Supplementary Materials for

Changes in Cytoplasmic Volume Are Sufficient to Drive Spindle Scaling

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Other Supplementary Material for this manuscript includes the following:
available at www.sciencemag.org/content/342/6160/853/suppl/DC1

Movies S1 to S5
**Materials and Methods**

**Spindle assembly in Xenopus egg extracts**

Cytostatic factor (CSF)-arrested egg extracts were prepared as described previously (12, 26). Following the addition of de-membranated sperm nuclei, extract was incubated at 18°C for 1 h to allow for DNA replication and the formation of interphase nuclei. During this incubation step, fluorophores were added to the extract to aid in subsequent visualization. The extract was then driven into metaphase by addition of an equal volume of CSF-extract just before loading into microfluidic devices. Metaphase-arrested spindles were visualized by incorporation of bovine tubulin labeled with Alexa Fluor 488 or 568 dye (Sigma, prepared in house; (27, 28) and DAPI. To facilitate spindle length measurements, pole localization of NuMA was visualized using anti-NuMA antibodies directly labeled with Alexa Fluor 488 (e.g. (22))

**Microfluidic device fabrication**

Microfluidic devices were cast in polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) using well-established soft lithography techniques, which are described in detail elsewhere (29, 30). Briefly, a microchannel network was designed in AutoCAD (Autodesk, Inc) drafting software. The photomask was output to film (CAD/Art Services, Bandon, OR) and used to lithographically expose the network in a photoresist film (SU-3025, MicroChem, Newton, MA) spun onto a silicon wafer at 30 µm. Polydimethylsiloxane (PDMS) elastomer was then poured over the patterned wafer, cured at 70°C, and removed, producing an imprinted channel network. Fluid inlet and outlet ports were punched into the PDMS with a sharpened, unbeveled syringe tip (Brico Medical, Dayton, NJ). The PDMS channel network was exposed to oxygen plasma (Harrick Plasma, Ithaca, NY) and placed in contact with a cover glass slip (#1.5, Thomas Scientific) to form an irreversible bond. A simplified microchannel network design is shown in Figure 1A.

**Microfluidic encapsulation of Xenopus egg extract**

Xenopus extract encapsulation experiments were carried out in single-use PDMS microfluidic devices utilizing classic T-junction microfluidic droplet generators (29, 31). To limit liquid permeability through the PDMS device walls, devices were soaked for at least 10 h in CSF-XB buffer prior to generating extract droplets. Xenopus extract and carrier oil (Novec 7500 with 2% Pico- Surf surfactant, Dolomite Microfluidics, Royston, UK) were loaded into separate syringes, and both connected via Tygon® microbore tubing (0.010” ID x 0.030” OD, Saint-Gobain Performance Plastics) to the respective channel inlets. Fluid flow to the device was established using a syringe pump. Continuous phase, oil flow was initiated first at a rate of 1-15 (µl/min) followed by dispersed phase, extract flow at 0.1-5 (µl/min). Flow rates were adjusted to reach necessary droplet volume. Following droplet production, droplets were stored into microfluidic reservoirs of varying depths and geometries for imaging.

**Imaging and spindle length measurements**

Images were acquired using either a scientific-grade CMOS camera (Orca Flash 2.8, Hamamatsu) mounted on an inverted epifluorescence microscope (IX71 stand,
Olympus) or an EM-CCD camera (ImagEM, Hamamatsu) mounted on an IX71 stand equipped with a spinning-disc confocal head (CSU-X1; Yokogawa). Confocal illumination was provided by an LMM5 laser launch (Spectral Applied research). Integration of all imaging systems components was provided by Biovision Technologies (Exton, PA). All image acquisition and analysis was performed using Metamorph 7.7 software (Molecular Devices). Images were acquired using Olympus objectives of varying magnification: 10x (0.24 NA), 20x (0.75 NA and 0.85 NA), 40x (1.30 NA), and 60x (1.49 NA). Multi-dimensional z-stack imaging of encapsulated spindles allowed precise determination of encapsulating droplet diameter and the centroid of spindle poles in x,y,z (as determined by NuMA staining). Spindle length was subsequently determined in Excel (Microsoft) using the distance formula between poles. For each condition, measurements were taken from at least 5 independently prepared extracts.

**Statistical analyses**

Unless otherwise stated, statistical analyses were carried out using Excel and MiniTab 16. Graphs were generated using IgorPro 6 (WaveMetrics).

**Measurement of spindle position in droplets**

To calculate spindle position, we determined the midpoint of the line connecting the two NuMA-antibody decorated poles based on three-dimensional coordinates obtained from image stacks through droplets. For droplets, the center of the cross-sectional droplet area was the same for every z-plane and its coordinates were used for x and y. Determination of the z-axis center was dependent on droplet geometry. For slugs, the background tubulin fluorescence and z-sectioning through their entire volume allowed us to determine the z-coordinate midpoint. For spheres, background fluorescence allowed us to choose the z-axis centroid coordinate based on the focal plane containing the largest cross-sectional droplet diameter. Once we had the coordinates for spindle midpoint and the droplet centroid, the distance formula was applied and the absolute value of the results plotted in Fig. 4. For experiments with spindles, measurements were taken from at least 5 independently prepared extracts. For experiments with polystyrene beads, measurements were taken from at least 2 independently prepared extracts.

