The liquid drop nature of nucleoli

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Nucleoli are prominent subnuclear organelles, and are known to be hubs of ribosome synthesis. A recent study of Brangwynne et al. reports that the nucleoli of Xenopus oocytes display "liquid drop" behavior, suggesting that nucleolar structure may be driven by rather simple physical principles.

Living cells have a high degree of spatial ordering of their contents. Eukaryote cells contain a prominent nucleus bounded by a polymerized shell of lamins and a double lipid bilayer, microtubules organized by a localized organizing complex, and a cyto-skeleton of polymerized actin and other protein filaments. The relation between structure and function of cellular components can often be understood in simple physical terms: for example, the nuclear envelope provides a sturdy barrier between nucleoplasm and cytoplasm, but a necessarily permeable one, with large nuclear pores to facilitate the selective transfer of macromolecules across it.

Many supramolecular objects in the cell have "a structure," in that the macromolecules within them are stuck together in some specific way that can be understood in terms of stereochemical interactions: again, microtubules and other cytoskeletal filaments provide prototypical examples. We can think of such objects as "solids," and indeed, the functions of the nuclear lamina and microtubules rely on their defined shapes and their mechanical stiffness,[1,2] the property by which they resist shear forces.

However, being a solid is not the only way to be organized. Indeed, eukaryote cells contain objects with well-defined geometries which nevertheless have the character of liquids. An important example is provided by cell membranes. Membranes are liquids[3] in the sense that the phospholipids within them are mobile and are able to diffuse throughout the two-dimensional extent of at least some portion of a membrane leaflet. The liquid mobility in the bilayer permits lipid membranes to act as transport conduits, allowing membrane-bound proteins to travel from place to place in the membrane and also allows membranes to change their topology (with some help from membrane-budding and membrane-fusing enzymes), facilitating the sending and receiving of vesicle-encapsulated cargos.

A new example of a cell component with liquid-like organization was reported by Brangwynne et al.,[4] who performed a series of experiments which indicate that the nucleoli in *Xenopus laevis* oocytes behave as liquid droplets. Nucleoli are easily observed in the nuclei of most eukaryote cells and in metazoan cells appear as roughly spherical subnuclear organelles. They are ribosome "factories," incorporating the rDNA genes and their transcripts together with a large variety of other RNAs and proteins connected with ribosome subunit production.[5,6] As prominent as they are during interphase, nucleoli dissolve as cells proceed through prophase chromosome condensation. Then, following cell division, nucleoli reassemble onto the chromosomal "nucleolar organizer regions" (NORs) containing tandem repeats of rDNA genes. A key point is that just after cell division, the nucleoli are smaller and more numerous than at the end of G2, suggesting that they might gradually combine together as the cell cycle proceeds.

Brangwynne et al. were motivated to try to understand the basic physical properties of nucleoli, so they chose as a model system the Xenopus oocyte, which in addition to being a huge cell with a giant nucleus (the "germinal vesicle" or GV), has roughly a thousand nucleoli, organized around thousands of duplicated, extrachromosomal rDNA gene loci. This army of nucleoli is necessary to provide the large number of ribosomes needed by the egg for its voyage into the creation of an embryo.

With a thousand nucleoli per cell, one can hope to collect some statistics, and Brangwynne et al. did so, finding that the nearly spherical nucleoli had a rather broad distribution of volumes, showing a power-law distribution, $P(V) \propto V^{-3/2}$, consistent with distributions found for colloidal aggregation processes.[7] The same power-law distribution was obtained by Brangwynne et al. in their own computer simulations of droplet aggregation.

Furthermore, Brangwynnet et al. directly observed volume-conserving fusion events for nucleoli in isolated GVs. The fusion dynamics are consistent with the behavior of viscous liquid droplets shaped by surface tension. Combining the measurements of the nucleolar fusion dynamics with a theoretical estimate of the surface tension allowed Brangwynne et al. to conclude that the liquid interior of nucleoli has a viscosity roughly one million times larger than water. Further experiments showed that depletion of ATP from the GV using apyrase led to slower fusion kinetics, suggesting that the rearrangement of the contents of nucleoli is at least in part dependent on an ATP-dependent process.

