Current Biology

Inverse Size Scaling of the Nucleolus by a Concentration-Dependent Phase Transition

Graphical Abstract



Highlights

- Nucleolar size scales directly with cell size during development
- Nucleolar size scales inversely with cell size across RNAi conditions
- Nucleolar size and assembly depend on the concentration of components
- Direct and inverse scaling regimes can be explained by a phase transition model

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In Brief

Weber and Brangwynne show that nucleolar size is determined by the concentration of maternally loaded components, which condense into nucleoli only above a threshold concentration. This work suggests that intracellular phase transitions may provide a general mechanism for organelle assembly that inherently couples organelle size with cell size.





Report

Inverse Size Scaling of the Nucleolus by a Concentration-Dependent Phase Transition

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Summary

Just as organ size typically increases with body size, the size of intracellular structures changes as cells grow and divide. Indeed, many organelles, such as the nucleus [1, 2], mitochondria [3], mitotic spindle [4, 5], and centrosome [6], exhibit size scaling, a phenomenon in which organelle size depends linearly on cell size. However, the mechanisms of organelle size scaling remain unclear. Here, we show that the size of the nucleolus, a membraneless organelle important for cell-size homeostasis [7], is coupled to cell size by an intracellular phase transition. We find that nucleolar size directly scales with cell size in early C. elegans embryos. Surprisingly, however, when embryo size is altered, we observe inverse scaling: nucleolar size increases in small cells and decreases in large cells. We demonstrate that this seemingly contradictory result arises from maternal loading of a fixed number rather than a fixed concentration of nucleolar components, which condense into nucleoli only above a threshold concentration. Our results suggest that the physics of phase transitions can dictate whether an organelle assembles, and, if so, its size, providing a mechanistic link between organelle assembly and cell size. Since the nucleolus is known to play a key role in cell growth, this biophysical readout of cell size could provide a novel feedback mechanism for growth control.

Results and Discussion

To characterize nucleolar size as a function of cell size, we utilized the stereotypical changes in cell size resulting from the reductive divisions of developing *C. elegans* embryos. We acquired 3D time-lapse images of early embryos expressing a GFP fusion of fibrillarin-1 (FIB-1), a well-conserved nucleolar protein [8]. In the earliest stages (1–2 cell), FIB-1::GFP remains diffuse throughout the nucleoplasm, and no nucleoli are observed (Figure S1A). Beginning at the 4-cell stage, two discrete foci, corresponding to the two nucleolar organizing regions found in diploid *C. elegans*, transiently appear in cell EMS and occasionally in P2 (8 out of 30 embryos; Table S1). In subsequent cell cycles, two bright foci assemble and disassemble in every cell except those of the P (germline) lineage (Figure 1A; Movie S1).

Nucleoli are typically brightest in the 8-cell stage, and their fluorescence intensity subsequently decreases as cells continue dividing (Figures 1A and 1B). We confirmed that this is not due to photobleaching, as images taken of embryos at different stages give similar intensity values. Since the absolute size of these organelles spans the diffraction limit, we used integrated intensity as a metric for nucleolar size (Figures

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S1D and S1F). Nuclear size scales with cell size in *C. elegans* embryos [9], such that the volume ratio is roughly constant: $\xi = V_n/V_{cell}$, where ξ is the karyoplasmic ratio [1, 2] (Figure S1H). Using nuclear size as a proxy for cell size, we find a significant correlation between the maximum nucleolar intensity, I_o , summed over all nucleoli in a given nucleus (Figure S1F), and nuclear volume, V_n , (Figure S1G) for embryos in the 8-cell to 64-cell stages (Figure 1C). Thus, for these embryonic stages, nucleoli tend to be larger in larger cells and smaller in smaller cells, consistent with previous reports of direct scaling of nucleolar size with cell size [2, 10, 11].

Previous models of organelle size scaling [4–6, 12, 13] have proposed that finite pools of components can couple organelle size to cell size. This is due to the fact that, for fixed concentrations, small cells have fewer components than large cells, resulting in proportionately smaller organelles. We found that the integrated intensity of FIB-1::GFP in the nucleoplasm decreases as nucleoli begin assembling (Figure 1D). This depletion suggests that the number of FIB-1 molecules in the nucleoplasm may be limiting for nucleolar assembly, consistent with previous models. However, even at the peak of nucleolar assembly, a significant nucleoplasmic pool remains.

To test whether nucleolar size scaling is indeed a consequence of cell-volume changes, we used RNAi to change embryo size (Figure 2A). Following knockdown of the anillin homolog ANI-2 [14], we observed embryos that are $\sim 25\%$ smaller than control embryos. Surprisingly, instead of a corresponding decrease in organelle size, we found a significant increase in maximum nucleolar intensity in small ani-2(RNAi) embryos compared to control embryos at the 8-cell stage (Figure 2B). ANI-2 plays a role in structurally organizing the syncytial gonad [14], and it is possible that this unexpected result arises from this, or some other, function of ANI-2. To rule out this possibility, we tested a different RNAi condition: knockdown of the importin α IMA-3 [15], which produces even smaller embryos (~55% smaller than control). These small embryos also assembled large nucleoli (Figure 2B). We next sought to increase embryo size using RNAi knockdown of the gene C27D9.1 [16], which results in embryos \sim 55% larger than control. Consistent with the inverse size scaling seen in ani-2(RNAi) and ima-3(RNAi), we found that nucleolar size decreases significantly in large C27D9.1(RNAi) embryos (Figure 2B). We observed similar behavior for DAO-5::GFP, another nucleolar marker [17] (Figure S2A). These RNAi results show that the size of the nucleolus is indeed sensitive to cell volume but in exactly the opposite manner predicted by a limiting component mechanism of direct size scaling.

