (*X. laevis*) [19,21,22], zebrafish [23], and humans [24,25]. Oocytes can remain dormant for up to 50 years depending on the species; thus, Balbiani bodies are thought to keep the organelles in a low-activity state so that they survive dormancy and remain healthy. However, the precise function of the Balibiani body in maintaining dormancy is unknown, nor is much known about how they assemble and disassemble.

The first systematic biochemical characterization of Balbiani bodies was performed in *X. laevis* oocytes, taking advantage of the large size of these oocytes [19]. On average, *Xenopus* early-stage oocytes and Balbiani bodies are 250 μm and 60 μm in diameter, respectively [21]. Velo1

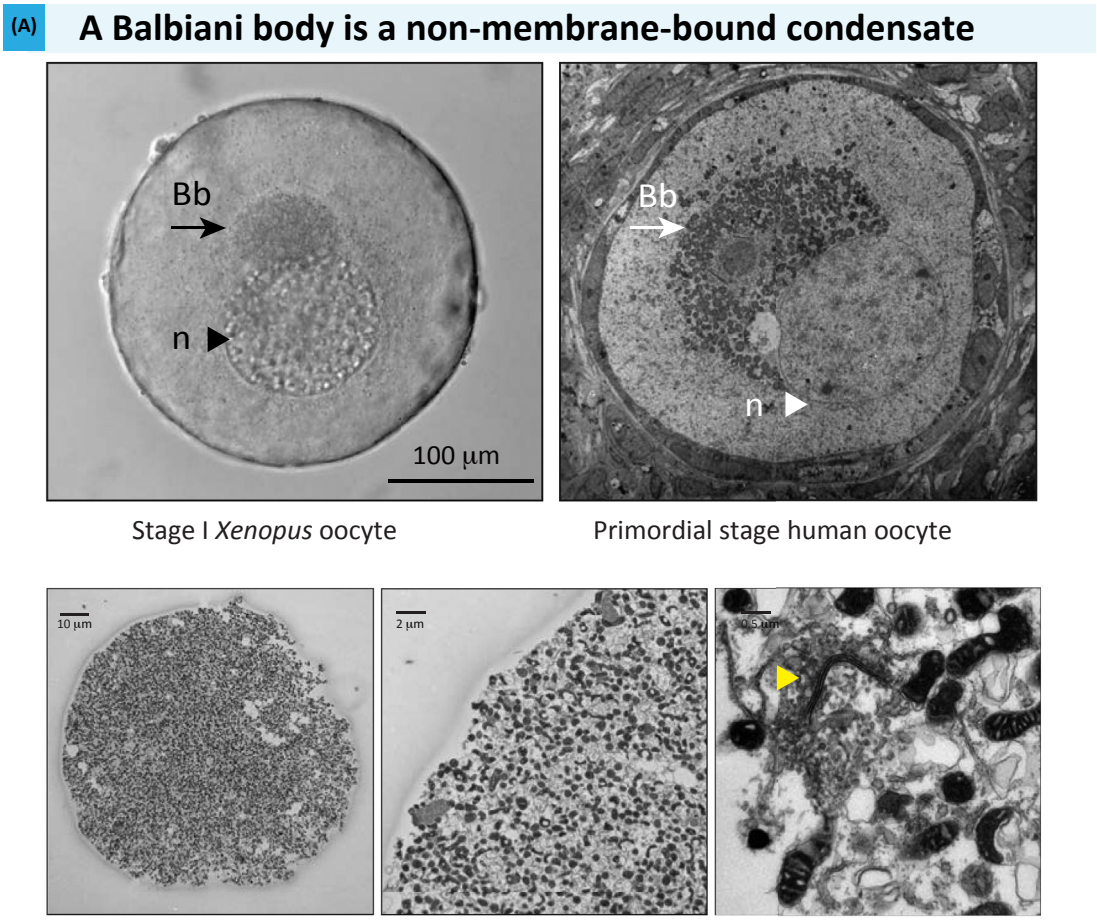

Figure 3. A Balbiani Body Is a Non-Membrane-Bound Condensate with Solid-Like Properties and Is Packed with Membrane-Bound Organelles. (A) Upper panel: Babiani bodies from *Xenopus laevis* (frog) and human oocytes [19,24]. Arrows point to the Balbiani body (Bb); arrowhead depicts the nucleus (n). Lower

