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Beyond Stereospecificity: Liquids and Mesoscale Organization of Cytoplasm

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The cytoplasm is not a homogenous solution but instead consists of large dynamic assemblies that arise from transient molecular interactions. Some of these structures have been shown to represent liquid droplets of concentrated protein and RNA. Liquid phase separation of cytoplasm may be a fundamental principle of cytoplasmic organization.

As biologists, we are generally interested in the following question: How does complexity arise from molecular interactions? The structures that we study, such as cells or tissues, are many orders of magnitude larger than the molecules that make them up. What are the rules by which individual molecules interact to make large structures of defined size and organization? One of the most commonly studied forms of molecular organization is that of protein complexes. Techniques for studying the structure and function of protein complexes are quite mature, involving, among others, X-ray crystallography, NMR, and (3D reconstruction by single particle) electron microscopy. All such techniques depend on the defined interactions between different proteins within complexes. Stereospecific interactions usually occur along defined protein faces, and their interaction can generally be disrupted through specific point mutations. These well-defined interactions have allowed us to understand these structures at below-nanometer scale.

While our image at the nanoscale sharpens, our understanding of the principles of structural organization on larger length scales remains hazy, even while ultimately it is the organization of these larger-length scales that interests us. Can we define unifying biophysical principles guiding the assembly of biomolecules into coherent, functional structures? Or must we be satisfied that large-scale structural features such as size and shape in some way emerge from the logic of complex interaction networks? The lack of an answer to these questions is apparent for the case of non-membrane-bound macromolecular assemblies found throughout the cytoplasm and nucleoplasm. These assemblies consist of large numbers of interacting macromolecular complexes and act as reaction centers, or storage compartments. Examples are RNA/protein bodies such as nucleoli, Cajal bodies (CBs), and germ granules. Other examples are structures such as kinetochores, centrosomes, or adhesion complexes. We have little idea how these compartments are organized. What are the rules that ensure that defined sets of proteins cluster in the same place in the cytoplasm? How does this clustering result in functional assemblies rather than, say, pathological aggregates such as amyloid plaques?

A central feature of these non-membrane-bound compartments is that they are very dynamic. For instance, if you label a γ-tubulin complex by tagging one of the components with GFP, allow GFP-tagged γ-tubulin complexes to incorporate in a centrosome, and then photobleach these complexes, they will exchange with cytoplasmic complexes within minutes. Thus, the γ-tubulin complexes have a short residency time in the centrosome. Similar fast turnover rates of complexes in compartments can be found throughout the cell. How do these remain as coherent structures of defined size and shape when their components completely turn over so quickly?

One way to think about non-membrane-bound compartments is as different liquid phases of cytoplasm. The concept of the cytoplasm as an emulsion of liquid protein phases actually has a long history: in the early 20th century, E.B. Wilson and other eminent biologists considered the cell densely packed with liquid "coacervates" (Figure 1) (Wilson, 1899). Alexander Oparin in the 1920s proposed that these structures represent the first reaction crucibles of life in the primordial soup. However, these ideas of liquid-like cytoplasmic states dropped out of favor with the molecular biology revolution, with its focus on crystals and stereospecific interactions between proteins.

The concept of liquid-like cytoplasmic states is an appealing way to think about mesoscale organization of the cytoplasm because many of the perplexing features of cytoplasmic compartments are also seen in liquid phases of nonliving materials; moreover, there is considerable understanding of the physical principles underlying liquid phases in nonliving systems (Poon, 2002). This work on nonliving systems underscores the importance of weak, long-ranged interactions in forming stable condensed liquid phases. Within cells, there are numerous potential sources of weak interactions, including hydrophobic attraction, hydrogen bonding, and electrostatic interactions. Another source is the depletion attraction, which nonspecifically induces an attraction in the presence of high concentrations of macromolecules. It is estimated that the fraction of the total cellular volume occupied by proteins is remarkably high, ~30% (Ellis, 2001), suggesting that the depletion attraction (also known as "macromolecular crowding") could play a significant role in structuring the cytoplasm by driving liquid phase separation. These numerous long-range weak interactions seen between macromolecules are ideal for forming liquid phases.

Anyone who has ever seen ice or snow is familiar with small molecules spontaneously generating structural order by phase

transitions, but the idea that proteins and other macromolecules can also undergo phase transitions may seem surprising at first glance. However, such phase transitions have been important for our detailed understanding of protein structure by X-ray scattering, which relies on the ability of concentrated protein solutions to undergo phase transitions to form protein crystals. Less well-known condensed liquid phases also readily form in concentrated protein solutions. Although X-ray crystallographers simply throw out liquid droplets that form in their solutions, protein liquids have important technological applications (Chilkoti et al., 2006), and condensation of protein liquid droplets may underlie lens opacification in cataracts (Annunziata et al., 2003). Thus, under certain conditions, liquid-phase protein droplets are known to form in concentrated protein solutions, both in vitro and in vivo.

