A nuclear F-actin scaffold stabilizes RNP droplets against gravity in large cells

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***Supplementary Note***

**Calculating nucleoplasmic viscosity from microrheology data**

A microscopic spherical particle within a purely viscous solution (i.e. water) at equilibrium will undergo Brownian motion caused by thermal energy fluctuations[1](#_ENREF_1). This Brownian motion is characterized by a random walk trajectory of particle position, such that the mean squared particle displacement, MSD, as a function of lag time, , is given by (see Methods) , where d is the number of spatial dimensions (typically two for fluctuating motion within the focal plane of the microscope), and *D* is the particle diffusion coefficient.

 If the system is truly in equilibrium, then the diffusion coefficient of a spherical Brownian particle of radius, R, diffusing within a solution of viscosity , obeys the Stokes-Einstein relation[2](#_ENREF_2), , where is Boltzmann’s constant, is the temperature. To demonstrate this, we tracked the fluctuating motion of our polystyrene probe particles undergoing Brownian motion in an aqueous buffer (PBS). The particles indeed exhibit a *1/R* dependence on *D* (black symbols, Fig. 2D); from a fit to this data, we obtain ηwater=0.001±0.004 Pa-s, (95% confidence interval), which is in good agreement with the known viscosity of water at 20oC, ηwater=0.0010019 Pa-s [3](#_ENREF_3).

 For 0.1-1 µm polystyrene particles within actin-disrupted GVs we found that by plotting their apparent diffusion coefficient vs. size, they also exhibited a size dependence,, consistent with the Stokes-Einstein relation. Assuming thermal equilibrium, we fit this data to determine the oocyte nucleoplasmic viscosity, 0.005 ± 0.002 Pa-s, approximately 5 times more viscous than water (colored symbols, Fig. 2E). We only included beads from actin-disrupted GVs for this fit. However, in Fig. 2E we include the smallest beads in native GVs (blue square) to illustrate its comparable diffusion coefficient; it is thus likely probing the background viscous nucleoplasmic fluid. The small difference in its diffusion coefficient from that in actin-disrupted GVs likely reflects an additional hydrodynamic drag contribution from the intact actin meshwork.

 As described in the next section, by monitoring the sedimentation velocity of metallic particles of known density, we were able to obtain an independent measure of the nucleoplasmic viscosity in agreement with the microrheology result, confirming the validity of the thermal equilibrium assumption we made by utilizing the Stokes-Einstein equation. We note that this equilibrium assumption may break down for particles that are not freely diffusing through the GV, such as those large particles that are constrained by the actin network and therefore subject to any non-equilibrium, actively generated forces within the network.

**Calculating the sedimentation velocity**

The sedimentation velocity, *v*, of a particle within a simple viscous liquid can be calculated by considering the sum of all forces acting on the particle: . Both probe particles and RNP droplets analyzed in this study are well-approximated as spheres of radius *R.* For a spherical liquid droplet of viscosity, λη, in a simple viscous liquid of viscosity, η, the drag force for low Reynolds number (Re) motion (for all cases here, Re<<1) is given by the Hadamard-Rybczynski formula:  [4](#_ENREF_4). We previously determined that RNP droplet viscosity is several orders of magnitude larger than water[5](#_ENREF_5), suggesting that λ>>1, and . The net force due to gravity is given by: , where Δρ is the density difference between the particle and the surrounding fluid, and *g* is the gravitational acceleration constant (*g*=9.8 m/s2). At steady state, the acceleration is zero, , and one obtains: .

 By tracking the sedimentation velocity of metallic probe particles of known density (1.8 g/cc), we determined a nucleoplasmic viscosity of 0.007 ± 0.002 Pa-s; although this estimate is less precise than our Brownian microrheology data due to the limited Z-resolution in tracking these rapidly sedimenting metallic beads and the density variability between metallic beads, the viscosity estimate is consistent with the more precise microrheology data described above. For RNP droplets of unknown density, we used the measured nucleoplasmic viscosity, 0.005 ± 0.002 Pa-s, to fit the RNP droplet sedimentation velocities as a function of size, , (Fig. 4K), to determine the density difference, Δρ. We can then proceed to use this measured density difference for nucleoli to determine the magnitude of the net gravitational force, *Fg*, on large nucleoli (R= 10 μm) in the *X. laevis* GV. We find, *Fg* ~ 1 pN. This is a relatively small force that the actin network must withstand, with corresponding small local stresses of order  ~0.01 Pa.

**Estimating the nucleoplasmic density**

To determine the average density of the entire GV, we performed sedimentation experiments by placing the GV in a graduated cylinder filled with mineral oil (Sigma) of known density (0.84 g/ml) at 18°C. The viscosity of the mineral oil was estimated by measuring the sedimentation rate of a drop of water in mineral oil; here λ<<1 and we assume a drag force of to determine the mineral oil viscosity, η= 0.057±0.005 Pa-s. The GV was dissected in mineral oil and imaged using a Zeiss stereoscope to measure its size. The GV was carefully pipetted into the graduate cylinder. Once the GV attained steady sedimentation, the time for the GV to fall a given distance was measured (typically between 20-65 mm) and used to estimate the average sedimentation velocity. The drag force on the GV was estimated by Stokes’s law for a hard sphere: . Using the known mineral oil density and measured viscosity, we obtained ρGV=1.12±0.03 g/cc.

