Aster migration determines the length scale of nuclear separation in the Drosophila syncytial embryo

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Abbreviation used in this paper: PDMS, polydimethylsiloxane.

In the early embryo of many species, comparatively small spindles are positioned near the cell center for subsequent cytokinesis. In most insects, however, rapid nuclear divisions occur in the absence of cytokinesis, and nuclei distribute rapidly throughout the large syncytial embryo. Even distribution and anchoring of nuclei at the embryo cortex are crucial for cellularization of the blastoderm embryo. The principles underlying nuclear dispersal in a syncytium are unclear. We established a cell-free system from individual Drosophila melanogaster embryos that supports successive nuclear division cycles with native characteristics. This allowed us to investigate nuclear separation in predefined volumes. Encapsulating nuclei in microchambers revealed that the early cytoplasm is programmed to separate nuclei a distinct distance. Laser microsurgery revealed an important role of microtubule aster migration through cytoplasmic space, which depended on F-actin and cooperated with anaphase spindle elongation. These activities define a characteristic separation length scale that appears to be a conserved property of developing insect embryos.

Introduction

In developing organisms, important spatiotemporal decisions are taken. Correct positioning of the nucleus and spindle in a dividing cell is important for the fate of the daughter cells (Gönczy, 2008). In embryonic cells, this can be a challenge because cells can be up to two magnitudes larger than their metaphase spindle (Grill and Hyman, 2005; Schenk et al., 2010; Wühr et al., 2010). Massive microtubule aster growth has been shown to position the nuclei in Xenopus laevis eggs in preparation for cytokinesis (Wühr et al., 2010). In the case of most insects, the fertilized egg initially develops in the absence of cytokinesis (Foe and Alberts, 1983; Fleig and Sander, 1986; de Saint Phalle and Sullivan, 1996). Nuclei undergo rapid successive divisions, and, therefore, a vast number of nuclei share the same intracellular space in a syncytium. They need to be distributed throughout a large cytoplasmic volume and brought to the cell cortex to form a blastoderm embryo. But how do they distribute throughout the large embryo, and what sets their density?

In Drosophila melanogaster, the first 2 h of embryonic development are characterized by 13 syncytial nuclear divisions (Foe and Alberts, 1983). During the first nine divisions, nuclei divide every 8–9 min (25°C) and spread from the center throughout the interior of the embryo (Baker et al., 1993). At the end of the preblastoderm stage, typically after the ninth division, a fraction of the nuclei arrive at the cell cortex (Baker et al., 1993), where they are anchored and prepared for cellularization (Mazumdar and Mazumdar, 2002). Regular arrangement of nuclei at the cell cortex is essential for proper development (Hatanaka and Okada, 1991; Callaini et al., 1992; Megraw et al., 1999) and relies on the efficient distribution of nuclei through the preblastoderm embryo (Vaizel-Ohayon and Schejter, 1999; Rodrigues-Martins et al., 2007). In comparison with other cell types, at half a millimeter, the embryo is huge (Markow et al., 2009). Within the first nine divisions (70–80 min), the spreading of nuclei 6–8 µm in diameter through such a large volume of viscous cytoplasm cannot be explained by diffusion. An active transport mechanism is required for such a process. Earlier studies suggested that the microtubule and actin cytoskeletons both play important
Results and discussion

Extracted preblastoderm cytoplasm supports autonomous nuclear division and distribution

To directly investigate the basic mechanism by which dividing nuclei distribute throughout the cytoplasm of the early Drosophila embryo (preblastoderm stage), we developed a cell-free assay that allows the observation of successive mitotic divisions using time-lapse fluorescence microscopy imaging. Cytoplasm was extracted from individual embryos in nuclear cycle 6 or 7 (Foe et al., 1993) during late telophase and interphase, when nuclei were intact, and extract was deposited in droplets of defined size (typically 80–100 µm in diameter and 10–30 µm in height; Fig. 1 A). Transgenically encoded fluorescent proteins marking DNA (Histone 2Av–mRFP) and microtubules (Jupiter–GFP, a microtubule-associated protein; roles (Zalokar and Erk, 1976; Hatanaka and Okada, 1991; Baker et al., 1993; von Dassow and Schubiger, 1994).