Interestingly, although nucleolar size scales inversely with cell size across RNAi conditions at a particular developmental stage (e.g., 8-cell stage embryos), within each RNAi condition, we still find direct scaling of nucleolar size with cell size during development (Figure 3A). However, the slopes of these data are not the same. Small embryos (*ima-3* and *ani-2 RNAi*) have greater slopes than large embryos (*C27D9.1 RNAi*). This slope is the ratio of maximum nucleolar intensity to nuclear volume, I_o/V_n , and thus represents an apparent concentration. When we plot I_o/V_n as a function of embryo volume, V, we find a strong inverse relationship (Figure 3B), suggesting that





Figure 1. Nucleolar Size Scales Directly with Cell and Nuclear Volume during Development of Early C. elegans Embryos

(A) Maximum intensity projections of 3D stacks of a control embryo expressing FIB-1::GFP at various stages. 8-cell, 16-cell, and 32-cell stages were taken from a time-lapse movie of a single embryo; 64-cell stage represents a different embryo. Lower panel shows individual nuclei with assembled nucleoli. The scale bar represents 10 µm for whole embryos and 5 µm for individual nuclei.

(B) Integrated intensity in arbitrary units of individual nucleoli as a function of time in a developing control embryo. Colors correspond to cell stage as indicated below. Time was measured relative to nuclear envelope breakdown in cells ABa and ABp.

(C) Direct scaling of maximum nucleolar intensity with nuclear volume for embryos at the 8-cell to 64-cell stages. Data from time-lapse movies (n = 10) and snapshot images (n = 10–15 per stage) are plotted together. Raw data (points) and mean \pm SD for each cell stage (8, circle; 16, square; 32, triangle; 64, diamond) are shown with a linear fit through the origin. $r^2 = 0.15$; p = 9.1 \times 10⁻²³ by two-tailed t test.

(D) The nucleoplasmic pool of FIB-1::GFP is depleted as nucleoli assemble. Mean \pm SD of the integrated intensity of the nucleoplasm and nucleoli are plotted as a function of time for the 8-cell stage AB-lineage nuclei in the embryo are shown in (A). See also Figure S1 and Movie S1.

the concentration of nucleolar components may not be fixed but may instead decrease as embryo size increases.

Prior to the onset of significant zygotic translation, the concentration of a typical protein in the early embryo is established by the concentration loaded into each oocyte. Oogenesis occurs in the syncytial gonad of C. elegans hermaphrodites by cellularizing gonad cytoplasm [18]. Thus, molecules dissolved in this cytoplasm should give rise to the same concentration in all embryos, regardless of embryo size (Figure S2E). However, using a cross between our FIB-1::GFP line and a line expressing mCherry::PH(PLC1 b1) to visualize cell membranes, we found that the nucleus and a single large nucleolus are loaded into oocytes while they are still fully assembled (Figure 3C). The nucleolus eventually disassembles as the oocyte matures, but it is intact when the oocyte closes off from the syncytium, typically around position 5 in wild-type (WT) control animals (Figure 3A, white arrow). The integrated intensity of the nucleolus loaded into the first cellularized oocyte was the same for all RNAi conditions (ani-2, ima-3, and C27D9.1) (Figure 3C, SF), suggesting that the number of nucleolar components loaded into each oocyte is fixed. This is in contrast to centrosomes and mitotic spindles, which are completely disassembled during oogenesis, leading to a fixed concentration of nucleolar components in each oocyte (Figure S2E). Furthermore, we found that the total fluorescence intensity within an embryo is equal to this maternal load until approximately the 128-cell stage (Figure S2G). This indicates that there is no significant zygotic contribution of FIB-1 protein in these early embryos.

The loading of a fixed number of components should result in concentration differences between embryos of different sizes (Figure 3D, inset). Since the total embryonic concentration of a given nucleolar component, C, is equal to the number of molecules, N, divided by the embryo volume, C = N/V, we predicted that small embryos would have a high concentration. To test this prediction, we directly measured FIB-1::GFP intensity in the nucleoplasm of embryonic cells, prior to nucleolar assembly. The average nuclear concentration, C_n, indeed decreases with increasing embryo size across all RNAi conditions (Figure 3D); DAO-5::GFP exhibits a similar, albeit weaker, concentration decrease (Figure S2D). We fit these data to the function C_n = N/(ξ V) to determine the number of FIB-1::GFP molecules loaded per embryo, N = 1.66 ± 0.11 × 10⁵. The



Figure 2. Nucleolar Size Scales Inversely with Nuclear Volume Following RNAi

(A) RNAi knockdown of select genes produces embryos of different size. n = 25 embryos for control; n = 10 embryos for each RNAi condition. Images depict 4-cell stage embryos following RNAi. The scale bar represents 10 µm.

(B) Inverse scaling of maximum nucleolar intensity with nuclear volume across RNAi conditions at the 8-cell stage. Raw data (points) and mean \pm SD for each condition (squares) are shown with the model prediction, $I_o = \alpha[(N/8) - C_{sat}V_n]$ (solid line). n = 25 embryos for control; n = 10 embryos for each RNAi condition. The means are all statistically different; p = 1.3×10^{-34} by ANOVA. Representative images of ABal nuclei are shown for each condition. The scale bar represents 5 μ m.

factor ξ arises because nucleolar components are concentrated within the nucleus, such that the nuclear concentration is scaled by the karyoplasmic ratio: $C_n = C/\xi$. These data show that the concentration of nucleolar components varies across different RNAi conditions, violating the underlying assumption of limiting component models of organelle size scaling—namely, that cells of different sizes have the same component concentration [6, 12, 13].

