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

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The size of a typical eukaryotic cell is of the order of ~10 μm. However, some cell types grow to very large sizes, including oocytes (immature eggs) of organisms from humans to starfish. For example, oocytes of the frog *Xenopus laevis* grow to a diameter ≥1 mm. They have a correspondingly large nucleus (germinal vesicle) of ~450 μm in diameter, which is similar to smaller somatic nuclei, but contains a significantly higher concentration of actin. The form and structure of this nuclear actin remain controversial, and its potential mechanical role within these large nuclei is unknown. Here, we use a microrheology and quantitative imaging approach to show that germinal vesicles contain an elastic F-actin scaffold that mechanically stabilizes these large nuclei against gravitational forces, which are usually considered negligible within cells. We find that on actin disruption, ribonucleoprotein droplets, including nucleoli and histone locus bodies, undergo gravitational sedimentation and fusion. We develop a model that reveals how gravity becomes an increasingly potent force as cells and their nuclei grow larger than ~10 μm, explaining the requirement for a stabilizing nuclear F-actin scaffold in large *Xenopus* oocytes. All life forms are subject to gravity, and our results may have broad implications for cell growth and size control.

Eukaryotic cells are subject to a variety of mechanical forces. These stresses often require structural stabilization by the cytoskeleton, which plays a role analogous to the structural scaffold of a building. However, unlike a building, gravitational forces are typically ignored in cells, because gravity is considered negligible at the small length scales, low Reynolds numbers and low variation in density characteristic of cells. Rather, attention has focused on forces such as those arising from molecular motor activity in the cytoplasm, where cytoskeletal actin (F-actin) plays a well-characterized mechanical role. Less is known about the mechanical organization of the nucleus. Some studies posit the existence of a nuclear scaffold of uncertain composition, whereas others suggest that only a nuclear lamina, or chromatin itself, comprises a structural element in the nucleus¹.² Potential roles for actin in the structural organization of the nucleus have been particularly controversial. Actin in a non-polymeric form is known to be a component of several transcription complexes³, but whether F-actin exists in a typical nucleus⁴, and its structural role, if any, are far from resolved⁵⁶. In small somatic nuclei, the actin concentration remains low owing to a dedicated nuclear export protein factor, Exp6 (ref. 7). Large amphibian oocytes, in contrast, have no detectable levels of Exp6 expression⁷, leading to high concentrations of nuclear actin⁸. This actin may play a role in RNA transport⁹ or in maintaining the structural stability of the germinal vesicle⁴ (GV). However, whether it is in the form of an F-actin network capable of supporting mechanical loads, and precisely what kinds of mechanical loads such an elastic network would need to bear, remain poorly understood¹⁰. To probe the microstructure of the nucleoplasm in *Xenopus* GV, we used a microrheology approach to study the fluctuating dynamics of PEG-passivated polystyrene particles micro-injected into the GV of stage V–VI oocytes (diameter, *L* cell = 1,000–1,300 μm; Supplementary Note). The particle mean-squared displacement (MSD) exhibited a power-law relationship with lag time, characterized by the diffusive exponent, α. The smallest beads studied, radius *R* = 0.1 μm, underwent diffusive-like motion through the GV (Fig. 1a,b and Supplementary Video S1). The MSD of a large ensemble of these beads exhibited α = 0.94 ± 0.04 (Supplementary Note), close to that expected for Brownian motion of a particle within a purely viscous fluid, α = 1 (ref. 12). However, beads with a slightly larger radius, *R* = 0.25 μm, showed a markedly different behaviour. They remained transiently stuck in place, before hopping to a new position and again remaining trapped there for some time (Fig. 1a,b and Supplementary Video S2). These had a smaller power-law exponent, α = 0.81 ± 0.07, as would be expected for such constrained dynamics. This suggests the presence of an elastic meshwork through which intermediate-sized (*R* = 0.25 μm) beads are hopping. Consistent with this, larger beads, *R* = 0.5 and 1.0 μm, exhibited highly arrested dynamics; the motion...
of these large beads exhibited correspondingly lower power-law exponents of $\alpha = 0.5 \pm 0.1$ and $\alpha = 0.4 \pm 0.1$, respectively (Fig. 1a,b and Supplementary Video S3). The decrease in $\alpha$ with increasing bead size suggests that the GV contains an elastic network, which is effective at constraining the mobility of objects greater than its apparent mesh size of $\sim 0.5 \mu$m (Fig. 2d). Indeed, very similar behaviour is observed with in vitro F-actin networks.$^{17}$

To determine whether actin is responsible for these constrained dynamics, we treated oocytes with the actin-disrupting drug latrunculin A (Lat-A; ref. 14). These GVs seemed to be more fluid when pipetted into the imaging chamber. Probe particles in GVs from Lat-A-treated oocytes were highly mobile, even at the largest bead sizes. The MSD of all bead sizes, $R = 0.1–1.0\mu$m, exhibited diffusive-like motion, with an average exponent of $\alpha = 0.94 \pm 0.04$ (Fig. 2a,d). We also tested another actin-disrupting drug, cytochalasin D (Cyto-D). The MSD of probe particles in GVs from Cyto-D-treated oocytes were also highly mobile at all particle sizes, with an average exponent of $\alpha = 0.9 \pm 0.1$ (Fig. 2b,d).