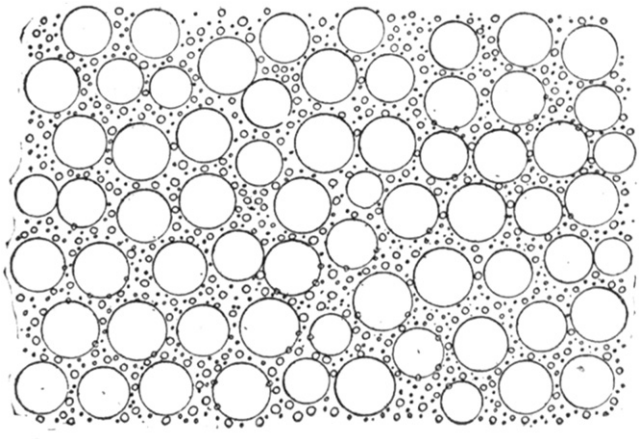

Figure 1. E.B. Wilson's Rendering of the Cytoplasm of a Starfish Egg, 1899

"…If the eggs of Ophiura be crushed by pressure on the cover-glass the protoplasm flows out, most of the alveolar spheres going in advance, while the granules and continuous substance lag behind. Meanwhile, the alveolar spheres often run together to form larger drops of all sizes, the origin of which is placed beyond question by their color … the granules are liquid in these forms also – a conclusion which I confess was a surprising result to me; for we are so accustomed from our studies on sections to regard the granules as solid bodies that we are apt to forget that sections show us only coagulated material." Adapted from Wilson, 1899.

The difference between a liquid and crystalline solid is that in a solid, atoms and molecules have well-defined relative positions, which makes it more straightforward to understand the structure of the solid. On the other hand, molecules within liquids are constantly undergoing internal rearrangement and do not have well-defined positions. One way of thinking of the difference between a liquid and a solid is to think of a school. When the kids are in a classroom, this is like a solid: their positions are predictable. When they are in the playground, this is like a liquid: they have a defined composition (e.g., they are all in the fourth grade), and they are in a boundary defined by the school premises, but you can never predict exactly where individual kids are, because their positions are constantly changing. The lack of reproducibility of molecular position in liquids means that the standard techniques we use in structural biology, such as crystallography, will not tell us the overall structural arrangement. We will have to think of new ways to assess such structures.

Recent evidence suggests that one class of structures, cytoplasmic bodies of RNA and protein, may indeed be liquid droplet phases. These are variously referred to as ribonucleoprotein particles (RNPs), RNA/protein bodies, or RNA granules. RNA/protein bodies represent an important class of structures in living cells, implicated in a variety of biological processes and diseases; they are typically involved in RNA processing and/or storage, for example, as found in CBs, processing bodies, germ granules, or stress granules. As with many other ribonucleoprotein assemblies, germ (P) granule components exist in a dynamic steady state with a pool of soluble components, suggesting that the constituent molecules have short residence times within the granules and that therefore the species must bind with relatively low affinity. Consistent with this, P granules were found to exhibit classic liquid-like behaviors, dripping under applied shear stress, fusing with one another upon contact, and "wetting" the nuclear membrane—much like water droplets on a windshield (Brangwynne et al., 2009). These behaviors were used to extract their apparent viscosity and surface tension, which are similar to values found in other macromolecular liquids formed from weakly attractive components, such as those formed from colloidal particles.

Most of the more than three dozen known proteins within P granules have RNA binding domains (Updike and Strome, 2010); generally, these domains are characterized by modularity, promiscuous binding (nonspecificity in the recognized RNA sequence), and low binding affinity (Lunde et al., 2007). These weakly "sticky" features of P granule components, and the liquid droplet behavior that likely results from these features, may thus be common to many other RNA/protein bodies within the cell. Indeed, recent work has shown that another protein/RNA body, the nucleolus, also exhibits liquid-like properties that appear to depend on ATPase activity (Brangwynne et al., 2011). In addition to multiple low-affinity RNA-protein interactions in RNA/protein liquid droplets, dynamic multivalent binding may occur with relatively disordered protein-protein interactions; such "allovalency" has been suggested to lead to the assembly of protein complexes that underlie cooperative effects important for cell cycle control (Klein et al., 2003).