Nucleoli behave as liquid phase ribonucleoprotein droplets [11, 19], and their assembly could be related to the emerging concept of intracellular phase transitions [20-23]. To test this hypothesis, we developed a simple mathematical model to describe nucleolar assembly, based on the physics of phase transitions [24, 25] (see Supplemental Experimental Procedures). At steady state, we assume that nucleolar components partition between a condensed droplet phase and a soluble pool with concentration C_{sat}. The saturation concentration Csat represents a threshold: if the nuclear concentration is below C_{sat}, nucleoli do not assemble, and FIB-1 remains soluble. When the nuclear concentration is above C_{sat}, molecules from the soluble pool condense into nucleolar droplets, depleting the nucleoplasm until its concentration reaches C_{sat}. The final size of the nucleolus is thus determined by the difference between the total concentration in the nucleus and this saturation concentration: $I_o = \alpha [C_n - C_{sat}]V_n$. Here, α is the intensity per molecule.

In a developing embryo, the nucleolus is disassembled during each mitotic cleavage, and nucleolar components are allocated proportionately to daughter cells. Therefore, C_n is fixed during early development, and the model predicts direct scaling of nucleolar size with nuclear and cell volume: $I_o \sim V_n \sim V_{cell}$. However, across RNAi conditions, C_n changes (Figure 3D). By expressing the nuclear concentration as $C_n =$ N/(ξ V), as above, we obtain a master scaling equation, $I_o =$ α [N/(V ξ) – C_{sat}]V_n. Using the relation V \approx mV_{cell} = mV_n/ ξ , where the parameter m indicates a particular developmental stage (e.g., 8-cell stage: m = 8), we can write $I_o = \alpha[(N/m) - C_{sat}V_n]$. Thus, across RNAi conditions, where N is fixed, the model predicts an inverse scaling relationship at a given cell stage, with larger nuclei assembling smaller nucleoli and vice versa. A schematic diagram illustrating the model's prediction of direct versus inverse scaling regimes is shown in Figure 3E.

To quantitatively test this model, we compared both the direct and inverse scaling data with the prediction of our master equation. From independent experiments, we directly measured the value of each parameter: α , N, ξ , and C_{sat} (see Supplemental Experimental Procedures). The model predictions show good agreement with experiment for both the direct scaling regime, $I_o/V_n = \alpha[N/(V\xi) - C_{sat}]$ (Figure 3B), and the inverse scaling regime, $I_o = \alpha[(N/m) - C_{sat}V_n]$ where m = 8 (Figure 2B). This agreement is remarkable, given that the prediction involves zero free parameters rather than a fit to the model.

The role of the saturation concentration, C_{sat} , can be highlighted by plotting the maximum nucleolar intensity for a given size nucleus, $I_o(V_n = 200 \ \mu m^3)$, as a function of nuclear concentration for all RNAi conditions. As nuclear concentration decreases and approaches C_{sat} , nucleoli become smaller (Figure 4A, circles).

The dependence of nucleolar size and assembly on nuclear concentration can be summarized in a phase diagram (Figure 4B). Here, C_{sat} represents the boundary between nucleoplasm consisting of a single phase of dissolved nucleolar components and nucleoplasm that has phase separated to form condensed nucleoli that coexist with a dissolved phase of concentration C_{sat} . The nuclear concentration for each RNAi condition falls above C_{sat} , within the phase-separated region, consistent with the fact that nucleoli always assemble in 8-cell to 64-cell stage embryos. Although we were unable to experimentally reduce nuclear concentration below C_{sat} , our model predicts that nucleoli would not assemble when $C_n < C_{sat}$ and nucleolar components would remain dissolved in the nucleoplasm.

Interestingly, nucleoli indeed do not assemble in very early embryos, suggesting that there is some developmentally regulated parameter, which we call χ (see Supplemental Experimental Procedures), that shifts the phase boundary and thus increases the saturation concentration. Specifically, the anterior cells ABa and ABp do not assemble nucleoli in 4-cell stage control embryos (Figure S1A; Table S1). Remarkably, however, we could induce nucleolar assembly in these early blastomeres by decreasing embryo size and thus increasing the concentration of nucleolar components. Experimental measurements of C_{sat} in the 4-cell stage, $C_{sat}^{4-cell} = 0.18 \pm$ 0.04 µM, indicate that the nuclear concentrations in our RNAi conditions span this phase boundary, such that nucleoli do not assemble in embryos where $C_n < C_{sat}^{4-cell}$ (C27D9.1(RNAi) and control), while they do assemble in embryos where C_n > C_{sat}^{4-cell} (ima-3 RNAi) (Figure 4B). The ani-2(RNAi) condition presents an interesting case where $C_n \approx C_{sat}^{4-cell}$. Consistent with a close proximity to the phase boundary, nucleoli in ABa cells of ani-2(RNAi) embryos are either very small or not detected at all (13 out of 28 embryos; Table S1).

To further test our model, we sought to change nuclear concentration by manipulating the maternal load of nucleolar



Figure 3. Maternal Loading of an Intact Nucleolus Results in Concentration Differences that Explain Direct and Inverse Scaling Regimes

(A) Direct scaling of maximum nucleolar intensity with nuclear volume during development in each RNAi condition; inverse scaling across RNAi conditions. Raw data (points) and mean \pm SD across 50-µm bins (squares) are shown for embryos at the 8-cell to 64-cell stages. Raw data for each RNAi condition were fit to a line through the origin to determine the slope, I_o/V_n . n = 20-25 embryos per stage for control; n = 8-15 embryos per stage for each RNAi condition. (B) Fitted slopes from (A) are plotted as a function of mean embryo volume for each RNAi condition. Error bars indicate 95% confidence intervals. The master scaling equation, $I_o/V_n = \alpha[N/(V\xi) - C_{sat}]$, is plotted with zero-free parameters (solid line). Dashed lines represent the range of uncertainty in model parameters.

(C) Nucleoli are loaded into oocytes intact. Integrated intensity (mean \pm SD) of nucleoli in the first cellularized oocyte in the hermaphrodite gonad for each RNAi condition (n = 10 oocytes per condition) is shown. Wild-type (WT) RNAi conditions are not statistically different; p = 0.73 by ANOVA. *ncl-1* is statistically different from all WT RNAi conditions; p = 0.0038 by ANOVA. Image shows WT control gonad expressing fluorescent markers for cell membranes (red) and nucleoli (green). White arrow indicates the intact nucleolus loaded into an oocyte.