All of this leads to an intriguing model of nucleolar organization, whereby nucleoli are organized around rRNA genes, by clustering of the attendant RNA and protein molecules but with a surrounding and surprisingly amorphous structural organization driven by the relatively weak association of those molecules. According to this model, the spherical nucleolar shape follows as the result of minimization of surface area at fixed volume. Thus, as Brangwynne and colleagues propose, the nucleolus behaves as a liquid droplet of nucleolar components, with its contents continuously agitated by enzymes dependent on energy-rich nucleotide

triphosphates, perhaps transcription or RNA helicase activity. As a cell goes through the cell cycle, the nucleoli tend to aggregate together and grow in size. Then when the chromosomes condense, the nucleoli dissolve. Following cell division, nucleolar components reassemble, by nucleating onto the new NORs.

This simple idea—that nucleoli are organized by aggregation and phase separation of their components from the surrounding cytoplasm onto a site of "nucleation"—rests on observations in the Xenopus GV. Given that chromatin organization might affect the liquid-drop behavior,[4] it would be very interesting to extend these experiments to somatic cell nuclei. An additional constraint on nucleoli might come from nucleoskeletal filaments; experiments of Brangwynne et al. where GVs were treated with the actin-polymerization-inhibiting drug cytochalasin-D showed "perturbed" fusion dynamics.[4]

Brangwynne et al. note that nucleoli do have internal structure,[6] with "cores" rich in fibrillarin, known to associate with newly transcribed rRNAs. In addition, the amplified, extra-chromosomal nucleoli they studied have a surface "coating" of the protein NO145, which Brangwynne et al. suggest might act as a surfactant, forming a layer between the nucleolar contents and the nucleoplasm. Conceivably, perturbation of NO145 might change the nucleolar surface tension and therefore the driving force and the rate of the fusion dynamics. Somatic cell nucleoli do not have such a proteinaceous coat, which is to be borne in mind.

A small loose end is the lack of a direct determination of the nucleolar surface tension. One way to remedy this might be to measure the force needed to statically deform a nucleolus from a spherical to an ellipsoidal shape (which would cost surface energy due to the increased surface area). Such a measurement would complete the physical characterization of the basic liquid drop phenomenology of nucleoli. It is worth noting that force measurements can provide crucial information about energetics which cannot be obtained from observations involving length, shape and rate measurements alone.

Brangwynne et al. note that germ-line P granules have also been shown to behave

like liquid droplets.[8] This, plus the fact that other RNA-protein nuclear suborganelles are highly dynamic, suggests that this general type of "emulsion droplet" mode of self-organization of organelles might be fairly common. One is reminded of the rather general tendency for phase separation to occur in mixtures of molecules or particles, via a variety of types of nonspecific interactions.[9] Phase separation effects become stronger for larger macromolecules[10] or colloidal particles (colloids are often stabilized by adsorption of macromolecules to the colloidal particle surfaces which act as a barrier to collisions of particles[11]). A second very general mechanism for driving phase separation is purely entropic in origin—for a mixture of two species of particles of dissimilar size, phase separation can occur solely due to increased entropy of the separated phases relative to a mixture.[12]