To confirm that these data do not reflect off-target drug effects, we micro-injected into the GV high concentrations of a human homologue of the protein Exp6, XPO6, to decrease the nuclear actin concentration. After $\sim 1$ h of incubation, we find the same effect as in Lat-A- or Cyto-D-treated oocytes: both small and large beads exhibited simple diffusive-like motion, with $\alpha = 0.91 \pm 0.08$ (Fig. 2c,d). The data from actin-disrupted GVs exhibited a size dependence of the diffusion coefficient, $D \sim 1/R$, consistent with the Stokes–Einstein equation describing Brownian motion in a purely viscous liquid (Fig. 2e and Supplementary Note). We fitted this data to the Stokes–Einstein equation to obtain the nucleoplasmic viscosity, $\eta = 0.005 \pm 0.002$ Pa s, approximately 5 times more viscous than water (black symbols, Fig. 2e).

Interestingly, the diffusion coefficients of small ($R = 0.1 \mu$m) beads in actin-disrupted GVs were similar to that of small diffusive beads in untreated GVs (blue square, Fig. 2e), suggesting that the latter are largely probing the background nucleoplasmic fluid.

To visualize the nuclear F-actin network, we constructed a GFP fusion of the small actin-binding peptide Lifeact$^{15,16}$. GVs expressing Lifeact::GFP revealed a dense, three-dimensional network of F-actin (inset Figs 1, 2f and 3a–c); quantitative image analysis was consistent with our bead microrheology data (Supplementary Fig. S1). Visualization of the network using a different actin-binding construct, utrophin::GFP (ref. 6; Supplementary Note), revealed similar structural features (Supplementary Fig. S2). Microrheological measurements of all bead sizes were unchanged in GVs expressing these constructs (Supplementary Fig. S3), confirming that these probes do not perturb F-actin structure. Moreover, consistent with our bead microrheology data, GVs treated with either Lat-A, Cyto-D or XPO6 exhibited a significantly fragmented actin network (Fig. 2G, Supplementary Fig. S1 and Videos S8 and S9).

These probes allowed us to visualize the mechanical response of the live actin network. Using a microneedle, we found that the network could withstand repeated compressive deformations, each time elastically recovering after removal of the force (Fig. 3a). We found similar results on application of tensile forces to the network; each time the network exhibited an elastic response, largely recovering its pre-deformation structural organization (Fig. 3b); interestingly, we frequently observed apparent actin polymerization in response to force application (arrowhead, Fig. 3a), suggesting that the network architecture is mechano-sensitive. These experiments were conducted at the nuclear periphery, where nuclear lamins are known to assemble a thin cortical shell$^{17,18}$. Using an RFP::lamin B3 construct, we found that the lamin cortex deformed in concert with the actin network. Even
under large deformations, we were unable to sever the lamin/actin mechanical connection (Fig. 3a,b), suggesting that the actin network is mechanically anchored to the lamin cortex.

Nuclear actin may have structural features that could alter its binding to conventional actin-binding proteins. It is thus unclear whether this nuclear actin network could be regulated by mechanisms similar to those functioning within the cytoplasm. As a first coarse perturbation, we depleted ATP using the enzyme apyrase. The particle MSD decreased (Fig. 3f), and the GV seemed stiffer on dissection, suggesting a possible role for ATP-dependent processes. We next examined the effect of proteins known to regulate cytoplasmic actin. Injecting the actin-filament-stabilizing protein tropomyosin into the GV led to a decreased particle MSD, comparable to the values seen on ATP depletion (Fig. 3f). Visualization of tropomyosin-injected GVs revealed a more dense, compacted actin network structure (Fig. 3d). These data suggest that the structure and mechanics of this nuclear actin network could potentially be regulated by molecular mechanisms similar to those at play in the cytoplasm.

Figure 2 Actin disruption leads to purely viscous nuclear properties. (a) In Lat-A-treated oocytes, the MSD for all bead sizes exhibited diffusive-like behaviour. Green: $R = 0.1 \mu m$ (19 $z$-positions from 9 GVs, 20,224 particles identified); blue: $R = 0.25 \mu m$ (28 $z$-positions from 15 GVs, 8,950 particles identified); black: $R = 0.5 \mu m$ (23 $z$-positions from 14 GVs, 3,261 particles identified); and red: $R = 1.0 \mu m$ (23 $z$-positions from 11 GVs, 1,642 particles identified). (b) Similar behaviour was observed in Cyto-D-treated oocytes. Green: $R = 0.1 \mu m$ (13 $z$-positions from 6 GVs, 7,754 particles identified) and red: $R = 1.0 \mu m$ (9 $z$-positions from 5 GVs, 382 particles identified). (c) Similar behaviour was observed in GVs injected with XPO6 to deplete actin. Green: $R = 0.1 \mu m$ (21 $z$-positions from 10 GVs, 42,411 particles identified); and red: $R = 1.0 \mu m$ (13 $z$-positions from 5 GVs, 1,218 particles identified). (d) Under actin-disrupting conditions (Lat-A, Cyto-D and XPO6), $\alpha \approx 1$ for all bead sizes, whereas $\alpha < 1$ for large beads in native GVs. Error bars represent standard error of the slope. (e) The diffusion coefficient, $D$, of beads in actin-disrupted GVs exhibits a roughly $R^{1}$ dependence expected from the Stokes–Einstein equation; small beads in untreated GVs have a comparable value of $D$ (blue square). Solid black symbols are beads diffusing in water. The colour scheme is as in d. Inset shows the scaled MSD $\times R$ against lag time. (f) Lifeact::GFP image showing the native actin network in a live GV. (g) Lifeact::GFP image of the fragmented actin network after Lat-A treatment. Scale bars, 5 $\mu m$. particles, on actin disruption, the motion of large nucleoli and HLBs became significantly less constrained, with an average exponent of $\alpha = 0.7 \pm 0.2$ (Fig. 4a). However, a minority population of small micronucleoli, of size $R \leq 1 \mu m$, seem to diffuse more freely even in an intact actin meshwork (Fig. 4b); their dynamics are correlated with their size, with the smallest micronucleoli exhibiting nearly freely diffusive motion (Fig. 4b and Supplementary Video S4), comparable to the micro rheology of inert microspheres of similar size (Fig. 1a). In addition to diffusive-like motion within the focal plane, we found that nucleoli and HLBs in Lat-A-treated GVs move to the bottom of the GV; nucleoli moved more quickly, over a timescale of $\sim 5$–10 min, versus $\sim 30$ min for HLBs. They were observed to collect at the bottom of the GV and undergo homotypic (nucleoli–nucleoli or HLB–HLB) fusion events on contact, consistent with a recent study; for nucleoli, this ultimately resulted in a small number of large nucleoli of $R \approx 25$–50 $\mu m$ (Fig. 4d–h and Supplementary Fig. S4 and Videos S5–S7). Similar behaviour was observed with Cyto-D-treatment (Supplementary Video S8) or XPO6-injection (Supplementary Fig. S5). This shows that both HLBs and nucleoli exhibit liquid-droplet-like behaviour previously described for several types of RNP bodies. Our protocol for these experiments involved incubation of oocytes in Cyto-D or Lat-A, or injection of XPO6 for 1–2 h, during which time the oocytes were in a small tube that was gently rotated, such that the gravitational force vector with respect to the animal–vegetal axis constantly changed, averaging to zero. As soon as the GVs were isolated from the oocyte and placed on a coverslip in a constant orientation, RNP droplets began settling towards the bottom. In contrast, after