(D) Nuclear concentration decreases with increasing embryo volume. Raw data (points) and mean \pm SD for each condition (squares) are shown with a fit to the equation $C_n = N/(\xi V)$ for WT embryos (filled markers; solid line) and *ncl-1* mutant embryos (open markers; dashed line). n = 15 embryos for WT control; n = 10 embryos for WT, *ima-3(RNAi)*; n = 14 embryos for WT, *ani-2(RNAi)*; n = 11 embryos for WT, *C2TD9.1(RNAi)*; n = 10 embryos for *ncl-1* control; n = 12 embryos for *ncl-1*, *ima-3(RNAi)*; n = 16 embryos for *ncl-1*, *ani-2(RNAi)*; n = 18 embryos for *ncl-1*, *C2TD9.1(RNAi)*. Inset: schematic diagram of nucleoli loaded into occytes of different size that subsequently disassemble to yield different concentrations in the embryos.

(E) Schematic diagram illustrating the direct and inverse scaling regimes. See also Figure S2.

components (i.e., the parameter N), in addition to the embryo volume (V). Mutants of the BRAT homolog NCL-1 exhibit enlarged nucleoli throughout the body [26]. Indeed, the size of the nucleolus loaded into *ncl-1(e1942)* oocytes is nearly 2-fold larger than WT (Figure 3C). The nuclear concentration of

FIB-1::GFP in *ncl-1* mutant embryos also depends on embryo volume (Figure 3D), and we fit this data to the equation $C_n^{ncl1} = N^{ncl1}/(\xi V)$ to determine the number of molecules loaded per embryo: $N^{ncl1} = 4.45 \pm 0.45 \times 10^5$, approximately 2.7 times greater than WT embryos. As in WT embryos, nucleolar size



Figure 4. A Concentration-Dependent Phase Transition Controls Nucleolar Size and Assembly (A) Maximum nucleolar intensity increases with nuclear concentration above C_{sat} for a given nuclear volume, $V_n = 200 \ \mu m^3$. Circles correspond to nucleoli at the 8-cell stage; squares correspond to nucleoli at the 8-cell stage; squares correspond to nucleoli at the 4-cell stage. Solid line is the model's prediction, $I_o = \alpha [C_n - C_{sat}]V_n$, for the 8-cell stage. Dashed line is the model's prediction, $I_o = \alpha [C_n - C_{sat}^{4-cell}]V_n$, for the 4-cell stage.

(B) Phase diagram for nucleolar assembly. Asterisks mark the measured saturation concentration at the 4-cell and 8-cell stages. Circles correspond to embryos at the 8-cell stage; squares correspond to embryos at the 4-cell

stage. Open symbols indicate no nucleolar assembly. Lines from (A) correspond to horizontal lines on the phase diagram. Representative images of ABal nuclei are shown for 8-cell stage embryos; ABa nuclei are shown for 4-cell stage embryos. See also Figure S3 and Table S1.

peaks at the 8-cell stage, but the maximum integrated intensity in *ncl-1* mutants is more than twice that of WT embryos (Figure S1B). Consistent with our finding that decreasing embryo size induces nucleolar assembly in early blastomeres, ABa and ABp assemble nucleoli in *ncl-1* embryos of any size. Thus, by increasing nuclear concentration through changing either N or V, we could cross the phase boundary and induce nucleolar assembly (Figures S1A and S3).

Our results demonstrate that nucleoli assemble in a cellsize-dependent manner, which has important implications for cell growth and size control. The connection between organelle size and cell size is mediated through the concentration of nucleolar components. Below a threshold concentration, nucleoli do not assemble. Above this threshold, the higher the concentration, the larger the size of the assembled nucleolus. Threshold concentrations are a hallmark of phase transitions [24, 25], strongly suggesting that nucleolar assembly represents an intracellular phase transition. Such concentration-dependent phase transitions may represent a general biophysical framework for understanding organelle assembly and scaling [27].

Experimental Procedures

C. elegans strains were maintained using standard techniques.

Imaging

Embryos were dissected from gravid hermaphrodites and imaged on M9-agarose pads. Images were acquired on a two-photon laser scanning system custom built around an upright Olympus BX51 microscope. Emitted light was collected with a 40×/NA 0.8 water immersion objective and an NA 1.3 oil immersion condenser and detected with high quantum efficiency GaAsP photomultiplier tubes (Hammamatsu). 3D volumes were acquired using an objective piezo controlled by ScanImage software [28].

Image Analysis

Images were analyzed with custom software in MATLAB. Nucleolar intensity was calculated by summing the fluorescence intensity within objects detected with a 3D band-pass filter.

Concentration Estimates

Pixel intensities in the nucleoplasm were calibrated using purified Histagged FIB-1::GFP.

Model Parameters

All model parameters were measured independently to produce a zero-free parameter prediction. For details on the model and parameter estimation, please refer to the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, one table, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.01.012.

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Current Biology Supplemental Information

Inverse Size Scaling of the Nucleolus

by a Concentration-Dependent

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Supplemental Data





Figure S1, related to Figure 1.

Quantifying nucleolar size and nuclear volume in early C. elegans embryos.

(A) Maximum intensity projections of 3D stacks of wild-type (WT) and *ncl-1(e1942)* mutant embryos expressing FIB-1::GFP at the pronuclear, 2-cell and 4-cell stages. Note that nucleoli assemble in all nuclei in *ncl-1* mutants, but not until cell EMS (Table S1) and only robustly in the 8-cell stage in WT. Scalebar = $10 \mu m$.

(B) Integrated intensity in arbitrary units of individual nucleoli as a function of time in a developing ncl-1(e1942) embryo. Colors correspond to cell stage as indicated below. Time was measured relative to nuclear envelope breakdown in cells ABa and ABp.