Different, partly exclusive and untested models for early nuclear dispersal have been proposed, suggesting important roles of cortical factors (Bearer, 1991; Hatanaka and Okada, 1991; Reinsch and Gönczy, 1998), collective transport by a cytoplasmic streaming process (von Dassow and Schubiger, 1994), or mutual repulsion by elements of the microtubule cytoskeleton (Baker et al., 1993; Foe et al., 1993). Hence, the mechanism by which the cytoskeleton determines the correct positioning of nuclei in a syncytium is unclear, largely because visualizing nuclear movements and the associated cytoskeletal rearrangements deep inside living embryos is challenging, and tools to perturb nuclear spreading mechanically are lacking. Thus, a quantitative understanding that explains how nuclei faithfully reach the cortex at the proper density after the correct number of divisions is missing.
anaphase, and spindle poles associated with reforming nuclei continued to move apart (Fig. 1 E, green line). In parallel, the distance between daughter nuclei increased (Fig. 1 E, red line). Typical for syncytial divisions (Kellogg et al., 1988; Callaini and Riparbelli, 1990), centrosomes duplicated in telophase, and the new spindle poles migrated around the nucleus in interphase (Fig. 1 E, dashed bold lines) after the central spindle had disappeared (Fig. 1 F, bottom). The nuclei continued to move apart slowly even in the absence of central spindle microtubules (Videos 1 and 2). During this time, their associated microtubule asters were larger (Fig. 1, G and H). Finally, daughter nuclei reached a mean separation distance of \(28 \mu m\) (Fig. 1 E, red line) concomitant with a decrease in astral microtubule length (Fig. 1 G). This nuclear separation distance was independent of the division cycle for noncrowded extract droplets.

Preblastoderm nuclear separation length is insensitive to spatial constraints

To investigate whether this specific distance is a hard-wired property of the preblastoderm division machinery or whether the machinery scales with available space, we exploited a major advantage of the extract assay. We confined a single nucleus within the rigid boundaries of a microchamber of dimensions similar to the separation distance (Fig. 2 A). Under these spatial constraints, individual nuclei divided, and spindles often aligned their axis along the longest chamber diameter (Fig. 2 B). The spindle substructures deformed rather than size adapted. In telophase, the central spindle appeared bent, whereas the nuclei rotated, and the astral microtubules contacting the chamber walls buckled.
role of astral microtubules, whose length reaches a maximum at the end of telophase (Fig. 1 F), we treated extract with low doses of microtubule-destabilizing drugs. Reduced astral microtubule density without a visible change of the central spindle (Figs. 3 [A and B] and S2 A and Video 4) led to a reduced separation velocity and decreased the final distance of nuclear separation after anaphase (Fig. 3 C), in contrast to treatment with buffer alone (Fig. S2 B). This suggests that in addition to the established role of central spindle microtubules in anaphase, astral microtubules contribute to the separation of the genetic material after anaphase in the preblastoderm cytoplasm despite the absence of cortical anchoring.

To dissect the contributions of the central spindle and the microtubule asters to postanaphase nuclear separation directly, we performed laser microsurgery. Remarkably, ablation of the central spindle midzone in telophase (Figs. 3 D and S3 A), even when repeated several times, did not affect subsequent nuclear separation (Fig. 3 E and Videos 5 and 6). Destruction of one centrosome, initiating rapid disassembly of the associated microtubule aster, caused its associated nucleus to pause and led to later midzone deformation (Fig. S3 B). Thus, after anaphase, nuclear separation appears to be mainly driven by microtubule aster migration and supported by midzone elongation. This predicts that only multiple ablations can stop nuclear separation. Indeed, simultaneous ablation of the midzone and one centrosome (Figs. 2 B and S1 B). This is indicative of compressive forces, most likely a consequence of the midzone generating outward-pushing forces. Consequently, DNA separation was markedly slowed down and essentially stopped at ~10 µm after anaphase (Fig. 2 C). Neighboring nuclei in the next interphase were unnaturally densely packed. Nevertheless, the nuclei continued their mitotic program and entered a second round of division; however, the spindles exhibited severe morphological defects (Video 3). This demonstrates that preblastoderm spindles cannot adapt to reduction of available space. Their division program is presumably set to ensure that a domain of ~28 µm is occupied by each nucleus. This differs from later-occurring divisions, when spindles are anchored at the cortex (blastoderm stage), and the available space for each nucleus decreases with each division (Figs. 2 D and S1 C). At that developmental stage, spindles reduce their size progressively (Fig. 2 E; Brust-Mascher and Scholey, 2007).