![Image](https://example.com/image.png)
overnight incubation of oocytes in a Petri dish with a stable orientation, the RNP droplets had undergone massive fusion events, having already settled to the bottom of the stationary GV (Fig. 4h).

Unlike RNP droplets, the polystyrene microrheology probe particles (Figs 1 and 2) were not observed to sediment to the bottom of the GV, owing to their relatively low density, $\rho \approx 1.06 \text{ g ml}^{-1}$, similar to that of water, and small size. Indeed, we determined the average density of the GV, $\rho_{GV} = 1.12 \pm 0.03 \text{ g ml}^{-1}$ (Supplementary Note), which was slightly larger than that of the probe particles. In Lat-A-disrupted GVs, when we micro-injected metallic beads ($R = 0.5 \mu$m) with a significantly higher density, $\rho \approx 1.8 \text{ g ml}^{-1}$ (Supplementary Note), we observed rapid sedimentation. The velocity, $v$, of sedimentsing spherical particles will be given by a balance between the drag force and the buoyant force, with an expected dependence, $v = 2R^2\Delta\rho g/9\eta$, where $\Delta\rho$ is the density difference between the particle and surrounding fluid, and $g$ is the gravitational acceleration constant. By measuring the velocity of sedimenting metallic beads, we determined a nucleoplasmic viscosity, $\eta = 0.007 \pm 0.002 \text{ Pa s}$ (Fig. 4k and Supplementary Note), consistent with the value from diffusive microrheology data (Supplementary Note).

By tracking the vertical motion of settling RNP droplets, we found that larger RNP droplets settled at higher velocities (Fig. 4k), consistent with the expected $R^2$ dependence of the velocity. Using our measured value for the nucleoplasmic viscosity, we plot the sedimentation velocity, $v$, as a function of normalized RNP droplet size, $2R^2g/9\eta$, to determine $\Delta\rho$. For HLBs, we find $\Delta\rho_{HLB} = 0.011 \pm 0.002 \text{ g ml}^{-1}$. For nucleoli, we obtain a higher density, $\Delta\rho_{NUCLEOLUS} = 0.035 \pm 0.07 \text{ g ml}^{-1}$. HLBs and nucleoli thus have a macromolecular density $\sim 10\%$ and $40\%$ larger, respectively, than the surrounding nucleoplasm (Supplementary Note). These values are roughly consistent with a previous density measurement using an optical interferometry technique. Thus, the directed movement of RNP droplets towards the bottom of actin-disrupted GVs agrees quantitatively with a gravitational driving force that arises from the density difference between RNP droplets and the surrounding nucleoplasm.

The need for a nuclear F-actin scaffold to stabilize internal structures can be understood by considering the sedimentation length, $\ell_{sed}$. This defines the length scale beyond which gravitational effects begin to dominate over random, diffusive motion associated with the thermal energy scale, $k_BT$ (Supplementary Note):