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was identified as the most enriched protein in *Xenopus* Balbiani bodies that was not part of the membranous organelles. Velo1-GFP localizes to Balbiani bodies after injection into oocytes and fills the gaps between mitochondria. Velo1 forms a stable matrix in the Balbiani body, judged by its very slow turnover after photobleaching (Figure 3B). These observations, combined with the very high local concentration of Velo1 (which exceeds 500 μM in Balbiani bodies), suggest that Velo1 acts as a structural glue to hold the organelles together in the Balbiani body [19].

How can a protein act as a glue to bring organelles together in a stable but reversible matrix in the cytoplasm? Velo1 is a highly disordered protein with a prion-like domain (PLD) in its N terminus and a positively charged C terminus that binds to RNA. On stimulation PLDs have the capacity to form amyloids, which have a self-templating capacity. However, many RNA-binding proteins (RBPs) with PLDs are shown to form liquid-like condensates under physiological conditions [1]. Mutations in these RBPs are associated with neurodegenerative diseases, as they can trigger aberrant transitions of the functional liquid-like condensates to pathological solid aggregates, which could have amyloid-like properties [26–28]. Although the majority of our knowledge on amyloids comes from these pathological forms, physiological amyloids have been recently identified [29–32]. Understanding how these physiological amyloids are regulated could provide insight into how to prevent the formation of pathological amyloids.

In vivo and *in vitro* experiments have demonstrated that Velo1 is a physiological amyloid that forms cages around organelles. Pure protein experiments showed that Velo1 self-assembles into amyloid-like condensates in a PLD-dependent manner. Moreover, Velo1 could cluster mitochondria in a cell-free system, simulating aspects of Balbiani body function in the oocyte [19]. Velo1 self-assembly is a fast and highly specific process, as other RBPs were excluded from Velo1 amyloid-like condensates. *In vivo* experiments show that endogenous Velo1 has amyloid-like features in early oocytes but not in mature eggs. This suggests that Velo1 assemblies are solubilized during oocyte maturation [19,33].

Balbiani bodies have a spherical shape in many species [2,3,34] (Figure 3A). Other spherical compartments in the cell, such as vesicles and dynamic biomolecular condensates like nucleoli and germline granules, derive their shape largely from surface tension [2,3,34], a cohesive force among liquid molecules that minimizes surface area. This would suggest that Balbiani bodies might have liquid-like properties. However, mechanical isolation of Balbiani bodies from *Xenopus* oocytes proved that they behave as solids with a stable structure [19]. Previous electron microscopy studies in human oocytes also noted the presence of coarse and fine fibrils in Balbiani bodies, indicating that the solid, amyloid-like nature of Balbiani bodies is conserved [24,25]. One possible explanation for the round shape of a Balbiani bodies is that they are initially liquid-like but then rapidly mature into solid, amyloid-like structures, similar to centrosomes as discussed above. However, technical challenges in obtaining non-mature oocytes have made this idea difficult to test.

Amyloid Bodies

Nuclear amyloid bodies (A-bodies) were recently discovered as nuclear foci with amyloid-like properties. Like the Balbiani bodies discussed above, A-bodies comprise physiological and reversible amyloids and are thought to promote cellular dormancy [19,35]. They form under

panel: Electron microscope (EM) images of isolated Balbiani bodies from *X. laevis* stage I oocytes. Mitochondria are visible in all panels; yellow arrowhead points to a Golgi stack in higher-magnification image [19]. (B) Velo1-GFP localizes to the Balbiani body in *X. laevis* oocytes after injection of the recombinant protein or mRNA encoding it (left panel). The nucleus is outlined in white; the plasma membrane is outlined in magenta. On photobleaching, Velo1-GFP signal has not recovered after 1.5 h, the area corresponding to the yellow broken rectangle in the image [19].