Nuclear separation is defined by actin-dependent migration of centrosome-nucleated microtubules

Central spindle microtubules are crucial for the elongation of anaphase B spindles (Sharp et al., 2000; Glotzer, 2009). It is less clear what drives the movement of syncytial nuclei after anaphase (Baker et al., 1993; Foe et al., 1993). To test a potential role of astral microtubules, whose length reaches a maximum at the end of telophase (Fig. 1 F), we treated extract with low doses of microtubule-destabilizing drugs. Reduced astral microtubule density without a visible change of the central spindle (Figs. 3 [A and B] and S2 A and Video 4) led to a reduced separation velocity and decreased the final distance of nuclear separation after anaphase (Fig. 3 C), in contrast to treatment with buffer alone (Fig. S2 B). This suggests that in addition to the established role of central spindle microtubules in anaphase, astral microtubules contribute to the separation of the genetic material after anaphase in the preblastoderm cytoplasm despite the absence of cortical anchoring.

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dramatically reduced nuclear separation, except for some residual movement caused by the still-intact aster of the second nucleus (Fig. 3 F, dashed lines). Finally, when the midzone and both asters were removed, separation stopped completely, and the nuclei collapsed back to the spindle center (Fig. 3 G).

What do asters pull on in the syncytium? In the absence of a cortex, astral microtubules may anchor to an actin filament network within the cytoplasm (Hatanaka and Okada, 1991; Foe et al., 1993; von Dassow and Schubiger, 1994; Reinsch and Gönczy, 1998). We visualized both actin and microtubules in extract by mixing cytoplasm from actin-RFP and Jupiter-GFP embryos. Actin concentrated around the spindle and its asters from metaphase to telophase (Fig. 4 A and Video 7). Inhibition of F-actin turnover with latrunculin A reduced DNA separation after anaphase (Fig. 4 B and C) and Video 8), whereas chromosome segregation in anaphase is unaffected (P = 0.111). Each curve represents an independent experiment (gray; from Fig. 1 E). (D) The aster size in latrunculin A–treated extract (black; n ≥ 20, representative out of three repeats) was essentially as in untreated extract (gray; from Fig. 1 G).

Single-embryo extract observations compared with previously proposed models

Visualizing nuclear movements in living preblastoderm embryos is challenging as a result of the large size of the embryo and the considerable amount of yolk. Past studies using either live differential interference contrast or fluorescence microscopy reported two phases of collective nuclear movements during the early syncytial cleavages. Spreading along the anterior–posterior embryo axis (axial expansion, cycles 4–6; von Dassow and Schubiger, 1994) required the actin cytoskeleton (Zalokar and Erk, 1976; Hatanaka and Okada, 1991) and was thought to be driven by cytoplasmic flow (von Dassow and Schubiger, 1994). Nuclear displacement toward the cortex (cortical migration, cycles 7–9; Foe and Alberts, 1983; Baker et al., 1993) occurred episodically, during telophase and interphase of each division (Foe and Alberts, 1983), and required microtubule function (Baker et al., 1993). Thus, separation of daughter nuclei by the central spindle machinery during telophase (Foe et al., 1993) and additional mutual repulsion of nondaughter nuclei by plus end–directed kinesins pushing astral microtubules apart (Baker et al., 1993) were proposed to drive cortical migration. Whether or not the cortex is key for both phases has remained unclear.