(C) Intensity profiles of a 1 μm bead in the xy and xz planes. 2D and 3D Gaussian fits are shown.

(D) Comparison of the integrated intensity (mean \pm SD) beneath a 2D or 3D Gaussian fit with our bandpass/threshold method for 0.2, 0.5, 1 and 2 μ m beads.

(E) A montage of maximum intensity projections of a single 8-cell stage nucleus (ABpl from Figure 1A) over time.

(F) The integrated intensity, calculated using a bandpass filter and threshold, of individual nucleoli (blue) and the sum over all nucleoli and FIB-1::GFP foci (black) as a function of time for the nucleus shown in Figure S1E. We define the maximum nucleolar intensity, I_o, as the maximum value of the sum.

(G) Maximum intensity projections of the original and segmented images of the 8-cell stage embryo expressing FIB-1::GFP shown in Figure 1A. These images were acquired on a two photon laser scanning microscope. Nuclei were segmented using Otsu thresholding and their diameters (blue lines) were used to calculate nuclear volume.

(H) The karyoplasmic ratio is approximately constant for 8-cell to 64-cell stage embryos across all RNAi conditions. Nuclear volume of the AB lineage in 8-cell to 64-cell stage embryos imaged on a two photon laser scanning microscope is plotted as a function of average cell volume. The x-axis is an approximation of cell size, assuming that the embryo volume V is divided into m cells of equal size. The dashed line represents the karyoplasmic ratio calculated independently from the ratio of concentrations in the cytoplasm following nuclear envelope breakdown and the nucleoplasm prior to nucleolar assembly: $\xi = C_{cell}/C_n = 0.061$. Large embryos at the 8-cell stage exhibit a slightly smaller karyoplasmic ratio, consistent with a previous report [S1].

(I) Maximum intensity projections of the original and segmented images of an 8-cell stage embryo expressing both FIB-1::GFP (nucleoli) and EMR-1::mCherry (nuclear membrane). These images were acquired on a spinning disk confocal microscope, which enables dual-color acquisition but suffers from attenuation such that only ~half the embryo can be visualized. Nuclei were segmented using Otsu thresholding for FIB-1::GFP images and a marker-based watershed algorithm for EMR::mCherry images.

(J) A comparison of the nuclear volume (mean \pm SD) calculated using each marker for n = 10 AB, 8-cell stage nuclei.





Figure S2, related to Figure 3.

Maternal loading of an assembled nucleolus gives rise to direct and inverse scaling regimes for FIB-1::GFP and additional nucleolar marker DAO-5::GFP.

(A) Maximum intensity projections of 3D stacks of embryos expressing DAO-5::GFP at the 8-cell stage following RNAi treatment.

(B) Direct scaling of maximum nucleolar intensity with nuclear volume during development in each RNAi condition; inverse scaling across RNAi conditions for embryos expressing DAO-5::GFP. Raw data (points) and mean \pm SD across 50-µm bins (squares) are shown. Raw data for each RNAi condition were fit to a line through the origin to determine the slope, I_0/V_n . n = 3 embryos per RNAi condition.

(C) Fitted slopes from panel B are plotted as a function of mean embryo volume for each RNAi condition. Error bars are 95% confidence intervals. Raw data was fit to the master scaling equation, $I_o = \alpha [N/(V\xi)-C_{sat}]V_n$, with αN and αC_{sat} as free parameters (solid line).

(D) Nuclear concentration of DAO-5::GFP decreases with increasing embryo volume. Raw data (points) and mean \pm SD for each condition (squares) are shown with a fit to the equation $C_n = N/(\xi V)$.

(E) Schematic diagram illustrating the consequences of organelle loading. When an organelle is disassembled during oogenesis, the concentration of components is fixed across oocytes of different size. When an organelle is assembled during oogenesis, the number of components is fixed across oocytes of different size, leading to differences in concentration.

(F) Maximum intensity projections of oocytes in the hermaphrodite gonad for each RNAi condition and the *ncl-1(e1942)* mutant. Red channel is mCherry::PH(PLC1 δ 1); green channel is FIB-1::GFP. White arrows indicate the intact nucleolus loaded into an oocyte.

(G) The fluorescence intensity of FIB-1::GFP loaded into oocytes ("maternal load") can account for the total fluorescence intensity found throughout the embryo from the 1-cell to 64-cell stages. Evidence of zygotic expression can be seen beginning at the 128-cell stage, where fluorescence intensity dramatically increases. n = 10-15 embryos per stage.

(H) Analytical results from the concentration-dependent phase transition model. With no partitioning ($C_{sat} = 0$, such that the organelle is an infinite sink for components), the fixed concentration constraint leads to direct scaling with nuclear volume, while the fixed number constraint results in a constant organelle size, independent of nuclear volume. Color corresponds to embryo size.

(I) When $C_{sat} > 0$, the nucleoplasmic pool limits organelle assembly in large nuclei, giving rise to an inverse scaling regime.

(J) The apparent concentration, I_o/V_n , decreases as a function of embryo volume. The decreases occurs more dramatically when $C_{sat} > 0$.



Figure S3, related to Figure 4.

Phase diagram for nucleolar assembly. *ncl-1* embryos fall above the phase boundary in all developmental stages and all RNAi conditions.

Table S1, related to Figure 4.

Condition	ABa/ABp	EMS	P2
L4440 (RNAi),	0/30 (0%)	30/30 (100%)	8/30 (27%)
negative control	0/50 (0/0)	50/50 (100/0)	0/50 (27/0)
ani-2 (RNAi)	15/28 (53%)	28/28 (100%)	16/28 (57%)
ima-3 (RNAi)	26/26 (100%)	26/26 (100%)	26/26 (100%)
C27D9.1 (RNAi)	0/25 (0%)	7/25 (28%)	3/25 (12%)
ncl-1(e1942)	24/24 (100%)	24/24 (100%)	24/24 (100%)

Fraction of embryos (percent) with nucleoli assembled in each lineage at the 4-cell stage.