certain stress conditions, like heat shock and acidosis, and sequester proteins involved in cell-cycle progression and DNA synthesis. Similar to other cellular stress granules with liquid-like properties (e.g., cytoplasmic stress granules, Cajal bodies, nuclear speckles), A-bodies can appear spherical in shape (Figure 4). However, A-bodies are distinct from other stress granules in that they accumulate amyloid-specific dyes such as Thioflavin S, Congo Red, and Amylo-Glo (Figure 4A). Photobleaching experiments showed that the proteins in A-bodies are immobile, further supporting the idea that mature A-bodies are solid structures and differ from typical stress granules.

We have proposed that other solid-like condensates form initially through liquid–liquid phase separation followed by maturation into a solid-like state. It is not yet clear whether A-body assembly follows the same pathway. A-bodies are proposed to be seeded by a long noncoding RNA, rIGSRNA, since silencing of rIGSRNA impaired or delayed the formation of A-bodies [35]. Once seeded, A-bodies then accumulate proliferative factors like cyclin-dependent kinase,

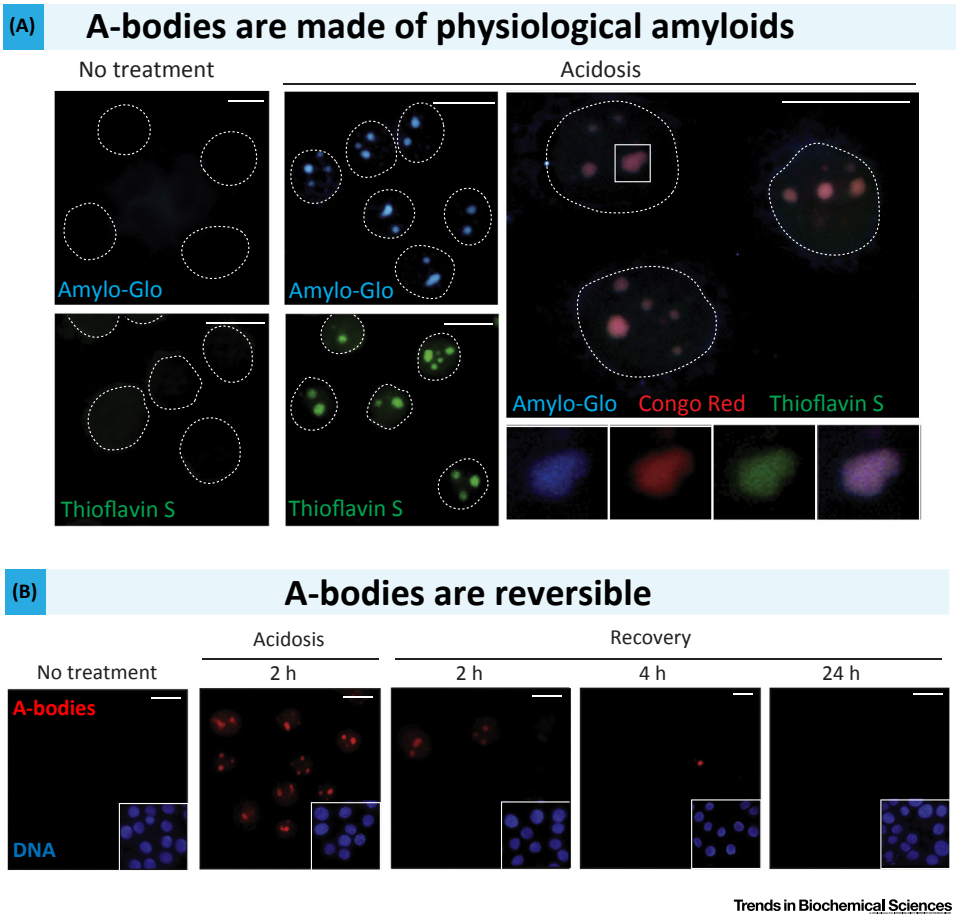

Figure 4. A-Bodies Are Reversible Amyloids That Form in the Cell Nucleus on Stress. (A) A-bodies stain with amyloid-specific dyes. Left panel depicts cells without the stress treatment; right panel shows cells subjected to acidosis (pH 6.0 + 1% O_2) for 2 h. Cell nuclei are outlined with broken circles. Untreated or acidotic MCF-7 cells were stained with Amylo-Glo (blue), Thioflavin S (green), or Congo Red (red). Image reproduced from [35]. (B) A-bodies disappear after the removal of stress. MCF-7 cells were subjected to acidosis (pH 6.0 + 1% O_2) for 2 h then returned to standard conditions for the indicated times. Cells were stained with a dye that recognizes amyloids, Congo red, and Hoechst (insets). Bars, 20 μm. Image reproduced from [35].

histone deacetylases, and ubiquitin ligases, leading to cell-cycle arrest. Following removal of stress, A-bodies completely disappear and release their complement of proteins back into the nucleoplasm (Figure 4B). The rapid dissipation of A-bodies is driven by elements of the heat shock machinery, including Hsp70 and Hsp90, which are typically required to disaggregate amyloid fibrils [36,37]. Thus, it is clear that A-bodies resemble solid amyloids in their mature state. It will be important to conduct time-resolved analyses of A-body formation to see whether they undergo a liquid-to-solid transition.

Conclusions and Outlook