Using single-embryo extract, we have revealed the existence of an autonomous mechanism for nuclear spreading independent
of cytoplasmic streaming and cortical cues (Fig. 1 B and Videos 1 and 2). Yolk particles were pushed away by separating nuclei (Video 2), arguing against cytoplasmic flow transporting nuclei (von Dassow and Schubiger, 1994). Astral microtubules contribute to nuclear separation in telophase and provide the only source of movement in interphase (Fig. 3). This is in line with the reported episodic cortical migration (Foe and Alberts, 1983) and reports of centrosomes and associated microtubules migrating to the embryo cortex in the absence of nuclear divisions (Freeman et al., 1986; Raff and Glover, 1989). However, autonomous aster movement argues against nuclear migration driven solely by central spindle pushing (Foe et al., 1993). The reported ringlike arrangement of the most outer nuclei seen in fixed embryos (Baker et al., 1993) might be a consequence of an increasing density of the actin cytoskeleton toward the cell cortex (von Dassow and Schubiger, 1994; Foe et al., 2000; Riparbelli et al., 2007), possibly generating an exclusion zone that promotes ordered, directional expansion (Field and Lénárt, 2011). Gradual disassembly of an actin gel in this exclusion zone might assist the final stages of cortical migration (Hatanaka and Okada, 1991), requiring additional mechanisms, such as microtubule-based actin remodeling (Waterman-Storer et al., 2000), that are absent in our simplified cell-free system. Improved in vivo imaging should resolve this question in the future.

The principle of constant nuclear domain size in syncytial development

The early Drosophila embryo extract exemplifies how the length scale for the distance between multiplying nuclei is set in a syncytium. The nuclear division machinery does not adapt to space constraint. Instead, the biochemical activities of the preblastoderm cytoplasm define a characteristic separation distance of nuclei, or a nuclear domain size (Fig. 5 A), reminiscent of a concept formulated earlier for plants (Mazia, 1993; Baluska et al., 2004). It is interesting to compare the characteristic nuclear domain size of \( \sim 28 \mu m \) diameter in early Drosophila embryo extract (Fig. 1 E) with the total available embryo volume assuming ellipsoidal shape (0.51 mm long and 0.18 mm wide; Markow et al., 2009). Assuming an even distribution of spherical nuclear domains in the embryo volume (Fig. 5 B), our calculations show that after nine divisions, these domains fill the embryo volume completely (see Materials and methods). At this stage, some nuclei remain in the interior of the embryo (for example, see Fig. 1 in Baker et al. [1993]), whereas the majority is in contact with the cortex. Remarkably, now the division program changes, and nuclei continue to divide while anchored at the cortex of the blastoderm embryo (Schejter and Wieschaus, 1993). Hence, the length scale of nuclear separation appears to be perfectly adjusted to the requirements of nuclear distribution before the blastoderm stage, setting the basis for later cellularization.

Different insect species may have embryos with different sizes. How does embryo size relate to nuclear separation distance? This distance could either scale with embryo size if the number of nuclear cycles was constant for embryos of different size or the number of divisions required for nuclei to reach the cortex could scale with embryo size, keeping the nuclear separation distance constant. Comparison of our data with the literature argues strongly for the latter scenario. Assuming a constant separation distance of \( \sim 28 \mu m \) (as measured in Drosophila extract), our simple model does not only correctly predict the number of divisions required to fill the Drosophila embryo but also larger and smaller insect embryos that are known to have fewer or more division cycles before cortical anchoring, respectively (Table 1, middle columns; Anderson, 1962; Raminiani and Cupp, 1975; Bull, 1982; Fleig and Sander, 1986; Perondini et al., 1986; Sommer and Tautz, 1991; de Saint Phalle and Sullivan, 1996). Inversely, relating embryo dimensions and the observed number of divisions for nuclei to arrive at the cortex, our model predicts a roughly constant nuclear domain size of \( \sim 29 \mu m \) for several species (Table 1, last column), inferred from a remarkably linear correlation between the logarithm of embryo volume and the reported number of preblastoderm divisions (Fig. 5 C). Therefore, the length scale of nuclear separation in the early syncytial embryo appears to be conserved, whereas the number of divisions required for nuclei to reach the cortex scales with embryo size. This shows how the scaling of a subcellular structure relates to the developmental program of an entire organism. Our simple model appears to reflect a general principle of nuclear positioning in a syncytium.