Supplemental Experimental Procedures

Construction of transgenic worm lines:

GFP-tagged fosmid lines were kindly provided by Tony Hyman (MPI-CBG) [S2]. To visualize the nuclear envelope, *emr-1* was amplified from genomic DNA and cloned into plasmid TH313 [S3], which contains the *pie-1* promoter and a C-terminal mCherry tag, using SpeI and SmaI restriction sites. This construct was randomly integrated into the worm genome by microparticle bombardment [S4].

Strain Name	Genotype	Source	
	fib-1::gfp	[S2]; clone 445429768005125 D03	
Crbuui	https://transgeneome.mpi-cbg.de/transgeneomics/public/clone.html?wellId=32516341		
CF2218	ncl-1(e1942)	CGC	
OD70	mCherry::PH(PLC1δ1)	CGC	
CPB008	CPB001xCF2218	cross	
CPB009	CPB001xOD70	cross	
CPB011	dao-5::gfp	[S2]; clone 37513532900044066 A01	
	https://transgeneome.mpi-cbg.de/transgeneomics/public/clone.html?wellId=32556468		
CPB065	ptnIs035[Ppie1::emr-1::mCherry + Cbunc-119(+)]		
CPB064	CPB001xCPB065	cross	

Transgenic C. elegans lines

Worm maintenance and imaging:

C. elegans lines were maintained at 20° C on NGM plates seeded with OP50 bacteria. Embryos were dissected from gravid hermaphrodites and imaged on M9-agarose pads. Oocytes were imaged in adult hermaphrodites anesthetized with 1% levamisole hydrochloride in M9.

Timelapse movies and still images were acquired on a two-photon laser scanning system custombuilt around an upright Olympus BX51 microscope. An excitation wavelength of 960 nm was used to visualize GFP while maintaining embryo viability. Emitted light was collected with a 40X/NA0.8 water immersion objective and an NA1.3 oil immersion condenser and detected with high quantum efficiency GaAsP photomultiplier tubes (Hammamatsu). 3D volumes were acquired using an objective piezo controlled by ScanImage software [S5].

Images of dual-labeled worm lines were taken on an inverted Zeiss Axio Observer Z1 microscope equipped with a Yokogawa CSU-X1 confocal spinning disk (Intelligent Imaging Innovations) and a QuantEM 512SC camera (Photometrics). Oocytes were imaged with a 40X/NA1.4 oil immersion objective; embryos were imaged with a 100X/NA1.4 oil immersion objective.

RNAi experiments were performed by picking L4 larvae onto NGM plates containing 1 mM IPTG and 100 μ g/mL ampicillin, seeded with feeding clones from the Ahringer library [S6]. The

empty vector L4440 served as a negative control for all experiments. Worms were allowed to feed and grow at 20°C for 36-48 hrs before embryos were harvested for imaging. Knockdown of *ima-3* results in embryonic lethality [S7]. After 36 hrs on *ima-3* RNAi feeding plates, hermaphrodites contain small embryos that successfully complete early divisions in which FIB-1::GFP and DAO-5::GFP enter and exit the nucleus. However, all of these embryos fail to hatch.

RNAi feeding clones

Gene Name	Sequence
negative control	L4440
ani-2	K10B2.5
ima-3	F32E10.4
-	C27D9.1
ncl-1	ZK112.2

Image analysis:

Images were analyzed with custom software in Matlab. Nucleoli were detected by first convolving the raw z-stacks with a 3D bandpass filter. A Gaussian kernel was used to smooth high-frequency noise. A low-frequency boxcar kernel was used to remove objects larger than the largest nucleolus typically observed in *ima-3(RNAi)* embryos. The difference between the low-pass and high-pass images was thresholded; the threshold value was determined empirically to retain small/dim nucleoli while minimizing detection of background/autofluoresence as much as possible. The same kernel sizes and threshold value were used for all RNAi conditions acquired on the same microscope (i.e. same values for all movies taken on the two photon; different values for all images taken on the spinning disk). The integrated intensity of a nucleolus was calculated by summing the fluorescence intensity within each detected object. Comparison of our method to 2D and 3D Gaussian fits of beads is shown in Figure S1D. 3D Gaussian fitting of nucleoli was not robust and frequently failed to converge or expanded to fit the entire nucleus.

The maximum nucleolar intensity per nucleus, I_o, was obtained by taking the maximum value of the sum of integrated intensities for all nucleoli within a nucleus (see Figure S1F).

Nuclei were segmented using Otsu thresholding of FIB-1::GFP or DAO-5::GFP fluorescence. To validate this approach, we crossed an EMR-1::mCherry line into our FIB-1::GFP line to directly visualize the nuclear envelope. We used a marker-based watershed algorithm to segment the nuclear envelope. Nuclear volume was calculated using the diameter (d) extracted from the image plane with the largest nuclear area and assuming spherical geometry: $V_n = 4\pi (d/2)^3/3$. Results from both markers are shown in Figure S1J. FIB-1::GFP measurements are lower than EMR-1::mCherry, but these more accurately reflect the volume within which the soluble pool of nucleolar components resides.

The integrated intensity in the nucleoplasm was determined by subtracting the sum of the integrated intensities of all nucleoli and FIB-1::GFP foci (as shown in Figure S1F) from the total intensity inside segmented nuclei.

Embryo volume was calculated using the length (l) and width (w) of the eggshell at the midplane and assuming ellipsoidal geometry: $V = \pi l w^2/6$. These measurements were made at the 4cell stage; however, embryo volume does not change with time or cell stage.

Nuclear concentration, C_n , was measured by calculating the average pixel intensity in nuclei of 4-cell stage embryos prior to nucleolar assembly (if any). Pixel intensity was converted to concentration units as described below.