$$\ell_{sed} = \frac{k_BT}{\frac{4}{3}\pi R^3\Delta\rho g}$$

GVs in mature Stage V–VI oocytes are very large, $L_{GV} \approx 450 \mu$m. Using our measured value $\Delta\rho_{NUCLEOLUS}$, we calculated $\ell_{sed}$ for different sized nucleoli, finding that it becomes comparable to $L_{GV}$ for nucleoli of size, $R \approx 0.2 \mu$m. Thus, for nucleoli larger than $\sim 0.2 \mu$m, $\ell_{sed} < L_{GV}$,
Figure 4 Actin disruption results in sedimentation and fusion of RNP droplets. 
(a) Two-dimensional MSD of RNP droplets shows highly constrained motion in native GV (circles). Red: nucleoli (R > 2 μm; 11 z-positions from 5 GVs, 320 droplets identified); and green: histone locus bodies (HLBs) (16 z-positions from 10 GVs, 232 droplets identified). In Lat-A-treated GVs (squares), these RNP droplets are more mobile. Red: nucleoli (R > 2 μm; 10 z-positions from 6 GVs, 442 droplets identified) and green: HLBs (9 z-positions from 5 GVs, 149 droplets identified). Inset shows a few large nucleoli embedded in an F-actin meshwork visualized by Lifeact::GFP. Scale bar, 10 μm. (b) A sub-population of small nucleoli (micronucleoli, R < 2 μm) are more mobile, and occasionally exhibit intermittent dynamics (cage hopping) in native GVs. Inset shows micronucleoli inside a meshwork labelled with Lifeact::GFP. (n = number of displacements at a given lag time per nucleolar size from 11 z-positions from 7 GVs, 240 droplets identified). Error bars = s.e.m. Scale bar, 10 μm. (c) Left column shows example X–Y trajectories for a representative micronucleolus (top) and a typical large nucleolus (bottom). The right column shows corresponding temporal changes in position, X(t) and Y(t). d–h, Top images, a maximum intensity projection of a 100-μm-thick section of nucleoli labelled with NPM1::RFP (red) and HLBs labelled with GFP::coillin (green); bottom images, a three-dimensional rendering in the X–Z plane. (d) Nucleoli and HLBs are suspended in an untreated GV. For e–g, time-lapse images are from the same GV from a Lat-A treated oocyte; time refers to minutes after dissection. (h) Large RNP droplets that form overnight after actin disruption by Lat-A. Scale bar, 50 μm. Three-dimensional grid size = 50 μm. (i) Three-dimensional representation of nucleoli (top) and HLB (bottom) trajectories after actin disruption in the GV shown in e–g. (j) The vertical position of RNP droplets (red: nucleoli R > 2 μm; and green: HLBs) from i as a function of time. (k) The sedimentation velocity plotted against normalized size. Black: metallic R = 0.5 μm beads (n = 16 movies of 16 GVs, 237 tracks analysed); red: nucleoli (R > 2 μm; n = 17 movies of 16 GVs, 394 tracks analysed); and green: HLBs (n = 18 movies of 12 GVs, 149 tracks analysed). Larger circles represent binned data points and solid lines are linear fits of the data, with the slope representing the buoyant density. Error bars represent s.d.

and gravity is no longer negligible. The structure of the nuclear F-actin network, of mesh size ~0.5 μm, is therefore well suited to mechanically stabilize sedimentation-prone RNP droplets.

Small somatic cells do not exhibit high actin concentrations in the nucleus, suggesting either gravitational sedimentation is not problematic in somatic nuclei, or that another structure provides a stabilizing scaffold. As cells grow, there is often a concomitant scaling in the size of their organelles. During oocyte growth from small Stage I oocytes, L<sub>o</sub> ≈ 200 μm, to large mature Stage VI oocytes, L<sub>o</sub> ≈ 1,300 μm, the GV grows proportionally, from L<sub>GV</sub> ≈ 100 μm to 450 μm (ref. 29; Fig. 5a,c). As gravity becomes dominant when L<sub>GV</sub> > L<sub>o</sub>, gravity becomes increasingly significant as the oocyte and GV grow. Moreover, RNP droplets also scale with cell and GV size, with the average size of nucleoli exhibiting a roughly linear scaling relationship (Fig. 5b); HLBs seem to exhibit similar scaling behaviour (Fig. 5d). As the sedimentation length has an inverse dependence on RNP volume,
Our findings reveal a new role for nuclear F-actin, which becomes essential for mechanical stabilization against gravity in large cells. However, even for the largest nuclei in the X. laevis GV, gravitational forces are relatively small, of the order of \( \sim 0.01 \, \text{Pa} \), so they can be in the regime \( \ell_{\text{sed}}/L < 1 \), where gravitational forces do not play a disruptive role in intracellular organization. Although oocytes are generally large, and there exist numerous examples of cells of varied size, eukaryotic cell size is typically of the order of \( 10 \, \mu\text{m} \), for reasons that are still not well understood. Our results suggest an intriguing possibility: cells are typically no greater than \( 10 \, \mu\text{m} \), because beyond this size gravity is increasingly disruptive, and additional stabilization mechanisms become necessary.

**Methods**

Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
C.P.B. and M.F. designed the study, discussed results, and wrote the paper. M.F. performed the experiments and analysed the data.

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The authors declare no competing financial interests.

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METHODS

Oocyte collection. Adult female *X. laevis* frogs were anaesthetized for 15 min in 0.1% MS-222 solution. Oocytes were surgically isolated following an IACUC-approved protocol and incubated at 18°C in OR2 solution, as previously described. To remove the follicular layer, oocytes were separated using forceps and incubated for 1.5 h at 18°C. Stage V and VI oocytes were used for all experiments and were identified on the basis of their diameter of 1–1.3 mm (ref. 29). The oocyte and the subsequently dissected GV were imaged using a Zeiss stereo microscope to confirm size.

DNA and mRNA constructs. Cloning of DNA constructs was generally completed using either carboxy-terminal EGFP–pcS2+ or RFP–pcS2+ vectors. A vector Lifeact::GFP construct was cloned using codon-optimized oligonucleotides for the small peptide Lifeact (5′-ATGGGAGGTGCTATGCTTGAAGATTTGATTTCTAA-GGAAGA-3′) with a nuclear localization signal (5′-CCAAAGAAGAAAGAGG-GTG-3′) and later digested with Xho1 and Xmal. Oocytes were separated using forceps and incubated for 2 h under constant slow rotation. Three times with PBS, and incubated with a 25:75 molar ratio of biotin-PEG (Sigma) and later digested with Xho1 and Xmal. Urophin-230 and uropein-261 were cloned from complementary DNA containing urophin (gift from D. Mullins) into EGFP–pcS2+ vectors with a nuclear localization signal. The full-length version of urophin (urophin-230) was used to visualize the filaments; the shorter version urophin-230 did not express correctly. Collin was cloned from a GFP::collin–pcS2+ vector (gift from J. Gall). In the case of visualizing the lamin, a RFP::lamin B3 construct was made using *X. laevis* cDNA from lamin B3 (Accession Number: BC078034, Thermo Scientific) and where the RFP was on the amino terminus. NPM1::RFP, NPM1::GFP, and fibrillarin::GFP were used as previously described. NPM1 was produced using the 5S-based capped RNA transcription kit from linearized DNA and later purified with RNasey spin columns and stored at −80°C.

