

# Phase transitions and size scaling of membrane-less organelles

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The coordinated growth of cells and their organelles is a fundamental and poorly understood problem, with implications for processes ranging from embryonic development to oncogenesis. Recent experiments have shed light on the cell size–dependent assembly of membrane-less cytoplasmic and nucleoplasmic structures, including ribonucleoprotein (RNP) granules and other intracellular bodies. Many of these structures behave as condensed liquid-like phases of the cytoplasm/nucleoplasm. The phase transitions that appear to govern their assembly exhibit an intrinsic dependence on cell size, and may explain the size scaling reported for a number of structures. This size scaling could, in turn, play a role in cell growth and size control.

## Cell size, and size-dependent function

Cell growth and size control are critical aspects of a wide range of physiological and pathological processes, including embryonic development, wound healing, and cancer. Cell growth is usually coupled to division, such that cells reach sizes typically no greater than  $\sim 10\ \mu\text{m}$ . However, there are numerous examples of cells of larger sizes, both prokaryotic and eukaryotic. These include the 750- $\mu\text{m}$ -diameter sulfur bacteria *Thiomargarita namabiensis* (Schulz and Jorgensen, 2001), to meter-long neurons of an adult human (Marshall et al., 2012). Moreover, during development, cell growth and division are often uncoupled, leading to changes in cell volume spanning several orders of magnitude within the same organism (Marshall et al., 2012). For example, before fertilization, many oocytes undergo significant growth without division, leading to mature oocytes with sizes reaching 1–2 mm or more (Costello and Henley, 1971). Subsequently, during early embryogenesis, cells undergo rounds of division without growth, leading to increasingly smaller cells.

It is remarkable that cell size can vary by several orders of magnitude without adversely affecting cell function. Indeed, the

enormous variation in the spatial extent of cells should strongly impact processes occurring within the cytoplasm. For example, the rate of diffusive transport is intrinsically size-dependent, suggesting that the efficiency of biological processes such as lysosomal degradation, protein translation, or RNA metabolism may vary in cells of different size. Moreover, many structures that mediate intracellular processes, such as mitochondria, flagella, and the nucleus, are frequently observed to be larger in large cells, and smaller in small cells, a phenomenon known as “size scaling” (Chan and Marshall, 2012). Finally, the cytoplasm/nucleoplasm itself is not a uniform solution of soluble molecules, but is a complex fluid consisting of membrane-less assemblies of RNA and protein. Here we discuss an increasing body of evidence which suggests that the “mesoscale” organization of this fluid is also cell-size dependent, underscoring the potentially significant impact of organelle size scaling on biological function.

## Phases of the cytoplasm/nucleoplasm

The high degree of microscale organization inherent in the cytoplasmic and nucleoplasmic fluid has become increasingly apparent from the study of a class of membrane-less cytoplasmic organelles. These structures are typically comprised of both RNA and protein, and are known as ribonucleoprotein (RNP) granules/bodies, or more recently RNP droplets. Examples include germ (P) granules (Brangwynne et al., 2009), processing bodies (Decker et al., 2007), stress granules (Wippich et al., 2013), Cajal bodies (CBs; Strzelecka et al., 2010), and nucleoli (Shav-Tal et al., 2005). Other macromolecular assemblies such as the purinosome (An et al., 2008), or centrosome (Decker et al., 2011), are comparable structures in that they appear to function in controlling the rate of intracellular reactions by colocalizing molecules at high concentrations within a small cellular micro-domain. These structures are highly dynamic, with components in constant flux with the surrounding nucleoplasm or cytoplasm (Phair and Misteli, 2000; Pederson, 2001). Moreover, studies using fluorescently tagged dextrans or optical interferometry have found that such structures are typically only slightly more dense than the rest of the nucleoplasm or cytoplasm (Handwerker et al., 2005; Updike et al., 2011). RNP