All of the biomolecular condensates we have discussed in this review have solid-like features in their mature form. Other condensates, like germ granules, nucleoli, FUS droplets, and PML bodies, have liquid-like properties in cells. There also exist condensates that exhibit intermediate behaviors: centrosomes are initially liquid like and then rapidly mature into gels. Thus, cells have developed organelles spanning a wide spectrum of material states, raising several questions. (i) Why do cells require both liquid-like and solid-like condensates? (ii) What is the physiological purpose of condensate maturation? (iii) How do cells disassemble the solid-like condensates when they are no longer needed?

Are the Material Properties of Biomolecular Condensates Tuned for Function?

A major question in the field of biological phase separation is how material properties of biomolecular condensates relate to their diverse functions. It is likely that cells employ liquid-like and bioreactive-gel-like condensates to concentrate RNAs, enzymes, and substrates to promote biochemical reactions. A condensed liquid phase or porous gel phase would allow diffusion of reactants and at the same time provide a crowded environment that raises the effective concentration of those reactants. Such an environment would enhance reactions that are transition-state limited or that depend on inefficient enzymes (i.e., high K_m) [38]. By contrast, cells are likely to use solid condensates to shut down reactions. Both A-bodies and Balbiani bodies have been proposed to promote dormancy. Many RNA-processing and cell-cycle-related proteins that are essential for cell proliferation, such as RNA helicase HAT1 and DNA polymerase catalytic subunit (POLA1), are sequestered in A-bodies upon stress [35]. Furthermore, the organelles in the Balbiani body are sequestered and immobile [19]. Such a structure could in theory limit the access of chemical reactants, perhaps by acting as an exclusive phase and decreasing enzymatic reaction rates. Indeed, mitochondria in Balbiani bodies have low membrane potential and are proposed to be in a dormant state [39]. A sequestration mechanism has also been shown to promote yeast sporulation: in response to starvation in diploids, Rim4 aggregates and sequesters mRNAs encoding cyclin B and other proteins, thus repressing their translation [29]. While the broad functions of bioreactive gels and functional amyloids are clear, how these functions are determined at the molecular level is an open question. A key physical difference between these condensates could be porosity, which would affect the diffusion of enzymes and reactants. Centrosomes and the nuclear pore exhibit nanometer-scale pore sizes, allowing the entry and diffusion of small molecules and even proteins (microtubule polymerase and mitotic kinase exchange between centrosomes and the cytoplasm, and proteins <5 nm readily pass through nuclear pores). However, Audas *et al*. demonstrated that five different proteins in A-bodies do not exchange with the surrounding nucleoplasm. Thus, it is possible that functional amyloids exhibit small pores that prevent the diffusion of protein substrates.