Calibrating pixel intensity:

His-tagged FIB-1::GFP was purified from *E. coli* using standard Ni affinity chromatography procedures. Protein concentration was determined by a Bradford assay and confirmed spectrophotometrically. A dilution series of purified protein was imaged with the same acquisition settings as embryos and used to generate a standard curve, I = a[FIB-1::GFP]. The slope of this curve, $a = 3.1x10^3$ intensity/pixel/µM, was used to estimate the nuclear concentration, C_n ; the number of FIB-1::GFP molecules per embryo, N; and the saturation concentration, C_{sat} (see below).

Phase Transition Model:

We consider the nucleus as a two-phase system containing a mixture of the bulk nucleoplasm and the nucleolus. At the beginning of each cell cycle, these two liquid phases are fully mixed, with nucleolar components dissolved throughout the nucleoplasm. As the cell cycle progresses, the nucleoplasm undergoes a de-mixing phase transition in which nucleolar components condense to form a distinct phase: the nucleolus. The maximum size of the nucleolus is reached when the concentration of nucleolar components remaining in the nucleoplasm is reduced to the saturation concentration, C_{sat} .

We make two simplifying assumptions before examining the scaling predictions of this model. First, the surface tension, γ , of nucleolar droplets is small and can be ignored. Surface tension represents an energetic cost of creating an interface, and can contribute to both the kinetics of droplet assembly and the equilibrium size of droplets. However, γ is expected to be small for macromolecular liquid phases; γ for nucleoli in *X. laevis* germinal vesicles was estimated to be ~10 μ N/m [S8], which is 10⁴-fold smaller than the air-water interface. Thus, here we ignore the effect of the surface energy on nucleolar assembly.

Second, this model assumes that the de-mixing phase transition in the nucleoplasm approaches equilibrium. The cell cycle in early embryos progresses rapidly, ~20 min for the AB lineage at the 8-cell stage, and we do not observe a long plateau in nucleolar intensity for these early stages (Figure 1B). This raises the possibility that the nucleoplasm does not have sufficient time to reach equilibrium before the cell divides. However, we frequently see plateaus in nucleolar intensity in the posterior lineages of the 16-cell stage and all lineages of the 32-cell and 64-cell stage, suggesting that equilibrium has been reached in these later, longer cell cycles. If nucleoli in the 8-cell stage have not fully equilibrated, then the observed maximum nucleolar intensity

will be less than that predicted by our model. This could contribute to the variability observed in nucleolar size at the 8-cell stage.

Under these assumptions, the maximum size of the nucleolar phase is determined by the supersaturation of the nucleoplasm. When the nucleoplasm is undersaturated, $C_n < C_{sat}$, nucleoli do not assemble and nucleolar components remain dissolved throughout the nucleoplasm. When the nucleoplasm is supersaturated, $C_n > C_{sat}$, nucleolar components in the nucleoplasm condense into nucleolar droplets, depleting the nucleoplasm until its concentration reaches saturation, $C_n \approx C_{sat}$. Thus, the size of the nucleolus depends on the degree of supersaturation: $C_n - C_{sat}$. To compare directly with experiments, we express the size of the condensed phase in terms of intensity, such that $I_o = \alpha [C_n-C_{sat}]V_n$, where α is the intensity per molecule.

Now we examine the scaling behavior of the nucleolar phase as a function of nuclear volume under two different constraints. First, we fix the *concentration* of nucleolar components, C, to simulate development. Second, we fix the *number* of nucleolar components, N, to simulate maternal loading of an intact nucleolus into oocytes of different size following RNAi.

1. Within a developing embryo, nucleolar components are dispersed at mitosis and allocated proportionately to daughter cells, yielding a constant concentration of nucleolar components within cells of different size. Thus, the total concentration of molecules within any given blastomere is the same as the total concentration in the embryo, $C_{cell} = C$. However, these molecules are concentrated into the nucleus, yielding a nucleoplasmic concentration, $C_n = C/\xi$. The nucleolus then assembles to a size given by

$$I_o = \alpha \left[\frac{C}{\xi} - C_{sat} \right] V_n. \quad \text{Eq. 1}$$

Since C, ξ and C_{sat} are roughly constant from the 8-cell to 64-cell stage in a developing embryo, we predict a direct scaling between nucleolar size and cell size: $I_o \sim V_n \sim V_{cell}$.

2. Across RNAi conditions, N is fixed, rather than C. We can express C in terms of N and V to obtain a master scaling equation,

$$I_o = \alpha \left[\frac{N}{\xi V} - C_{sat} \right] V_n . \text{ Eq. } 2$$

Since N is fixed, the scaling proportionality depends inversely on embryo volume. Thus, for a given cell/nucleus volume, larger embryos (larger V) will have smaller organelles and vice versa. The inverse scaling prediction can be seen more clearly when we look at a particular developmental stage. For example, $V = m^*V_{cell} = m(V_n/\xi)$, where m = 8 for the 8-cell stage. Substituting into Eq. 2, we find

$$I_o = \alpha \left[\frac{N}{m} - C_{sat} V_n \right], \text{ Eq. 3}$$

yielding a linear relationship between I_o and V_n with a negative slope, $-\alpha C_{sat}$.

In Figure S2H, we plot the results of our analytical model under the fixed C and fixed N conditions. Previous work has modeled organelle scaling using a limiting component model that treats the organelle as an infinite sink, into which all components eventually assemble [S9, S10]. In our model, this corresponds to the case where $C_{sat} = 0$. For this limit, organelle size scales

directly with cell (nuclear) size when C is fixed. However, there is no scaling when N is fixed: organelle size is the same in all cells, regardless of their size (Figure S2H, Eq. 3). Therefore, the simple limiting component model cannot account for the inverse size scaling observed experimentally across RNAi conditions.