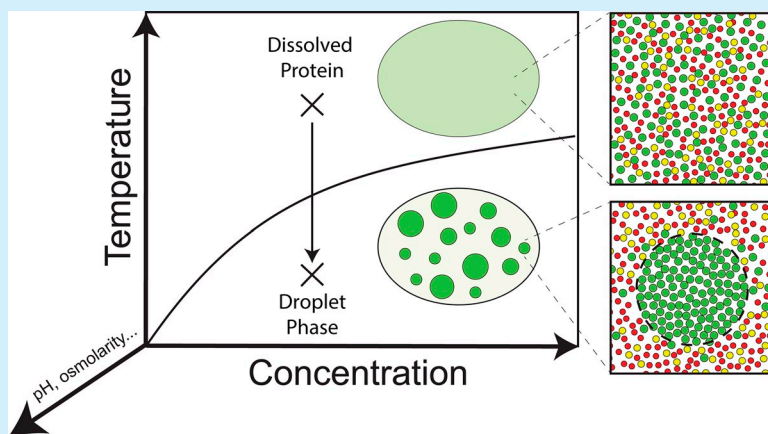
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Abbreviation used in this paper: CB, Cajal body.

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## Box 1. Phase transitions

Phase transitions are a well-defined and important concept in physics (Goldenfeld, 1992). Broadly, a “phase” of matter is a type of configuration of large collections of molecules, and phase transitions are the changes that occur as the molecules transition from one configuration to another. The most well-known phase transitions are those involving water, which can form dilute vapor, condensed water, or condensed solid (ice) phases. However, proteins, lipids, and other macromolecules also undergo phase transitions (Asherie, 2004; Vekilov, 2010). Liquid droplet phases are commonly seen in concentrated protein solutions in vitro (Asherie, 2004), and their assembly represents the same physics underlying the condensation of dew droplets from concentrated water vapor.

For the aqueous phase transitions common from everyday experience, we typically think about temperature as the controlling variable; for example, ice forms on cold winter days and water evaporates in a hot pan. Such phase transitions may be represented graphically by a phase diagram, in which the different regions of the diagram indicate the distinct phase(s) which are favored under given conditions, such as temperature and molecular concentration. A schematic phase diagram is shown here, with two regions: one representing a single phase of soluble molecules (upper region), and one representing a two-phase coexistence of droplets and soluble molecules (lower region). Although the phase diagram typically shows just two parameters, this is only a slice of a multi-dimensional phase diagram that can include other parameters such as pH or osmolarity.



granules are thus biophysically similar to the rest of the intracellular fluid, and yet appear to represent a different “state” of cytoplasm, comprised of a locally distinct molecular ensemble.

These biophysical studies are consistent with the emerging concept that phase transitions play a key role in structuring the cytoplasm/nucleoplasm (see Box 1; Walter and Brooks, 1995; Brangwynne, 2011; Weber and Brangwynne, 2012). Indeed, within living cells, an increasing number of membrane-less intracellular structures behave like liquid-droplet phases of the cytoplasm/nucleoplasm (Fig. 1; Brangwynne et al., 2009, 2011; Li et al., 2012; Aggarwal et al., 2013; Feric and Brangwynne, 2013; Wippich et al., 2013). Multi-domain proteins, and/or intrinsically disordered, low complexity sequence domains appear to play a central role in the phase transitions underlying cytoplasmic droplets, as well as amyloid-like fibers and gels (Ader et al., 2010; Kato et al., 2012; Li et al., 2012; Weber and Brangwynne, 2012; Aggarwal et al., 2013). These weak, repetitive interaction domains facilitate the formation of a coherent structure in the absence of a membrane, while still enabling a fluid-like micro-environment similar to that within conventional membrane-bound organelles.

## Phase transitions, concentration, and container size

For intracellular phase transitions, protein and/or RNA concentration are particularly important variables. For example, in vitro protein droplets only condense from soluble RNA and protein above a certain concentration threshold (Li et al., 2012), while dynamic amyloid-like fibers appear to assemble only at relatively high protein concentrations (Kato et al., 2012). The concentration dependence of phase transitions has other, biologically important consequences. For example, when a droplet condenses in a container of finite size, the droplet grows until the surrounding molecular concentration is depleted, establishing an equilibrium with the dilute phase at a given droplet volume fraction. The total droplet volume thus depends on the size of the container (and the precise location within the phase diagram), which is a generic feature of such phase transitions. Indeed, studies that use microfluidics to study protein crystallization find that the size of the microfluidic drop controls the size of the crystal (e.g., longest special dimension) that forms within it (Yamaguchi et al., 2013). Interestingly, container size can play a role not only in the final structure, but also in the kinetics of its assembly: the characteristic time required to nucleate

amyloid fibers depends on microfluidic drop size (Knowles et al., 2011).