Materials and methods

Fly strains

\( w^{118S}; P(\text{PTT-GA}) \) Jupiter\( ^{2004}i \) flies (a gift from A. Debec, Université Pierre et Marie Curie, Paris, France; Moore et al., 2001; Karpova et al., 2006) were crossed with \( w^{118S}; P(\text{His2Av–mRFP1}) \) (stock no. 23650; Bloomington Stock Center) to generate \( w^{118S}; P(\text{PTT-GA}) \) Jupiter\( ^{2004}i ; P(\text{His2Av–mRFP1}) \) recombinant progeny. The resulting stock was homozygous viable.
### Table 1. Summary of dimensions and division cycles in the syncytial embryo of eight different species

<table>
<thead>
<tr>
<th>Species</th>
<th>References</th>
<th>Embryo size (length; width)</th>
<th>Nuclei at cortex after (divisions)</th>
<th>Domain size calculated&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Calculated&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Observed&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Sciara coprophila&lt;sup&gt;c&lt;/sup&gt;</td>
<td>de Saint Phalle and Sullivan, 1996</td>
<td>200; 110</td>
<td>6.35</td>
<td>6</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>Bradysia tritici</td>
<td>Perondini et al., 1986</td>
<td>250; 130</td>
<td>7.16</td>
<td>7</td>
<td>29.0</td>
<td></td>
</tr>
<tr>
<td>Mormoniella vitripennis</td>
<td>Bull, 1982</td>
<td>370; 140</td>
<td>7.93</td>
<td>8</td>
<td>27.6</td>
<td></td>
</tr>
<tr>
<td>Drosophila melanogaster</td>
<td>Foe and Alberts, 1983; Markow et al., 2009</td>
<td>510; 180</td>
<td>9.12</td>
<td>9</td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td>Aedes aegypti&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Raminani and Cupp, 1975</td>
<td>620; 160</td>
<td>9.06</td>
<td>9</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td>Dacus tryoni&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Anderson, 1962</td>
<td>975; 190</td>
<td>10.21</td>
<td>10</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td>Musca domestica</td>
<td>Sommer and Tautz, 1991</td>
<td>1,000; 260</td>
<td>11.2</td>
<td>11</td>
<td>29.0</td>
<td></td>
</tr>
<tr>
<td>Apis mellifera</td>
<td>Fleig and Sander, 1986</td>
<td>1,400; 300</td>
<td>12.05</td>
<td>11</td>
<td>35.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Assuming a domain size of 28 µm, as measured in this study.

<sup>b</sup>Assuming the observed number of divisions for nuclei to reach the cortex.

<sup>c</sup>Embryo dimensions were measured in the pictures presented in reference.

<sup>d</sup>Number of divisions estimated from cycle time and time for arrival of nuclei at the cortex.

<sup>e</sup>Number of divisions deduced from point of appearance of pole buds and formation of pole cells.

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**Micromanipulation**

The frame of an inverted, motorized light microscope (IX-81; Olympus) was fixed on a vibration-free optical table. Independent of the motorized stage (H117 ProScan; Prior Scientific), a second platform was connected to the microscope frame and to the optical table (Thorlabs, Inc.). Two three-axis motorized manipulators (MP-285; Sutter Instrument) were mounted on this platform close to the optical axis of the microscope, leaving space for the upright condenser. Micromanipulators were operated with a three-axis rotational handle (ROE-200; Sutter Instrument) linked to the control unit (MPC-200; Sutter Instrument). Manipulations using the glass pipettes were performed in transmission mode using a 20x UPlanApo 0.8-NA objective (Olympus), a long working distance 0.55-NA condenser (Olympus), and a polarizer and analyzer in crossed configuration.