Fluorescent microspheres for passive microrheology. Green and red fluorescent microspheres (Invitrogen) of 0.1, 0.25, 0.5 and 1 μm in radius with carboxyl surface chemistry were passivated with PEG–amine (M2, K2 or M5, K5, as purchased; Rapp Polymere) to prevent non-specific binding. The PEylation reaction was completed using EDC chemistry in MES buffer. To test the efficacy of the reaction, PEylated beads were compared with carboxyl beads and beads with BSA adsorbed to the surface with incubation of fluorescent BSA overnight. PEylated beads had significantly reduced fluorescent BSA adsorption and were deemed passivated. Before micro-injection, the microspheres were sonicated for 1–2 min.

Metallic microspheres for sedimentation experiments. Metallic Dynabeads MyOne Streptavidin C1 (Invitrogen) were used in the sedimentation experiments, with a nominal density of 1.8 g ml⁻¹ and radius of 0.5 μm. The beads were washed three times with PBS, and incubated with a 25:75 molar ratio of biotin–4–fluorescein and biotin–PEG (relative molecular mass 5,000 (M, 5K), as purchased; Rapp Polymere) to prevent non-specific binding. The PEylation reaction was completed using EDC chemistry in MES buffer. Most experiments were performed on a Zeiss microscope. Micronedmed beads were pulled from borosilicate glass with O.D. 1 mm and I.D. 0.78 mm using a PicoPump PV820 on a Zeiss dissecting microscope. Micronedmed beads were pulled from borosilicate glass with O.D. 1 mm and I.D. 0.78 mm using a Sutter Instrument Model P-97. After sonication, approximately 3–10 nl of fluorescent microspheres with modified surface chemistry was micro-injected directly into the germinal vesicle of the oocytes. Approximately 20–50 nl of NPM1::GFP, NPM1::RFP, fibrillarin::GFP, Lifeact::GFP, urophin-261::GFP or RFP::lamin B3 was injected into the cytoplasm of oocytes. All oocytes were incubated overnight for accumulation of translated protein and for the recovery of the GV on micro-injection of microspheres. After overnight incubation, the GVs were manually extracted in mineral oil using forceps and a hair loop. The GV was then transferred using a pipette into an imaging chamber, consisting of a glass coverslip and slide separated by a 1-mm-thick silicone well (Grace Bio-labs).

Actin disruption. Oocytes were incubated in 2 μg ml⁻¹ of latrunculin A (Sigma) and 0.8% dimethylsulphoxide (Sigma) for approximately 1–2 h under constant slow rotation. For cytochalasin-D experiments, oocytes were incubated in 2 μg ml⁻¹ of cytochalasin D (Sigma) and 0.4% dimethylsulphoxide for 2–3 h. For XPO6 experiments, approximately 10 nl of human XPO6 (gift from D. Gorlich) was micro-injected into the GV of oocytes, and after 1 h of incubation, nuclei were dissected and prepared for imaging. After actin disruption, nuclei became significantly more difficult to dissect and nuclei were seen to exhibit fusion and sedimentation events. To stabilize the actin filaments, tropomyosin (Sigma) was dissolved in ultrapure water at a concentration of 5 mg ml⁻¹ and was micro-injected (approximately 3–10 nl) into the nucleus. To bundle the actin filaments, fascin, a gift from G. Koenderink, was micro-injected into the GVs at 1.54 mg ml⁻¹ or 28 μM and incubated for 1–2 h. To crosslink the actin filaments, alpha-actinin (Sigma) was micro-injected into the GVs at 6 mg ml⁻¹. To remove ATP, the ATPase apryrase (Sigma) was micro-injected into the GV at a concentration of 2 mg ml⁻¹ and incubated for 30 min to 1 h. In all of these micro-injections, rhodamine dextran was co-injected to confirm that the protein was micro-injected before completion of the experiment.

Deformation of GV. To visualize the location of the microneedle tip, glass microneedles were loaded with 0.02 μm fluorescent blue microspheres (Invitrogen) and imaged with 405 nm light. The needle was mounted on an Eppendorf micromanipulator. The GV was placed in a pool of mineral oil contained by a silicon well and on top of a glass coverslip. The microneedle was placed near the edge of the live GV in immersion oil. During the experiment, the needle was used to deform the edge of the GV under low and high strain repeatedly and brought back to its original location.

Spinning-disc confocal imaging. Most experiments were performed on a Zeiss inverted microscope equipped with a Yokogawa CSU-X10 confocal spinning disc (Intelligent Imaging Innovations, 3) using ×10 or ×20 dry, or ×40, ×63 or ×100 oil objectives. Time-lapse images were acquired from a QuantEM 512SC camera (Photometrics) using time intervals that were four or five times the exposure time. All experiments were done at room temperature at approximately 20°C. For passive microrheology using microspheres, the beads were imaged ≥20 μm above the coverslip, to ensure that all beads were at least five bead diameters above the surface.