Intracellular phase transitions also occur within a container of finite size: the cell. The resulting condensed phases should thus exhibit a cell-size dependence. Consistent with this, P granules in *Caenorhabditis elegans* embryos condense from soluble components into droplet phases that cease growing once the concentration of soluble components is depleted (Brangwynne et al., 2009; Lee et al., 2013). As a result, larger cells should contain larger/more numerous RNP droplets. However, this assumes that the concentration of molecular components is maintained. This may be the case in embryonic systems, which undergo successive rounds of cell divisions by partitioning the same cytoplasm. But we note that for non-embryonic cells whose size increases during growth, this may not necessarily be the case. If a cell were to grow by increasing its volume without producing new RNA/protein components of the droplets, the total concentration of these components would decrease during cell growth. This would ultimately cause the concentration to fall below the phase transition threshold, and the droplets to dissolve into their components (Fig. 2 A).

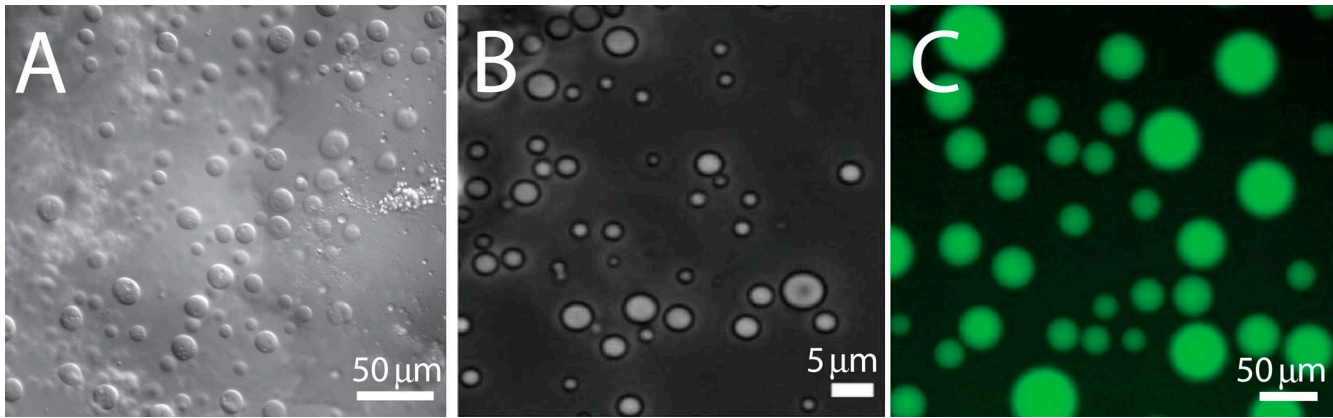


Figure 1. **RNA/protein droplets.** (A) Nucleoli (and other RNP droplets) within the nucleus of an *X. laevis* oocyte. Adapted from Brangwynne et al. (2011). (B) In vitro droplets formed from myelin basic protein (MBP). Adapted from Aggarwal et al. (2013). (C) In vitro droplets of fluorescently labeled multi-domain SH3<sub>4</sub>/PRM<sub>4</sub> proteins. Adapted with permission from Macmillan Publishers Ltd.: *Nature* (Li et al., 2012).