**Supplementary Text**

**Growth curve fits**

To facilitate statistical comparisons, spindle length data from our work in spherical droplets and from Wuhr et al. (2) were each fit with a growth model curve that explicitly asymptotes. For this purpose, we choose the Mitscherlich Law. Using this equation, the relationship between mean spindle length (L) and droplet diameter (D) takes the form

\[ L = \theta_1 - \theta_2 e^{-\theta_3 D} \]

As D grows, \(e^{-\theta_3 D}\) approaches zero, so the asymptote is \(\theta_1\); the other two parameters set the stage for speed of ascent (\(\theta_3\)), and abruptness of approach to the asymptote (\(\theta_2\)). The method of least squares was used to estimate the three parameters and the results of
the best fits are shown below for both the *in vitro* (droplets) and *in vivo* data (Figs S1 and S2 respectively).

**Determination of the average uniform random position expected in droplets**

*For spheres:* the random number generator in Excel was used to produce random values between +/- 1 for each coordinate, x, y, and z. All resulting points that lied outside a unit sphere centered on the origin were discarded (because the volume of the cube is approximately twice that of the sphere, about half of these positions were discarded). The mean value of the remaining points was calculated producing a result of 0.75. For each sphere, the following equation was used to calculate expected average position for uniform random positioning within a spherical volume:

\[
\text{average position} = 0.75 \times (\text{radius} – \text{excluded distance})
\]

In spherical droplets, we set the excluded distance equal to half the spindle width. In the absence of direct width measurements, we estimated this to be 1/4th the spindle length.

*For slugs:* each slug was modeled as a rectangle with a cross sectional area (width x height) equal to that of the confining channel. A "Monte Carlo"-type analysis, similar to that described above for spheres, was used to generate random coordinates within a rectangular volume defined by the dimensions of the rectangle minus the excluded distance (e.g. channel height – 1/4th spindle length, channel width – 1/4th spindle length, end-to-end slug length – 1/4th spindle length). The average value of all random positions generated in this way was used to produce the red plots in the slug graphs. This analysis was repeated for each slug.
Fig. S1. Fitted data for in vitro spindle scaling.
Spindle length plotted as a function of encapsulating droplet diameter (black circles). The data are overlaid with the best Mitscherlich fit and shown as a solid red line. The equation describing the best three parameter fit is shown above the plot (described in Supplementary Text).
Fig. S2 Fitted data for in vivo spindle scaling.
Spindle length plotted as a function of cell size (black circles). As in Fig. S1, the in vivo data are overlaid with the best Mitscherlich fit and shown as a solid red line. The equation describing the best three parameter fit is shown above the plot (described in Supplementary Text).
Fig. S3 Spindle length in slugs does not correlate with slug elongation.

Percent change in spindle length in slugs plotted as a function of the extent of slug elongation (see “Δ” in caption for Fig. 3). The difference between the measured spindle length in a slug and the predicted spindle length in an isovolumetric sphere was calculated as a percentage of predicted spindle length. Predicted spindle length was estimated using the Mitscherlich fit to spindle lengths measured in spherical droplets (described in supplementary information).
Movie S1
Droplet formation at a T-junction droplet generating device. Xenopus extract and carrier oil were loaded into separate syringes and pumped into separate channel inlets. The continuous oil phase was initiated first at a flow rate of 1-15 (µl/min) followed by a dispersed extract phase at a flow rate of 0.1-5 (µl/min). The two discrete phases merge at a T-junction within the device to produce stable, monodisperse emulsions of extract droplets in a continuous oil phase. Relative flow rates of the phases dictate droplet size. Scale bar = 100 µm.

Movie S2
Microfluidic generation of elongated slugs. High speed movie showing the transition from spherical droplet to slug. Two-dimensional confinement of spherical droplets in channels results in the formation of elongated slugs - over the range of spherical droplet diameters used (40-60 µm), an up to 3-fold increase in slug long axis relative to an isovolumetric sphere diameter was achieved. Scale bar = 100 µm.

Movie S3
Spindle morphology and position within a spherical droplet over time. Time-lapse movie showing the position of an encapsulated spindle in a confining sphere over time. Atto-488 tubulin (pseudo-colored red) is used to visualize microtubules, and 568 α-NuMA primary (green) is to visualize spindle poles. Tubulin fluorescence decreases over time due to photobleaching. Scale bar = 25 µm.

Movie S4
Polystyrene bead movements within a confining slug. Time-lapse showing the position of a 15 µm polystyrene over time within a confining slug. To minimize heating of the sample and the introduction of convective flows, images were acquired at approximately 3 minute intervals. Scale bar = 25 µm.

Movie S5
Variable extract flows within confining slugs. 60 second streaming acquisitions (2 frames/sec) of beads encapsulated within confining slugs. Two representative acquisitions highlight the variability of extract flows in confining slugs. Top panel – bead movement caused by extract flows within a confining slug. Bottom panel – a slug of similar geometry as above, but with flows acting only minimally on the encapsulated bead. Scale bar = 25 µm.
References and Notes