The concept of protein bodies as liquid-phase droplets may thus be applicable to a wide range of intracellular structures. One feature important for recognizing liquid-phase droplets is their shape. There is usually an energetic cost associated with the interface between two materials, characterized by the surface tension at the interface. If a liquid droplet is deformed, surface tension quickly drives the material back into the spherical shape, which minimizes the interfacial area. For the same reason, two droplets that come into contact and begin fusing are also driven by surface tension into a single larger sphere. This property of liquids appears to underlie the observation that most protein/RNA bodies are surprisingly spherical, even though they are not membrane-bound; for instance, for many years CBs were referred to as "spheres," due to their geometric shape

and remarkably smooth contour. On the other hand, nonspherical bodies (e.g., focal adhesion complexes) may also possess liquid properties, but additional factors such as force-generating motor proteins influence their structural shape and organization; for example, the complex architecture of chromatin appears to deform nucleoli in the somatic nucleus, while the onset of large pulling forces during anaphase deforms initially spherical centrosomes into elongated ellipsoidal shapes.

Another defining feature of liquid-like bodies is that the molecular components must rearrange relatively quickly to facilitate the structural relaxations that give rise to liquid flow behavior (e.g., during fusion events). Thus, any body with liquid-like behavior will undergo rapid reorganization of its internal contents, which can be seen by photo bleaching. However, it is important to realize that whether materials will exhibit liquid- or solid-like dynamic behaviors depends on time scales. For instance, glass is structurally disordered like a liquid, but only exhibits significant flow over very long timescales. Thus, considering the timescale of a particular biological process is key to the question of whether biomolecular assemblies will exhibit dynamic, liquid-like features relevant to that process.

The concept of liquid-phase droplets may help us understand how cells assemble distinct intracellular microcompartments that are structurally indistinguishable from the rest of the liquid cytoplasm. Many non-membrane-bound protein bodies are not visible using techniques that depend on optical density changes, such as phase contrast microscopy. Rather, these bodies are defined by an increased local concentration of certain proteins; however, it is important to realize that the total concentration of protein may be comparable to that in surrounding cytoplasm (Handwerger et al., 2005), emphasizing the fact that these bodies represent a kind of cytoplasmic phase separation (demixing), rather than simply an aggregation process. This locally increased concentration of specific reactants will increase reaction kinetics by overcoming reaction energetic barriers. Concentrated reactants within a small-volume liquid droplet would also ensure that enzymes and reactants could rapidly diffuse around the liquid phase, thus increasing the speed of any reaction. Because of exchange between the droplet and the cytoplasm, reactants can be turned over within the liquid droplet, allowing constant sampling of the cytoplasm.

The physical picture that emerges from these studies is of a cytoplasm, or nucleoplasm in the case of nuclear bodies such as the nucleolus, that represents complex emulsions of dynamic liquid-phase droplets of RNA and protein. Beyond protein/RNA assemblies, there are many different compartments that form transiently in the cell that are very dynamic and have no obvious repeated structure. Besides the examples mentioned here, there are compartments such as those that form at the end of double-stranded breaks or those that form at telomere ends. We don't know the rules by which these compartments are put together, but it is tempting to speculate that condensation of liquid-like protein phases could be a general conceptual framework for understanding the formation of such compartments. By nucleation of certain factors around specific sites (e.g., double-stranded breaks), or by changing the interaction strengths of weakly interacting molecules locally, it is not hard to see how such phase transitions could be induced. Such local changes could be induced by, among other things, locally activated kinase activity, or gradients of RNA-binding molecules.

In conclusion, the dynamic organization of the cytoplasm by partitioning molecules into liquid-phase microcompartments represents a powerful mechanism for the spatiotemporal control of subcellular structure and function, for several reasons. First, simple rules can define these complex rearrangements. As an example, simple alcohols can transition between phases in a water-chloroform mixture because of the polar nature of the -OH group on the alcohol molecule. Similarly, simple rules governing the interactions of biological materials could define whether proteins transition into different phases within the cytoplasm. Second, the cell has the problem of how to create localized reaction centers whose assembly and disassembly must be dynamically controlled in space and time. A phase transition can promote a reaction by automatically concentrating the components, while the tendency for dynamic assembly and disassembly of reaction centers can be tuned by spatiotemporal control of regulatory molecules within the cell. Finally, there may be implications for fluidity of compartments and metabolic changes that take place during aging. Work on nucleoli has shown that the fluidity is dependent on the presence of ATP. It seems likely that a drop in metabolic activity and ATP availability as cells age could lead to changes in fluidity of such compartments, potentially leading to pathological states.

More generally speaking, phase transitions in the cytoplasm seem to represent a three-dimensional protein/RNA analog of the two-dimensional demixing phase transitions seen in lipid bilayers, which has been a powerful way to think about organization of membranes. Thus, this may be a general conceptual framework for subcellular organization. The important directions in the future will be to link these physical concepts to the underlying chemistry of the systems, especially in understanding the rules of molecular interaction that drive the formation of different phases, exactly as structural biology has done for macromolecular complexes. The techniques will be different, but the ultimate goal is the same: to understand the organization of complex structures from the interaction of their molecular components.

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