### Nucleation, growth, and size scaling of RNP droplets

Phase transitions could thus provide a simple mechanism by which the total volume of intracellular organelles could scale with cell size, given a constant concentration of components (Fig. 2 B). This will equate to size scaling if the number of organelles is fixed, for example by controlling the nucleation of droplets. Many RNP assemblies can indeed be nucleated by RNA, suggesting RNP nucleation can be tightly regulated (Shevtsov and Dundr, 2011). This appears to be the case for the nucleolus, a structure nucleated at the repeated ribosomal DNA (rDNA) genes; the size of the nucleolus has been reported to scale with cell size in human sensory ganglia neurons (Fig. 3; Berciano et al., 2007), although the biophysical origin and generality of this scaling are unclear. This is particularly interesting given the function of the nucleolus in ribosome biogenesis, and consequent role in cell growth and size control (Jorgensen et al.,

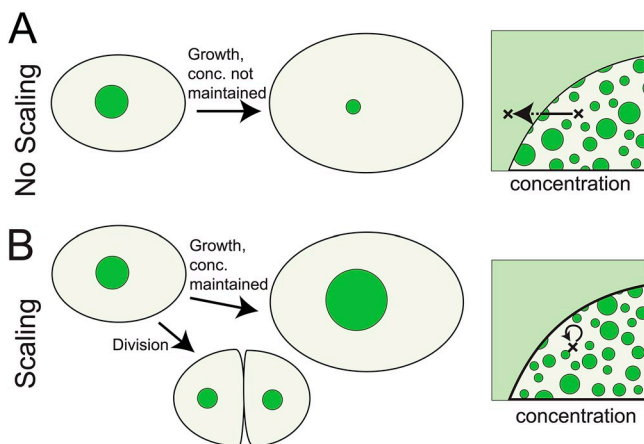


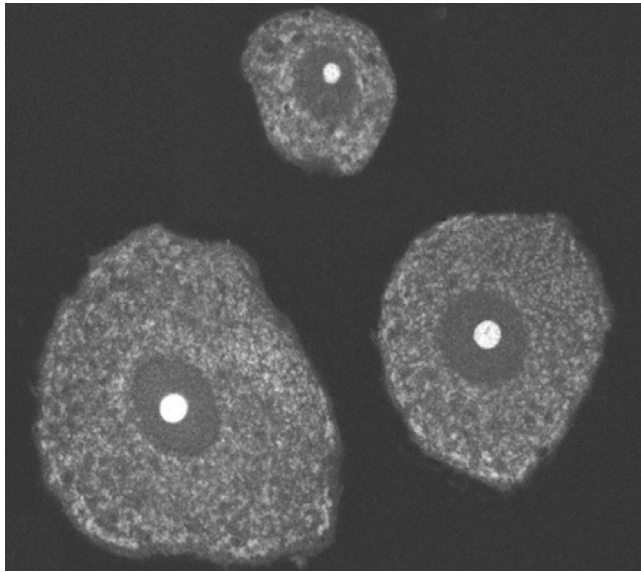
Figure 2. **Phase transitions and size scaling.** (A) Growth of a cell without the production of droplet component(s) will lead to decreased total component concentration (conc.), shifting the location within the phase diagram. In this case, a single nucleated droplet would dissolve (no scaling). (B) In a situation where cell size changes either through growth with a constant total component concentration or where cell size changes through division, the location in the phase diagram will not change, and the size of a single nucleated droplet will scale with cell size.

2002; Pederson, 2011). However, even for structures whose nucleation is not tightly controlled, there is a thermodynamic cost of having an interface (defined by its surface tension), which favors a single large droplet, rather than many small ones. Surface tension could thus lead to coarsening of condensed phases in a process known as Ostwald ripening (Voorhees, 1992).

Size scaling was recently shown for a novel inducible subnuclear organelle, which appears to represent an RNP droplet (Singer and Gall, 2011). Upon placing living *Drosophila* oocytes between two coverslips, applying a light pressure to gently compress the oocyte nucleus led to the spontaneous de novo assembly of subnuclear organelles, although it is unknown whether they are related to native subnuclear structures such as CBs or nucleoli. They have a highly spherical appearance and were observed to undergo fusion events as they condense out of the nucleoplasm over a timescale of several minutes, similar to other RNP droplets such as P granules and nucleoli (Brangwynne et al., 2009, 2011). This experiment was performed in cells at different stages of oogenesis, and the amount of material that condensed out of the nucleoplasm, and droplet size, roughly scaled with cell size. A similar behavior has recently been described in a microfluidic system in which a condensed phase of cytoplasm (“coacervate”) was found to condense from a cell lysate upon increased salt concentration (Sokolova et al., 2013). As expected for a phase transition, the volume of this cytoplasmic condensate scaled with the microfluidic droplet volume.