A third mechanism that may drive formation of aggregates of proteins and other molecules which are participants in a sequence of biochemical processing steps is localization mediated by the reactions themselves. A simple example of this is coupled transcription and translation in prokaryotes whereby translation of an mRNA begins while it is still in the process of being transcribed.[13] The resulting physical connection might coordinate the relative positions of gene, RNA polymerase, mRNA, and even the destination of the folded protein. This type of effect could also spatially organize molecules which are not physically connected together: for example, rapid production of RNAs will build up a higher concentration of the transcripts near to the gene; enzymes that bind to those transcripts will therefore also be concentrated near the gene, even if they have no direct interaction with the gene involved. The degree to which this localization occurs will depend on the diffusion rates of the transcripts and the reaction rates of the enzymes binding them and on the tendency for the resulting collection of molecules to form a "phase separated" droplet. This dynamic effect could drive the formation of droplet-like aggregates of enzymes and substrates into "factories" that accomplish a sequence of molecular

processing steps. All these basic physical effects favor phase separation, and may play roles in stabilizing aggregates of macromolecules that tend to associate together. Notably, this association might be nucleated by a chromosomal locus (as in the case of nucleoli) or by RNAs (as appears to the be the case for a number of other nuclear bodies[14]).

The study of Brangwynne et al. provides an illuminating example of how a physical analysis can lead to insights into self-assembly of a cell organelle, in this case showing how surface tension, viscosity and fusion dynamics select the size and number of nucleoli. Integrating this type of "physical biology" approach with biochemical and genetic perturbations can provide novel strategies for analyzing organization and function of a wide variety of cell structures and processes.

References

1. Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, et al. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. J Clin Invest 2004; 113:370-8; PMID:14755334

2. Gittes F, Mickey B, Nettleton J, Howard J. Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. J Cell Biol 1993; 120:923-34; PMID:8432732; http://dx.doi.org/10.1083/jcb.120.4.923

3. Simons K, Sampaio JL. Membrane organization and lipid rafts. Cold Spring Harb Perspect Biol 2011; 3:a004697; PMID:21628426; http://dx.doi.org/10.1101/cshperspect.a004697

4. Brangwynne CP, Mitchison TJ, Hyman AA. Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes. Proc Natl Acad Sci USA 2011; 108:4334-9; PMID:21368180; http://dx.doi.org/10.1073/pnas.1017150108

5. Hernandez-Verdun D, Roussel P, Thiry M, Sirri V, Lafontaine DL. The nucleolus: structure/function relationship in RNA metabolism. Wiley Interdiscip Rev RNA 2010; 1:415-31; PMID:21956940; http://dx.doi.org/10.1002/wrna.39

6. Nemeth A, Langst G. Genome organization in and around the nucleolus. Trends Genet 2011; 27:149-56; PMID:21295884; http://dx.doi.org/10.1016/j.tig.2011.01.002

7. Weitz DA, Lin MY. Dynamic scaling of cluster-mass distributions in kinetic colloid aggregation. Phys Rev Lett 1986; 57:2037-40; PMID:10033616; http://dx.doi.org/10.1103/PhysRevLett.57.2037

8. Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, et al. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 2009; 324:1729-32; PMID:19460965; http://dx.doi.org/10.1126/science.1172046

9. Israelachvili JN. Intermolecular and Surface Forces. San Diego: Academic, 1992.

10. de Gennes PG. Scaling concepts in polymer physics. Ithaca: Cornell, 1979.

11. Tadros T. Polymeric surfactants in disperse systems. Adv Colloid Interface Sci 2009; 147-148:281-99; PMID:19041086; http://dx.doi.org/10.1016/j.cis.2008.10.005

12. Vanduijneveldt JS, Heinen AW, Lekkerkerker HNW. Phase-Separation in Bimodal Dispersions of Sterically Stabilized Silica Particles. Europhys Lett 1993; 21:369-74; http://dx.doi.org/10.1209/0295-5075/21/3/019

13. English BP, Hauryliuk V, Sanamrad A, Tankov S, Dekker NH, Elf J. Single-molecule investigations of the stringent response machinery in living bacterial cells. Proc Natl Acad Sci USA 2011; 108:E365-73; PMID:21730169; http://dx.doi.org/10.1073/pnas.1102255108

14. Shevtsov SP, Dundr M. Nucleation of nuclear bodies by RNA. Nat Cell Biol 2011; 13:167-73; PMID:21240286; http://dx.doi.org/10.1038/ncb2157