Laser scanning confocal microscopy. Images of the Lifeact::GFP meshwork with nucleoli labelled with NPM1::RFP were acquired on the Leica SP5 laser scanning confocal microscope using a ×100 oil immersion objective with 1.46 numerical aperture. Images of the Lifeact::GFP meshwork with red beads of R = 1.0 μm were acquired using a ×63 oil immersion objective with 1.4 numerical aperture.

Image analysis. Slidebook software was used to acquire images and make three-dimensional renderings of the Z-stacks. ImageJ software was used to format the images, merge channels and create QuickTime files of image sequences. For some images, a de-speckling filter in ImageJ was used for smoothing. In all experiments, the images were analysed using custom-built Matlab code. The centroid positions and trajectories of the beads in these experiments were detected using Matlab Multiple Particle Tracking Code adapted from ref. 36 (see http://physics.georgetown.edu/~matlab/index.html). The particles selected from those identified were based on criteria for particle brightness and radius of gyration. The dynamics of the beads were then analysed using custom-built Matlab software.

Image integrity. Images of the actin meshwork visualized with Lifeact or urophin were highly reproducible, and showed on average a mesh size of ~1 μm (Supplementary Fig. S1). Images of actin meshwork after treatment were variable depending on concentration of agent and length of exposure (Figs 2g and 3c–e). Distribution of nucleoli and fusion depended on the incubation time of drug and amount of time under the constant force of gravity (Figs 4e–h and Supplementary Figs 4b–e and S5). All images were repeated at least several times to ensure reproducibility.

Passive microrheology analysis. The particle’s position was tracked in two dimensions (XY). The mean squared displacement (MSD) was calculated from time and ensemble averages for particles using overlapping intervals for each condition and fitted to a power law as a function of lag time, from which the power-law exponent was determined as the slope of the curve on a log-log plot and the apparent diffusion coefficient as the y-intercept. MSD(t) = (x(t + t) - x(t))² + (y(t + t) - y(t))²

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smaller than the number of microspheres identified, and the standard error of the means was obtained using the number of measurements for each time point instead of the number of experiments performed.

**Estimation of error in passive microrheology for large microspheres.** To determine the error in the MSD measurements, the largest beads (R = 1.0 μm) were immobilized on a glass coverslip. Time-lapse movies were acquired using exposure settings that were comparable to those from the passive microrheology settings yielding similar signal-to-noise ratios of intensity. The MSD of these immobilized microspheres was 0.0007 ± 0.0003 μm². Thus, any MSD measurement for these beads above 0.001 μm² is above the noise floor, and reflects real motion.

**Sedimentation experiments and analysis.** After actin disruption (for example, Lat-A treatment) under constant, slow rotation, the GVs were quickly dissected, carefully pipetted into an imaging chamber, and mounted onto a spinning-disc confocal microscope. This process took approximately 5 min. A four-dimensional time-lapse movie was acquired to capture the distribution of objects in Z for a 100-μm-thick section as a function of time. The positions (X, Y and Z) of the objects, either nucleoli, HLBs, or metallic microspheres, were tracked in time; we note that the tracking resolution in the Z-dimension is not sub-pixel and is significantly lower than that for our XY tracking data used to calculate MSDs. The nuclear body size was estimated as the full-width, half-maximum measured in the Z-plane with maximum brightness. Only objects whose Z-position was at least 5 diameters above the coverslip and showed a good linear fit were analysed in determining the sedimentation velocity and density differences.

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**Figure S1** Actin disruption with latrunculin A or Xpo6. 

**a,** Row shows how actin network, visualized with Lifeact::GFP, is disrupted at 15 minute intervals after incubation with latrunculin A. Scale bar = 10 μm. Each image is from a different GV. 

**b,** Row shows how nuclear actin structure is disrupted due to actin export at 15-minute intervals after Xpo6 microinjection. Each image is from a different GV. Scale bar = 10 μm. 

**c, d,** The probability distribution of F-actin mesh size for GVs under latrunculin A, Xpo-6, conditions, for each time point shown above (blue: 15 minutes, yellow: 30 minutes, and red: 45 minutes). Black data points are for the intact Lifeact::GFP structure with no actin disruption (13 z-stacks from 9 GVs). The exponential behavior of the distributions is consistent with a Poisson interval distribution, where the mesh size is ~1 μm for untreated GVs and ~10 μm for actin-disrupted GVs after 45 minutes of treatment.
Figure S2 Visualization of the nuclear actin network. 

**a,** Image of Lifeact::GFP labeled network within the GV. 

**b,** Image of Utrophin-261::GFP labeled network within the GV, showing similar structure as Lifeact::GFP. Scale bar = 10 µm.
Figure S3 Expression of Lifeact::GFP does not alter microrheology of the GV.  

**a**, MSD versus lag time of $R=0.1 \, \mu m$ (green) ($n=24$ z-positions from 9 GVs, 10,648 particles identified), $R=0.25 \, \mu m$ (blue) ($n=16$ z-positions from 8 GVs, 2,053 particles identified), $R=0.5 \, \mu m$ (black) $n=19$ z-positions from 6 GVs, 1,867 particles identified), and $R=1 \, \mu m$ (red) ($n=35$ z-positions from 14 GVs, 3,011 particles identified) microspheres in native GV (circles) compared with MSD versus lag time of $R=0.1 \, \mu m$ (green) ($n=4$ z-positions from 2 GVs, 7,639 particles identified), $R=0.25 \, \mu m$ (blue) ($n=18$ z-positions from 6 GVs, 7,250 particles identified), $R=0.5 \, \mu m$ (black) $n=10$ z-positions from 4 GVs, 702 particles identified), and $R=1 \, \mu m$ (red) ($n=5$ z-positions from 4 GVs, 237 particles identified) microspheres in Lifeact::GFP expressing GVs (triangles).