**Microscopy system**

Spinning-disk confocal imaging (Andor Technology) was performed using 491- and 561-nm laser lines for excitation, a confocal scanner (5,000 rpm; CSU-X1; Yokogawa Electric Corporation), an emission filter wheel (Lambda 10B; Sutter Instrument), and an electron-multiplying charge-coupled device camera (Ixon, DU-885; Andor Technology) with an 8-µm pixel size. We used either a 20x UPlanApo 0.8-NA oil objective or a 40x UPlanFL 1.3-NA oil objective (both Olympus) for time-lapse imaging (100-ms exposure; 5, 10, or 20 s between frames) of the nuclear divisions in extract and in intact blastoderm embryos. Except during UV laser ablation, we magnified the field of view with the built-in 1.6× optovar. iQ software (Andor Technology) was used to control the microscope and for image acquisition.

**Single-embryo extract assay**

The cell cycle stage of embryos was determined using confocal fluorescent microscopy at 20x magnification. Typically, preblastoderm embryos that were in late anaphase/telophase of nuclear cycle 6 or 7 were chosen for extraction (Foe and Alberts, 1983). Cycle 6 (32 nuclei) was detected by observing >16 Jupiter-GFP signal patches from telophase spindles in the interior of the embryo. In cycle 7, when nuclear migration starts, the spindles on the side of the embryo facing the objective became more clearly visible. We verified our method of cycle stage detection by counting cycles (without extraction) until nuclei reached the cortex (cycle 10). For extraction, the vitelline membrane of a selected embryo was punctured with a pipette, and suction from inside the embryo was started immediately. Thereafter, the microscope stage was moved, and small droplets of cytoplasm (volume of 10–500 pL) were placed on the glass surface. As extraction was performed during telophase or the following interphase, time-lapse imaging typically started in late interphase or prophase. For imaging both actin and microtubules, extract droplets from embryos expressing actin-RFP were mixed with cytoplasm from embryos expressing Jupiter-GFP at a ratio of roughly 1:1. All experiments were performed at 25°C.

**Production of microchambers**

Arrays of microchambers of 20 × 40 and 30 × 30 µm were made in a single layer of polydimethylsiloxane (PDMS) cast from a master mold that...
was fabricated using soft lithography techniques (Simonnet and Groisman, 2006). To make the PDMS microchambers, a layer of PDMS prepolymer (Sylgard 184; Dow Corning) was spin coated onto the master mold and baked in an oven at 70°C for 1 h. The thickness of the PDMS layer was chosen such that it covered minimally the feature height. The region containing the microchambers was cut out, peeled off the mold using forceps, and placed on a cover glass with the feature side up.

Extract dilution and drug treatment
Using a fine pipette, small droplets (20–50 µm in diameter) of buffer (20 mM Hepes, pH 7.8, 100 mM KCl, 2 mM MgCl2; and 1 mM EGTA) or buffer containing a drug (nocodazole, colcemid, and cytochalasin D) were diluted 1:1,000 and latrunculin A 1:100 from a stock in DMSO) were positioned next to the immobilized embryos. Then extract was added aiming at dilutions of ~1:1. Diluted extract containing up to 1% DMSO preserved nuclear cycling (Fig. 1 C).

Laser ablation
The laser ablation system was initially designed by G. Heuvelmann and L. Hufnagel (European Molecular Biology Laboratory, Heidelberg, Germany). In brief, a 355 nm pulsed laser (15 µJ, 1 ns pulse width, 2.5 Hz; FT 55-355-Q; CryLas GmbH) was coupled into the light path using a dichroic mirror placed between the microscope and spinning-disk unit. The intensity of the expanded beam was controlled with a rotational polarizer after passing through a half-wave plate. A scanning galvano-mirror system (Thorlabs, Inc.) allowed high-speed positioning of the beam in x and y directions. The system was controlled with an in-house written plug-in for iQ software. Laser power was adjusted and tested by irradiating nuclei or chromosomes in extract, causing the nucleus to burst and chromosomes to melt and arrest mitosis, which confirmed that we do not observe bleaching only.