### Centrosomes, spindles, and other structures

Centrosomes are membrane-less droplet-like assemblies that are nucleated at centrioles in a tightly controlled process important for proper mitotic spindle assembly (Nigg and Stearns, 2011). Spindles tend to be smaller in smaller cells, and have been shown to become smaller as cell size decreases with each round of *C. elegans* embryonic division (Decker et al., 2011). Moreover, when the size of embryos was decreased, the size of centrosomes also decreased. This size scaling behavior has been interpreted in terms of a “limiting component” hypothesis, in which a particular structural component is hypothesized to be



**Figure 3. Cell growth and RNP size scaling.** In this image, dorsal root ganglia neurons of different size are stained with propidium iodide to visualize a single large nucleolus (white spot) within each cell nucleus. The size of nuclei and nucleoli increases with cell size. Adapted from Berciano et al. (2007) with permission from Elsevier.

present in limiting amounts, and is therefore depleted as the structure assembles (Goehring and Hyman, 2012). But it is important to recognize that if an intracellular phase transition underlies this process, centrosome assembly must be “component limited” because such condensed phases continue growing until components are depleted to levels defined by the phase diagram. Moreover, numerous components could simultaneously be “limiting,” possibly explaining why, in the case of the centrosome, identification of a single limiting component has been elusive.

The biological importance of centrosome size scaling relates to their function in nucleating and growing spindle microtubules. If the mitotic spindle were a structure of fixed size, it would be difficult to coordinate the precise segregation of chromosomes in different-sized cells. Greenan et al. (2010) showed that larger centrosomes assemble larger spindles by controlling spindle microtubule dynamics. The cell may thus build upon the relatively simple mechanism of phase transition–mediated cell-size dependence of centrosome assembly, to build a more complex structure, the spindle, of the correct size. Interestingly, however, two recent papers used a microfluidics approach to show that the size of *Xenopus* spindles directly scaled with the volume of the cytoplasmic extract droplet, with tubulin as a limiting component (Good et al., 2013; Hazel et al., 2013); the spindle itself could thus represent a (liquid crystal-like) phase of the cytoplasm (Reber et al., 2013).

A number of membrane-bound intracellular structures also exhibit scaling of their size with cell size. Recently, the intracellular mitochondrial network was shown to scale with cell size in yeast (Rafelski et al., 2012), which could be important for meeting the increased metabolic requirements of larger cells. The cell nucleus has long been known to exhibit size scaling (Jorgensen et al., 2007), with work in *Xenopus* implicating nuclear transport factors in size regulation (Levy and Heald,

2010); several excellent reviews focusing on scaling in these and other membrane-bound structures are available (Chan and Marshall, 2012; Goehring and Hyman, 2012; Levy and Heald, 2012). Generally, these structures appear to be more complex than RNP droplets, and their scaling may involve correspondingly more complex mechanisms of assembly. However, it is possible that in some cases, as may be the case for the spindle, the inherent cell size dependence of phase transitions provides the base mechanism for size scaling.

### Droplet size and function

It is thus clear that the assembly of cytoplasmic/nucleoplasmic droplets and other intracellular structures is often sensitive to the size of the cell, and that the intrinsic concentration dependence of phase transitions provides a biophysical mechanism for coordinating the growth of intracellular structures with the growth of the cell. The size of some structures must scale with the size of the cell in order for proper functioning, as with spindle scaling for proper mitotic segregation in different-sized cells. For RNP droplets functioning in intracellular RNA processing or function, it remains an open question how well these droplets accomplish their biological functions when they are assembled at different sizes.