 Importantly, this measures the average density of the entire GV, which is a combination of the density of “nucleoplasm,” RNP droplets, chromatin, and any other nuclear structures. This measured average GV density will be a weighted average of these different densities. Although we anticipate that, by volume, most of the GV is “nucleoplasm,” the embedded RNP droplets, chromatin, actin filaments, and other structures are likely to increase the average density of the GV above that of the pure “nucleoplasm.” Thus, our measurement of the average density of the GV can be considered an upper bound on the density of the “nucleoplasm.” We can compare this measurement to that of the protein density of nucleoplasm precisely measured using optical interferometry. If we assume that water is the most abundant molecule by volume with density ρwater=0.999 g/cc, then the total density of the nucleoplasm is the sum of mass concentrations of its components (water and protein): . Based on the literate value of ρprotein=0.106 g/cc[6](#_ENREF_6), the total density of the nucleoplasm can be estimated as 1.105 g/cc. Consistent with this, our measurement, ρGV=1.12±0.03 g/cc, is slightly higher than the value of nucleoplasmic density.

**Importance of gravity on cellular length scales**

For microscopic spherical particles, we consider the relative importance of thermal energy () and gravitational potential energy, . The ratio of these yields a sedimentation length, , which reflects the length scale at which thermal forces are comparable to gravitational forces; on larger length scales gravity will dominate, while on smaller length scales, thermal forces will dominate. This approach is validated by our finding that freely diffusing particles appear to be in equilibrium (see previous section). However, this does not account for any non-equilibrium forces that likely play a role in the observed non-equilibrium distribution of RNP droplets embedded in the actin meshwork. 

 Since the sedimentation length scale depends strongly on the size of the particle, , we sought to measure how the size of a representative nuclear body, the nucleolus, scales with nuclear size. To determine the nucleolar diameter, we measured the size (full width, half max) of NPM1::GFP labeled nucleoli of a 100-micron Z-stack in Stage IV-VI oocytes using custom routines in Matlab and estimated the nuclear diameter in ImageJ. For Stage I and some Stage IV oocytes, we estimated the nucleolar and nuclear diameters in ImageJ. For comparison to smaller and different cell types, we estimated the nucleolar and nuclear diameters from available images and data in the literature for XL2[7](#_ENREF_7), primordial cells[8](#_ENREF_8), and oogonia[8](#_ENREF_8). As shown in Figure 4B, we found an approximately linear scaling relationship of the form: , where  is the average nucleolar radius, β a scaling coefficient, and δ is an apparent y-intercept; a similar scaling behavior of nucleolar size with cell size has been observed in human sensory ganglia neurons[9](#_ENREF_9). With *R* and *LGV* in units of microns, β was determined to be 0.0053±0.0009 (unitless) and δ was 1.3±0.1 μm; error bars reported as 95% confidence intervals.

 To determine the sedimentation length for nuclear bodies for smaller oocytes and somatic cells, we estimated their physical properties: we assumed that the density of the nuclear bodies was the same as the densities we measured for nucleoli and HLBs of Stage V-VI from the sedimentation experiments (Figure 4K); we approximated the size of CBs/HLBs to be R=0.15-0.25 µm, as found in somatic cells[10](#_ENREF_10), and we used the nucleoli sizes as determined in Figure 5B. These were plotted as open circles in Fig. 5D. The data for sedimentation length for nucleoli and HLBs whose size and density difference that we directly measured from the sedimentation experiments were plotted as filled circles in Fig. 5D. As a result of larger RNP droplets being found in larger cells, the sedimentation length associated with these particles decreases significantly in larger cells, as can be seen from the downward slope of the dotted lines in Figure 5D. The data for the sedimentation length of “small RNP complexes”, shown in Fig. 5D, is estimated using a size range of R=50-75 nm, together with a high density difference similar to that of nucleoli Δρ=0.04 g/cc.

 To relate the nuclear diameter to the cell diameter, Stage IV-VI oocytes were imaged at low magnification either on a stereomicroscope or an inverted wide-field microscope. Stage I oocytes were kept in OR2, manually separated from the follicular tissue, and imaged at 20X on an inverted microscope using DIC. The nuclei were identified from the intact oocyte, since Stage I oocytes are transparent. In Image-J, the diameter was estimated by averaging the long and short axis of the nuclei or oocyte. For comparison, the cell and nuclear size were estimated from literature for other *X. laevis* cell types: XL2[7](#_ENREF_7), primordial cells[8](#_ENREF_8), and erythrocytes[11](#_ENREF_11). We observed a roughly linear scaling of the form: *LGV*=χ*Lcell*, where χ is the karyoplasmic ratio, which we determined to be χ=0.374 ± 0.008.

 Since gravity is expected to dominate within cells when , we sought to identify the critical GV size, , at which gravity becomes important. For simplicity, we ignore the y-intercept (i.e. setting δ=0); a true non-zero y-intercept is non-physical since it implies a finite-sized organelle in an infinitesimally small cell. We then utilize the scaling relation ; from a fit to this, we obtain β=0.010±0.001 (unitless); we note that independent of how this fit is performed, within a factor of 2 we find that, 0.01, and is thus suitable for an order of magnitude estimate. We then substituted the scaling relation, , into the sedimentation length equation, to obtain: . Using our measured values (where  is taken for nucleoli), we obtain a crossover length scale of ≈40 microns, and a critical nuclear body radius of ≈0.4 microns. This suggests that gravity becomes increasingly important as nuclei grow beyond the typical size of a somatic nucleus (of order ~10 μm). However, we note that gravity may not be completely negligible even in small somatic cells, which suggests that another mechanism may be in place to support somatic nuclear bodies. Typically, somatic nucleoli are tethered to chromatin at the nuclear organizing region[12](#_ENREF_12), the site of rRNA transcription; this is in contrast to the nucleoli within the GV, which assemble around extrachromosomal NORs and are thus untethered to chromatin. Our results show that nuclear actin serves as a meshwork to support these and other nuclear bodies (of comparable density) above the critical size.

Supplementary References

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