Material properties of biomolecular condensates may also be tuned for permanence, or lack thereof. *C. elegans* P granules, which are also liquid like, rapidly dissolve and condense within seconds; this process promotes their asymmetric segregation to the posterior end of the one-

cell embryo in response to polarity cues [2,40]. However, the non-dynamic condensates, as their name implies, are intended to persist for longer periods of time. Centrosomes assemble and disassemble only once each cell cycle and are most stable during mitosis, which can last for minutes (embryonic cells) up to 1 h (adult cells). Nuclear pores are established during interphase and stay intact until nuclear envelope breakdown just before mitosis, which can take ~20 h in human cells [15]. Balbiani bodies may be the most extreme case, as they remain intact for the entire life of a dormant oocyte, which can be up to 50 years in humans [24,25].

Is There a Physiological Role for Organelle Maturation?

The process of maturation refers to the dynamic arrest of phase-separated material, such as a biomolecular condensate. Unwanted maturation of biomolecular condensates has been proposed as a mechanism of pathological amyloid fiber formation in certain neurological diseases [28,41,42]. However, there could also be a physiological role for organelle hardening. We propose that non-dynamic biomolecular condensates form first through liquid–liquid phase separation, followed by maturation into a solid-like state. This could explain the biophysical properties of *C. elegans* centrosomes [12] and grPB granules, which are considered to be the nematode equivalent of Balbiani bodies. During long-term oocyte dormancy, grPB granules change from liquid-like droplets to solid square sheets in a manner regulated by RNA helicases [43]. In a similar fashion, the Balbiani body might form as a liquid droplet in a primordial germ cell cyst and solidify on oocyte formation. Balbiani bodies grow in size between early and late stage I of oogenesis, changing from 5–10 μm to 60 μm in diameter. A solid structure cannot easily maintain a spherical shape during growth. Thus, a parsimonious explanation is that Balbiani bodies form initially as liquid droplets with a spherical shape and then solidify upon expression or inhibition of a developmental protein or a major physiological change in the cytoplasm after the formation of the oocyte. Additional growth of the Balbiani body might be achieved by isotropic incorporation of new organelles. Such a liquid-to-solid phase transition may also explain why *in vivo* A-bodies and reconstituted FG-containing condensates are also spherical.

Many solid protein complexes, such as kinetochores or ribosomes, assemble piece by piece and remain solid throughout. Why would biomolecular condensates instead solidify through a liquid-to-solid phase transition? These structures are seemingly amorphous and would not require precise assembly as needed for a supramolecular machine like the ribosome. Phase separation followed by liquid-to-solid phase transition could even be advantageous. *In silico* modeling predicts that proteins that are flexible and have multivalent interactions with a binding partner can spontaneously form gels if present in sufficiently high concentrations [44]. Demixing of proteins through phase separation can typically occur at much lower protein concentrations, depending on protein–protein affinities. The result is a localized increase in protein concentration that makes gelation possible. Thus, phase separation followed by maturation would allow localized formation of gels even when the cytosolic concentration of a scaffolding protein is far below the critical gelation point.

Another potential advantage of this assembly mechanism is the temporal specification of function: a condensate could perform different roles in the liquid state versus the solid state. This especially makes sense for centrosomes. The PCM of a centrosome must concentrate tubulin and enzymes that aid the assembly of tubulin into microtubules. A liquid environment would favor microtubule nucleation and, importantly, polymerization such that the microtubule could extend beyond the PCM. Microtubules are stiff filaments, and if they were embedded deep within in a solid matrix their polymerization length would be approximately restricted to the pore size of the matrix. This is not the case *in vivo*, as electron microscopy clearly shows microtubules longer than 1 μm spanning the PCM in *C. elegans* [45]. Thus, during microtubule

nucleation and polymerization the PCM must internally rearrange like a viscous liquid. However, during mitotic spindle assembly and chromosome segregation the PCM must resist microtubule-mediated pulling forces. A solid-like matrix would be better suited for anchoring microtubules. We propose that the PCM is initially liquid-like to promote microtubule nucleation and escape and then becomes gel-like to resist pulling forces generated by the same microtubules. Maturation has indeed been observed in reconstituted PCM *in vitro*, but remains unknown whether the material state of the *in vivo* PCM changes.