In many cases, RNP droplets appear to function as liquid-phase micro-reactors, concentrating RNA/protein components and accelerating cytoplasmic reactions. They thus could play roles similar to the higher order structured signaling scaffolds (Wu, 2013) and metabolons that function to “channel” multi-step enzyme reactions (Jørgensen et al., 2005). However, there are few examples of direct *in vivo* measurements demonstrating the ability of droplets to perform such functions. *In vitro*, an aqueous two-phase PEG/dextran system recently demonstrated that RNA concentration within biomimetic droplets can lead to a 70-fold increase in the rate of ribozyme cleavage (Strulson et al., 2012). This increase depended on the fraction of droplet phase present, although the actual droplet size dependence was not investigated. Peptide–nucleotide droplets can exhibit analogous acceleration of enzymatic activity (Koga et al., 2011), whereas N-WASP–stimulated assembly of actin by the Arp2/3 complex can be accelerated nearly fivefold within multivalent protein droplets assembled *in vitro* (Li et al., 2012). Cytoplasmic lysate droplets formed upon increased salt concentration were shown to promote mRNA production roughly 50-fold (Sokolova et al., 2013); importantly, this increase could not be accounted for solely by the local increase in concentration, suggesting that crowding effects play a role in enhancing the rate of reaction. *In vivo*, coilin-mediated assembly of CBs has been shown to be essential during early zebrafish embryogenesis, likely by concentrating snRNP components and thereby increasing splicing reaction rates (Strzelecka et al., 2010). This is consistent with kinetic data combined with theoretical analysis, suggesting that assembly of a key mRNA splicing factor is  $\sim 10$ -fold faster in CBs than the surrounding nucleoplasm (Novotný et al., 2011).

Diffusive molecular transport into and out of, and within a given droplet, will be key to its function as a liquid phase micro-reactor. Diffusive transport is a process whose rate intrinsically

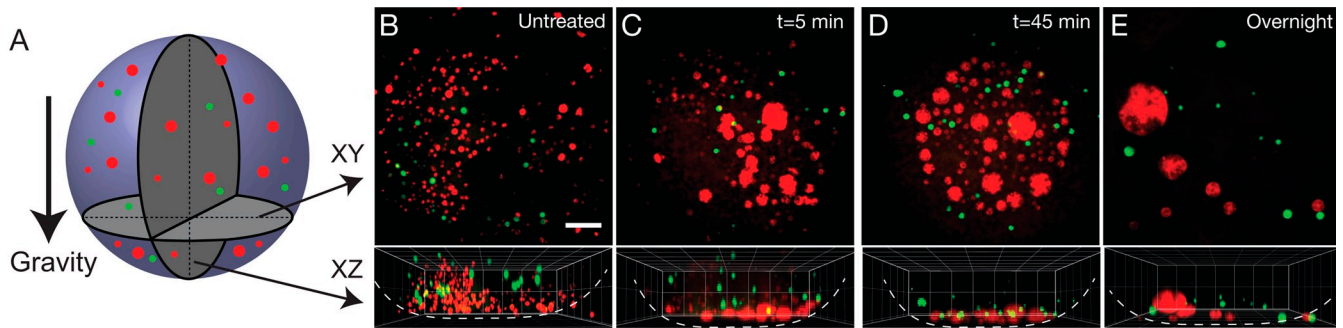


Figure 4. **Gravity and large droplets in large cells.** (A and B) An intact *X. laevis* oocyte nucleus (GV) contains hundreds of RNP droplets, including nucleoli (red) and histone locus bodies (green), distributed through the GV (XY projection shown in top panels, XZ projection shown in bottom panels). A nuclear actin scaffold keeps these droplets from fusing. Bar, 50  $\mu\text{m}$ . (C and D) Upon actin disruption, nucleoli undergo gravitational sedimentation to the bottom of the nucleus, where they undergo massive fusion events, ultimately resulting in a few large droplets (E). Panels B–E adapted from Feric and Brangwynne (2013), copyright Nature Publishing Group.