**b**, Diffusive exponent as a function of microsphere radius, with untreated case in blue and Lifeact::GFP in green.  

**c**, MSD at 5 s for each bead size, with untreated case in blue and Lifeact::GFP in green. Error bars = s.e.m.
Supplementary Information

Figure S4 Actin disruption leads to nucleolar sedimentation and fusion. Top images show a maximum intensity projection of a 100-micron thick section of nucleoli (labeled with NPM1::GFP & Fibrillarin::GFP) and bottom images show a 3-D rendering in the X-Z plane. a, Nucleoli are suspended in an untreated GV. For b–d, time-lapse images are from the same GV after Lat-A disruption of actin. e, Large nuclear bodies that form overnight after Lat-A treatment. Scale bar = 50 μm and grid size = 50 μm.
Figure S5 Actin disruption after Xpo6 microinjection also leads to formation of a few massive nucleoli at the bottom of the GV. Top row shows XY maximum intensity projection of an untreated GV (left) and one after overnight incubation after Xpo6 microinjection (right). Bottom row shows the XZ projection of a 100-μm thick section for the corresponding GVs. Scale bar = 50 μm and grid size = 50 μm.
Supplementary Video Legends

Supplementary Video S1 Diffusion of R=0.1 μm red microspheres within the Lifeact::GFP actin meshwork. These beads were the smallest bead size probed and showed diffuse-like behavior. Time reported as minutes : seconds.

Supplementary Video S2 Diffusion of R=0.25 μm red microspheres within the Lifeact::GFP actin meshwork. These intermediate beads showed cage-hopping behavior, during which the beads diffuse inside a pore and after some time, jump to a new pore. Time reported as minutes : seconds.

Supplementary Video S3 Diffusion of R=1 μm red microspheres within Lifeact::GFP actin meshwork. These beads were much larger than the average mesh size and exhibited highly-subdiffusive behavior, leading to their trapped dynamics. Time reported as minutes : seconds.

Supplementary Video S4 Diffusion of NPM1::RFP micronucleoli within Lifeact::GFP actin meshwork. The diameter of these micronucleoli was approximately equal to or smaller than the pore size, leading to intermittent dynamics and cage-hopping behavior. Time reported as minutes : seconds.

Supplementary Video S5 Increased mobility of GFP::coilin labeled HLBs upon actin disruption by latrunculin A. Top panel shows the XY projection of a 100-μm thick section. HLBs are more motile and show more diffusive like behavior than in unperturbed GVs. Bottom panel shows XZ projection of a 100-micron thick section. HLBs sediment to the bottom of the GV on the scale of ~1 hour. Time reported as minutes : seconds.

Supplementary Video S6 Sedimentation and fusion of NPM1::GFP & Fibrillarin::GFP labeled nucleoli upon actin disruption by latrunculin A. Top panel shows the XY projection of a 100-μm thick section. Nucleoli are more motile and show more diffusive like behavior than in unperturbed GVs. Bottom panel shows XZ projection of a 100-μm thick section. Nucleoli rapidly sediment to the bottom of the GV on the scale of ~15 minutes. Time reported as minutes : seconds.

Supplementary Video S7 Sedimentation of NPM1::RFP labeled nucleoli and GFP::coilin labeled HLBs upon actin disruption by latrunculin A. Top panel shows the XY projection of a 100-μm thick section. Nucleoli and HLBs are more motile and show more diffusive like behavior than in unperturbed GVs. Bottom panel shows XZ projection of a 100-μm thick section. Nucleoli rapidly sediment to the bottom of the GV on the scale of ~5-10 minutes, while HLBs sediment on a longer time scale of ~30 min. Time reported as minutes : seconds.

Supplementary Video S8 Actin disruption after 75-minute treatment with cytochalasin D. Actin is labeled in green with Lifeact::GFP and nucleoli are labeled in red with NPM1::RFP. Cyo-D causes the network to become disrupted and results in puncta formation of the actin. The nucleoli become more mobile and can be seen moving in and out of plane as they sediment. Time reported as minutes : seconds.

Supplementary Video S9 Actin disruption after 30-minute treatment with Latrunculin-A. Actin is labeled in green with Lifeact::GFP. Lat-A disrupts the actin meshwork and results in small, unconnected filaments that are diffusive. Dark bodies are unlabeled nucleoli that can be seen diffusing and moving in and out of plane as they sediment. Time reported as minutes : seconds.
A nuclear F-actin scaffold stabilizes RNP droplets against gravity in large cells
Feric and Brangwynne

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\(^1\). 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, \(\tau\), is given by (see Methods)

\[
MSD(\tau) = 2dD\tau,
\]

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 \(\eta\), obeys the Stokes-Einstein relation\(^2\),

\[
D = \frac{k_B T}{6\pi \eta R},
\]

where \(k_B\) is Boltzmann’s constant, \(T\) 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 \(\eta_{\text{water}}=0.001\pm0.004\) Pa-s, (95% confidence interval), which is in good agreement with the known viscosity of water at 20\(^\circ\)C, \(\eta_{\text{water}}=0.0010019\) Pa-s\(^3\).