How Are Stable Membraneless Organelles Disassembled?

Non-dynamic condensates rapidly mature into a stable state and, left on their own *in vitro*, would be likely to persist indefinitely. However, inside cells they are dissolved at specific times in a highly regulated manner. Centrosomes shed most of their PCM during mitotic exit only to rebuild it again in the next cell cycle, nuclear envelope breakdown triggers disassembly of the NPCs, and oocyte maturation triggers Balbiani body disassembly. What mechanisms drive the disassembly of these solid-like biomolecular condensates?

Much effort has been dedicated to the assembly of organelles while disassembly has been overlooked. However, efficient disassembly is often critical for organelle function and the survival of the organism. Such is the case for the mitotic spindle in budding yeast, where inhibition of spindle disassembly prevents spindle reassembly in the subsequent cell cycle [46]. Thus, it is unsurprising that yeast have employed at least three independent mechanisms to ensure full disassembly of the spindle and depolymerization of its constituent microtubules [47]. More to the point, centrosome disassembly is essential for female oocyte maturation in various organisms [48,49,50] and differentiation of cardiomyocytes in mammals [51]. It is unclear how failed disassembly of NPCs or Balbiani bodies affects cellular viability. The fact that their disassembly is stereotyped, rapid, and conserved across species indicates the importance of this process.

The principles that govern disassembly of biomolecular condensates are likely to depend on the unique material properties of each condensate. Dynamic condensates, such as P granules, FUS droplets, or nucleoli, will readily dissolve in response to changes in salt concentration or temperature or a decrease in protein concentration [3,27,28,30,31,40,52,53]. It is useful to represent the conditions that promote phase separation of biomolecular condensates with a phase diagram. Disassembly of dynamic condensates can be achieved simply by moving through the phase space of the diagram or by shifting of the phase boundary. This is not possible, however, for non-dynamic condensates in the solid-like state. For instance, aged SPD-5 condensates persist even when diluted far below the critical concentration needed for their assembly *in vitro* [12]. It is instead likely that active processes are needed to break apart the non-dynamic condensates. This is the case for A-bodies, whose disassembly depends on ATP-driven Hsp70 and Hsp90 chaperones. Other chaperones might also be involved, like the AAA+ ATP disaggregases (Hsp110 in mammals, Hsp104 in fungi), which are known to dissolve solid aggregates of protein [54,55].

Concluding Remarks

We have discussed how biomolecular condensates adopt different material states to achieve specific functions. To fully investigate these ideas, it will be first necessary to understand what kinds of molecular interactions drive the assembly of both dynamic and non-dynamic condensates. It was originally thought that intrinsically disordered regions and low-complexity sequences were necessary for liquidity. However, these motifs are found in liquid-like condensates (e.g., the QGSY-rich N terminus of FUS) and solid-like condensates (FG repeats in

Outstanding Questions

What determines the compositional specificity of non-dynamic condensates? What types of molecular interactions are involved?

How are the material properties of non-dynamic condensates determined and tuned?

Do Balbiani bodies, centrosomes, amyloid bodies, or nuclear pores ever exhibit liquid-like features *in vivo*?

How are non-dynamic condensates disassembled?

Nup tails). The presence of prion-like domains is also a poor indicator, as they are found in the main Balbiani scaffold protein Velo1 and also in RBPs that form liquid-like condensates. Low-complexity sequences and prion-like domains might simply drive interprotein interactions and the ultimate material state of the condensate is then determined by how these interactions are tuned by linker sequences. It is reasonable to assume that interaction strength correlates with the tendency to form stable complexes. Once the 'molecular grammar' rules have been uncovered, it will then be important to determine how modification of interprotein interactions affects the function of biomolecular condensates *in vivo*.

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