Data analysis
Basic image sequence analysis was performed in ImageJ (National Institutes of Health). For the measurement of unconfined nuclear separation (Fig. 1 D), experiments with one to three nuclei per extract droplet were considered (noncrowded situation). The centers of chromosome masses and nuclei were tracked manually during nuclear division and separation. Tracking error (±3 pixels < 0.5 µm) was estimated by repeated tracking and was smaller than the variability between experiments. The Euclidean distance between tracked objects was calculated and plotted as a function of time. For averaging curves from several experiments, we calculated the arithmetic mean and SD from data points that belonged to nonoverlapping time windows (bin size of 10 µs) and plotted mean ± SD against the center of the time window. The mean aster size (radius) was determined by manually measuring the length of all visible astral microtubules and pooling the data. For calculations and graphs, we used MATLAB (MathWorks). A heat map of the actin-RFP signal was generated using ImageJ (median filter with a width of 3 pixels).

Statistical test
To test for differences in postanaphase nuclear separation dynamics between control and test experiments [drug treatment and dilution], all DNA distance–time curves from individual experiments were pooled, and a multiple linear regression was performed between the time points 1 min (end of anaphase) and 5 min using the software package R. The abscissa was shifted so that time point 1 min was the new origin. The presence and absence of a treatment were described with a categorical factor g in the model

\[ y = b_0 + b_1x + g(b_2 + b_2x), \]

whereby \( g = 0 \) represents control, and \( g = 1 \) represents test conditions (x axis; time, y axis; distance). The statistical significance level of the parameter \( b_2 \) defines whether chromosome separation at the end of anaphase is different between the two conditions, whereas \( m_2 \) defines whether the slopes, and hence the dynamics, of the postanaphase separation are different. The significance level was set at \( P = 0.05 \) before testing.

Model calculation
Each nucleus occupies a nuclear domain represented as a sphere with diameter \( d \). The volume of an embryo is approximated as a rotation ellipsoid with length \( L \) and cross-sectional diameter \( D \). We assume tight packing of spherical domains; hence, the effective volume occupied by the spheres is \( V = \frac{4}{3} \pi L D^2 / \sqrt{3} \). Then, if \( n \) divisions produce \( 2^n \) nuclei, the calculated number of divisions to fill the entire embryo is

\[ n = \text{ln}(\pi LD^2 / \sqrt{3} / \text{ln}(2)), \]

where \( \text{ln} \) is the natural logarithm. Similarly, the nuclear domain size \( d \) can be calculated from the number of divisions, \( n \). Consequently, the logarithm of the embryo volume depends linearly on \( n \) for a constant nuclear domain size \( d \).

Online supplemental material
Fig. 1 compares nuclear separation in unconfined and confined extract as well as at the cortex in vivo. Fig. 52 shows that a small dose of nocodazole reduces nuclear separation distance and that buffer-diluted extract supports normal nuclear separation. Fig. 53 shows an intensity profile during UV laser ablation of the central spindle and illustrates the pausing of a nucleus whose associated aster has been removed. Video 1 shows a nucleus in extract undergoing five division cycles. Video 2 illustrates nuclear movement during interphase. Video 3 shows nuclear separation in confined space, and Video 4 shows its reduction in the presence of colcemid. Videos 5 and 6 show nuclear separation during single and repeated UV laser ablation, respectively, of the central spindle. Video 7 illustrates actin accumulation during a nuclear division, and Video 8 shows reduced nuclear separation when inhibiting F-actin turnover. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201204019/DC1.

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Author contributions: I.A. Telley and I. Gáspár initiated the extract assay and defined critical steps, I.A. Telley and T. Surrey conceived the project, I. Gáspár performed the recombinant fly cross and generated the ask-Gold transgenic fly, and I.A. Telley performed the experiments and analyses. All authors discussed the work and contributed to writing of the manuscript.

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