For 0.1-1 \(\mu\)m polystyrene particles within actin-disrupted GV\(s\) we found that by plotting their apparent diffusion coefficient vs. size, they also exhibited a size dependence, \(D \sim 1/R\), consistent with the Stokes-Einstein relation. Assuming thermal equilibrium, we fit this data to
determine the oocyte nucleoplasmic viscosity, $\eta = 0.005 \pm 0.002$ 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 micro rheology 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: $m \frac{\partial^2 x}{\partial t^2} = \sum Forces = F_{\text{drag}} - F_g$. 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, $\lambda \eta$, in a simple viscous liquid of viscosity, $\eta$, the drag force for low Reynolds number (Re) motion (for all cases here, $Re<<1$) is given by the Hadamard-Rybczynski formula: $F_{\text{drag}} = \frac{2\pi(2+3\lambda)}{1+\lambda} \lambda \eta R v^4$. We previously determined that RNP
droplet viscosity is several orders of magnitude larger than water\(^5\), suggesting that \(\lambda \gg 1\), and

\[ F_{\text{drag}} = 6\pi \eta R v. \]

The net force due to gravity is given by:

\[ F_g = \frac{4}{3} \pi R^3 \Delta \rho g, \]

where \(\Delta \rho\) is the density difference between the particle and the surrounding fluid, and \(g\) is the gravitational acceleration constant (\(g=9.8 \text{ m/s}^2\)). At steady state, the acceleration is zero, \(\frac{\partial^2 x}{\partial t^2} = 0\), and one obtains:

\[ v = \frac{2R^2 \Delta \rho g}{9\eta}. \]

By tracking the sedimentation velocity of metallic probe particles of known density (1.8 g/cc), we determined a nucleoplasmic viscosity of \(\eta = 0.007 \pm 0.002 \text{ Pa-s}\); 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, \(\eta = 0.005 \pm 0.002 \text{ Pa-s}\), to fit the RNP droplet sedimentation velocities as a function of size, \(\frac{2R^2 \Delta \rho g}{9\eta}\), (Fig. 4K), to determine the density difference, \(\Delta \rho\). We can then proceed to use this measured density difference for nucleoli to determine the magnitude of the net gravitational force, \(F_g\), on large nucleoli (\(R= 10 \text{ µm}\)) in the *X. laevis* GV. We find, \(F_g \sim 1 \text{ pN}\). This is a relatively small force that the actin network must withstand, with corresponding small local stresses of order \(\sigma_x \sim \frac{F_g}{R^2}\) \~ 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 $\lambda \ll 1$ and we assume a drag force of $F_{\text{drag}} = 4\pi \eta R v$ to determine the mineral oil viscosity, $\eta = 0.057 \pm 0.005$ 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: $F_{\text{drag}} = 6\pi \eta R v$. Using the known mineral oil density and measured viscosity, we obtained $\rho_{\text{GV}} = 1.12 \pm 0.03$ 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 $\rho_{\text{water}} = 0.999$ g/cc, then the total density of the nucleoplasm is the sum of mass concentrations of its components (water and protein): $\rho_{\text{GV}} = \rho_{\text{water}} + \rho_{\text{protein}}$. Based on the literate value of $\rho_{\text{protein}} = 0.106$ g/cc, the total density of the nucleoplasm can be estimated as 1.105 g/cc.
Consistent with this, our measurement, $\rho_{GV}=1.12\pm0.03 \text{ g/cc}$, 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 ($k_B T$) and gravitational potential energy, \( \left( \frac{4}{3} \pi R^3 \Delta \rho g \times \ell \right) \). The ratio of these yields a sedimentation length, \( \ell_{sed} = \frac{k_B T}{\frac{4}{3} \pi R^3 \Delta \rho g} \), 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, \( \ell_{sed} \sim R^{-3} \), 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 XL27, primordial cells8, and oogonia8. As shown in
Figure 4B, we found an approximately linear scaling relationship of the form: 

$$R = \beta L_{GF} + \delta,$$

where $\bar{R}$ is the average nucleolar radius, $\beta$ a scaling coefficient, and $\delta$ is an apparent y-intercept; a similar scaling behavior of nucleolar size with cell size has been observed in human sensory ganglia neurons\(^9\). With $R$ and $L_{GF}$ in units of microns, $\beta$ was determined to be $0.0053 \pm 0.0009$ (unitless) and $\delta$ was $1.3 \pm 0.1 \mu m$; 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 $R=0.15-0.25 \mu m$, as found in somatic cells\(^10\), 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 $\Delta \rho=0.04$ 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\(^7\), primordial cells\(^8\), and erythrocytes\(^11\). We observed a roughly linear scaling of the form: \( L_{GV} = \chi L_{cell} \), where \( \chi \) is the karyoplasmic ratio, which we determined to be \( \chi = 0.374 \pm 0.008 \).

Since gravity is expected to dominate within cells when \( \ell_{sed} < L_{GV} \), we sought to identify the critical GV size, \( L_{GV}^* = \ell_{sed} \), at which gravity becomes important. For simplicity, we ignore the y-intercept (i.e. setting \( \delta = 0 \)); 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 \( \bar{R} = \beta L_{GV} \); from a fit to this, we obtain \( \beta = 0.010 \pm 0.001 \) (unitless); we note that independent of how this fit is performed, within a factor of 2 we find that, \( \beta = 0.01 \), and is thus suitable for an order of magnitude estimate. We then substituted the scaling relation, \( \bar{R} = \beta L_{GV} \), into the sedimentation length equation, \( L_{GV} = \left( \frac{k_B T}{4 \pi \rho \bar{R}^3} \right) \) to obtain: \( L_{GV}^* = \left( \frac{3k_B T}{4\pi \beta^3 \Delta \rho g} \right)^{1/4} \). Using our measured values (where \( \Delta \rho \) is taken for nucleoli), we obtain a crossover length scale of \( \approx 40 \) microns, and a critical nuclear body radius of \( \approx 0.4 \) microns. This suggests that gravity becomes increasingly important as nuclei grow beyond the typical size of a somatic nucleus (of order \( \sim 10 \) \( \mu \)m). 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\(^12\), 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.

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