depends on length scale, suggesting that this function will depend on droplet size. The maximum diffusive flux of molecules reaching the droplet surface grows linearly with its size (Berg, 1993). Thus, with respect to transport into the droplet, large droplets will potentially be able to process more molecules from their environment. However, the time for a processed molecule within the droplet to diffuse to the surface for export increases with droplet size. For directed motion, the timescale for transport would grow linearly with droplet size. But for diffusive transport, this size dependence is even stronger, which suggests that transport efficiency is strongly diminished in larger droplets. This could explain their sizes in large *Xenopus laevis* oocytes, cells that reach sizes of  $>1$  mm with correspondingly large nuclei of  $\sim 450$   $\mu\text{m}$ . A scaling behavior is observed between the size of their RNP droplets, including nucleoli and histone locus bodies, and cell/nucleus size (Feric and Brangwynne, 2013). But droplets are stabilized against frequent contact and fusion events, resulting in many small droplets favored over fewer large droplets (Brangwynne et al., 2011).

#### Growing pains: Too much cytoplasm

*X. laevis* oocytes, with their coupled growth of cell, nucleus, and RNP droplets, provide a dramatic example of the scaling of biological form and function over more than an order of magnitude in size. However, it is not usually possible to significantly increase the size of a machine and all its parts, and still have it function properly. Indeed, as with diffusive transport, the physical forces at play within the cell (and in the nonbiological world) are all intrinsically scale-dependent (McMahon and Bonner, 1983). It may thus be surprising that the cell, as a complex biological machine with many interconnected parts, still functions when its size is increased over an order of magnitude.

There is a hint that important structural changes do occur during growth from a more typical sized cell ( $\sim 10$   $\mu\text{m}$ ) to the millimeter-sized oocyte: there is a significant increase in nuclear actin. In small somatic cells, nuclear actin concentration remains low due to the export factor Exp6. By contrast, large oocytes contain no detectable Exp6, leading to significantly elevated actin concentration in the nucleus (Bohnsack et al., 2006). However, the precise nature of nuclear actin has been controversial (Gall, 2006; Jockusch et al., 2006), and it was unclear why an

actin scaffold would be particularly necessary in large oocyte nuclei. A recent study used a particle-tracking microrheology technique to show that the large *X. laevis* oocyte nucleus indeed contains an elastic actin network, with a mesh size of  $\sim 1$   $\mu\text{m}$  (Feric and Brangwynne, 2013). Surprisingly, when this actin network was disrupted, RNP droplets underwent gravitational sedimentation to the bottom of the nucleus, where they fused with one another into extremely large spherical droplets, as shown in Fig. 4.

This finding was surprising because gravity is usually considered negligible within cells. This is because the random microscopic fluctuations due to Brownian motion usually are much more influential than the small, constant force due to the earth's gravitational pull. A single cell would have to be as big as a basketball for this gravitational force to give rise to a significant concentration gradient of molecules from the top of the cell to the bottom. But because the gravitational force depends strongly on size (mass), it becomes increasingly important for large macromolecular complexes, and even more so for large organelles such as RNP droplets. Moreover, as a result of RNP droplets scaling with cell size, large oocytes contain large nucleoli and histone locus bodies, and the problem of gravitational sedimentation increases further. The combined effects of cell growth and RNP size scaling leads to gravity becoming significant at the  $\sim 10$ – $100$ - $\mu\text{m}$  cell size range. This suggests that gravity could play a role in cell size control, and provides a fascinating example of the limits of organelle size scaling.

#### Conclusions

It is increasingly clear that the cytoplasm and nucleoplasm can exist in a variety of different phases. Repetitive, weak protein–protein and RNA-binding domains drive a spectrum of interactions that organize the cytoplasm, giving rise to liquid-like RNP droplets such as germ granules and nucleoli. Beyond the standard paradigm of membrane-bound organelles, these liquid-phase organelles represent a dynamic mesoscale organization of the cytoplasm/nucleoplasm, which can play a role in localizing and controlling biological reactions. Recent experiments suggest this assembly process is coupled to cell size through the intrinsic dependence of phase transitions on container size. This coupling between phase transition–driven assembly and cell

size could be key to elucidating the longstanding and fundamental problem of understanding how cells know how big they are.

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