Indeed, to obtain inverse scaling, C_{sat} must be greater than zero. When $C_{sat} > 0$, our model predicts direct scaling when C is fixed (albeit with a shallower slope) and inverse scaling when N is fixed (Figure S2I, Eq. 3), just as observed experimentally. The qualitatively distinct behavior that arises when $C_{sat} > 0$ highlights the novel feature of our model: namely, that the condensed organelle phase coexists with a soluble phase.

Finally, we plot the slope of the direct scaling curves as a function of embryo volume (Figure S2J). In both cases, the apparent organelle concentration, I_o/V_n , decreases with increasing embryo volume. However, I_o/V_n decreases more rapidly when $C_{sat} > 0$. This is because more components are required to maintain the saturation concentration in large nuclei compared to small nuclei, so fewer excess components are available to assemble into the organelle. Eventually, for very large embryos, the nucleoplasmic concentration falls below C_{sat} and no nucleolus assembles.

Estimating model parameters:

All model parameters were measured independently to produce a zero-free parameter prediction. Parameter values are reported in the following table and were estimated as described below.

Parameter	Units	Measured (mean \pm std)
α	intensity/molecule	39.4 ± 4.4
Ĩ	-	0.061 ± 0.004
N (method 1)	molecules	$1.66 \pm 0.11 \text{ x} 10^5$
N (method 2)	molecules	$1.52 \pm 0.65 \text{ x}10^5$
C _{sat}	molecules/µm ³	43 ± 11
C _{sat}	μM	0.071 ± 0.02
C_{sat}^{4-cell}	molecules/µm ³	107 ± 25
C_{sat}^{4-cell}	μM	0.178 ± 0.04
N ^{ncl1}	molecules	$4.45 \pm 0.45 \text{ x}10^5$

Model Parameters

 α , the intensity per molecule, was calculated from the slope of the standard curve of purified FIB-1::GFP, a. Voxel size was calculated using the axial width of the two-photon excitation volume [S11].

 $\alpha = a^{(1 \text{ pixel}/(0.22^{\circ}0.22^{\circ}2.7 \ \mu\text{m}^3) * 1 \ \mu\text{M}/(602 \ \text{molecules}/\mu\text{m}^3) = 39.4 \ \text{intensity/molecule}$

The karyoplasmic ratio was estimated using the ratio of FIB-1::GFP concentration in the cytoplasm following nuclear envelope breakdown (such that nucleolar components were dispersed throughout the cell) and in the nucleoplasm prior to nucleolar assembly: $\xi = C_{cell}/C_n =$

0.061. This value is consistent with data from Hara and Kimura [S1], from which we estimate $\xi = 0.04$, assuming spherical geometries for both nuclei and cells.

Two methods were used to estimate the number of FIB-1::GFP molecules loaded per embryo. First, we fit an inverse function, $C_n = N/(\xi V)$, to the nuclear concentrations measured in embryos of different size (Figure 3C, main text): $N = 1.66 \pm 0.11 \times 10^5$. Second, we directly measured the concentration in the cytoplasm following nuclear envelope breakdown and multiplied by embryo volume: $N = C_{cell}*V = 1.52 \pm 0.65 \times 10^5$.

To estimate C_{sat} , we directly measured the nucleoplasmic concentration in 8-cell stage embryos at the time point when nucleoli reached their maximum size, I_o. $C_{sat} = 43 \pm 11$ molecules/ μ m³ = 0.071 ± 0.02 μ M. Additionally, we measured C_{sat}^{4-cell} in cells ABa and ABp in all *ima-3(RNAi)* embryos and in *ani-2(RNAi)* embryos that assembled nucleoli. $C_{sat}^{4-cell} = 107 \pm 25$ molecules/ μ m³ = 0.178 ± 0.04 μ M.

Limiting component:

It is important to recognize that while C_n and C_{sat} refer to FIB-1::GFP concentrations, FIB-1 is not necessarily an essential nucleolar component. Indeed, as a consequence of intact nucleolus loading, the concentration of all nucleolar proteins in our experiments is expected to change correspondingly, i.e. $C_{n,i} \stackrel{=}{\prec} \boldsymbol{\zeta}_i C_n$, where i indicates a given nucleolar component whose concentration relative to that of fibrillarin is given by the stoichiometric coefficient $\boldsymbol{\zeta}_i$. However, it is possible that the composition of the nucleolus is not fixed throughout development or across RNAi conditions, such that the relative amount of a given component may vary with respect to fibrillarin. Indeed, the concentration of DAO-5 does not depend as strongly on embryo volume as expected (Figure S2D). Nevertheless, maternal loading of an intact nucleolus into oocytes of different size represents a useful experimental tool to tune the concentration of the majority of nucleolar components in a simple, concerted fashion.

Developmental parameter, χ:

We find qualitatively different nucleolar behavior at the 4-cell stage compared to subsequent stages. While nucleoli readily assemble in 8-cell stage nuclei and beyond, they rarely appear in WT embryos at the 4-cell stage (Table S1). When they do assemble, in cell EMS, nucleoli are small and do not scale with nuclear volume according to the same relationship as later stages. Nucleoli do not appear in cells ABa and ABp, despite the fact that $C_n > C_{sat}$. However, increasing the nucleoplasmic concentration further, either by decreasing embryo volume (*ima-3(RNAi)*) or by increasing the number of nucleolar components (*ncl-1(e1942)*), we can induce nucleolar assembly in these cells. Therefore, nucleolar assembly still depends on concentration but the saturation threshold is higher: $C_{sat}^{4-cell} > C_{sat}$.

Phase transitions are controlled by the thermodynamic properties of the system. For example, in the condensation of water vapor into droplets on a cold mirror, temperature is the relevant variable. However, in a biological context, molecular concentrations or the degree of post-translational modifications are more appropriate variables. Thus, we hypothesize that an interaction parameter, χ , and therefore C_{sat}, may be developmentally regulated through a post-

translational modification that renders key nucleolar components more or less likely to interact with itself or other nucleolar components.

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