Asymmetric division through a reduction of microtubule centering forces

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Asymmetric divisions are essential for the generation of cell fate and size diversity. They implicate cortical domains where minus end-directed motors, such as dynein, are activated to pull on microtubules to decenter asters attached to centrosomes, nuclei, or spindles. In asymmetrically dividing cells, aster decenteration typically follows a centering phase, suggesting a time-dependent regulation in the competition between microtubule centering and decentering forces. Using symmetrically dividing sea urchin zygotes, we generated cortical domains of magnetic particles that spontaneously cluster endogenous dynein activity. These domains efficiently attract asters and nuclei, yielding marked asymmetric divisions. Remarkably, aster decenteration only occurred after asters had first reached the cell center. Using intracellular force measurement and models, we demonstrate that this time-regulated imbalance results from a global reduction of centering forces rather than a local maturation of dynein activity at the domain. Those findings define a novel paradigm for the regulation of division asymmetry.

Introduction

During cell division, multiple cues are integrated to determine the relative position and size of daughter cells. Cell geometry, polar domains, or sites of cell–cell adhesion may, for instance, influence microtubule (MT) aster forces, which move and orient centrosomes, nuclei, and spindles to generate the wide variety of division types observed in different cells and tissues (Grill et al., 2001; Théry et al., 2005; Minc et al., 2011). One of the most striking examples of division positioning control is asymmetric division. During this process, asters, nuclei, and spindles are usually first centered in the cell and then become displaced to one side, leading to the unequal segregation of cytoplasmic and cortical contents and producing cells with distinct sizes (Dan, 1979; Kaltischmidt et al., 2000; Kaltischmidt and Brand, 2002; Kimura and Onami, 2007). Asymmetric division is a common mechanism to generate fate diversity from yeast to humans, particularly well-studied in lineage progenitors, in stem cells, and in the Caenorhabditis elegans zygote (Gönczy, 2008; Morin and Bellaiche, 2011). Upstream signals responsible for asymmetry have been largely identified in such model cell types, but how downstream mechanical forces can orchestrate a robust and reproducible asymmetric division still remains unclear.

In animal cells, aster positioning is dynamically controlled by MT forces (Reinsch and Gönczy, 1998; Minc and Piel, 2012; Mitchison et al., 2012). Cytoplasmic dynein is one of the main MT-associated motors responsible for aster positioning. Dynein may generate cytoplasmic pulling forces, from the viscous drag created by the motion of endomembranes, such as the endoplasmic reticulum, or other vesicle cargos, it drives toward asters’ centers (Hamaguchi et al., 1986; Kimura and Kimura, 2011). This process may result in MT forces that scale to their length, and ensure aster centration and orientation with respect to cell geometry (Hamaguchi and Hiramoto, 1986; Kimura and Onami, 2005; Wühr et al., 2010; Minc et al., 2011; De Simone et al., 2018; Haupt and Minc, 2018). Dynein motors may also be anchored at the cell cortex within specific polar domains to generate asymmetric pulling forces that decenter asters for asymmetric division (Grill et al., 2001; Colombo et al., 2003). Regulation of the balance between cytoplasmic and cortical dynein forces is most likely at the heart of most division geometries, like those found during cleavage or stem cell lineage (Kaltischmidt and Brand, 2002; Mitchison et al., 2012; Pierre et al., 2016). However, given the difficulty of manipulating an inherently asymmetric division in a controllable and quantitative manner, the generic mechanisms regulating this competition remain mostly unexplored.

We implemented magnetic cortical domains that spontaneously cluster dynein minus end activity in sea urchin (Para-centrotus lividus) zygotes. These domains transform the first symmetric cleavage typical of those cells into a marked asymmetric division. We find that centering asters are relatively insensitive to the domain and can be decentered only after they reach the...
cell center. Combined in vivo force measurements and computer simulations support a novel paradigm in which asymmetric divisions may be triggered by a reduction in centering forces under constant cortical pulling forces.

Results and discussion

Magnetic cortical pulling domains to control the asymmetry of cell division

Certain particles injected into neurons or marine embryos may spontaneously exhibit minus end–directed movement to cluster around centrosomes (Adams and Bray, 1983; Hamaguchi et al., 1986). By screening magnetic particles injected into sea urchin zygotes, we found one type of particle displaying highly persistent MT minus end motion that formed compact aggregates at the aster center in a dynein-dependent manner (Fig. S1, A–C; Tanimoto et al., 2018). Using calibrated magnetic probes, we assembled, relocated, and retained cortical domains made of those beads of ~5–10 μm in size (Fig. S1 D and Video 1). To test whether these domains loaded with dynein activity could pull on MTs to drive asymmetric divisions, we assembled them in nonfertilized eggs and added sperm. Strikingly, ~20–30 min after fertilization, the zygote nucleus, which is normally maintained in the cell center, became attracted by the domain and exhibited a net asymmetric displacement. As occurs during symmetric divisions in those cells, the mitotic spindle formed after the asymmetric location and elongation of the interphase nucleus (Mine et al., 2011) and was thus well aligned with the domain, thereby driving cytokinesis that cleaved the cell in a marked asymmetric manner (Fig. 1, A and B; and Video 2). Asymmetric cleavages were never observed in un-injected eggs, in injected eggs in which the magnetic probe was removed after fertilization allowing the cap to be seen (Fig. S1, A and B; and Video 2). This demonstrates that asters that begin centripetal motion of cortical magnetic beads (Fig. S2, D and F; and Video 7). This switch in aster directionality was observed in 100% of cases (n = 11 events), independent of the distance between the cap and the site of sperm entry (Fig. 2, A and B; and Video 6). In such eggs, immunostaining confirmed a clear interaction between centering asters and magnetic domains. In addition, cap release assays yielded a near-immediate centripetal motion of cortical magnetic beads (Fig. S2, D and F; and Video 7). Together, these results indicate that a change in the imbalance between centering and decentering forces may take place when asters are close to the cell center.

Requirement for an evolution of centering or decentering forces in asymmetric divisions

To understand the key elements driving consecutive centration and decentration phases, we developed a minimal 1D model (Fig. 2 E and Materials and methods). The aster was represented as a frontal MT of length $L_f$ and a rear MT of length $L_r$. Centering MT forces were scaled to the segment length through a constant $a$, and MT lengths were assumed to be limited by the cortex (Kimura and Onami, 2005; Tanimoto et al., 2016). The position $x$ of the aster center evolved with time following an overdamped force balance equation, with $\gamma$ the effective drag of the aster: $m \ddot{x} = \alpha(L_f - L_r) - F_{cap}$ in which $F_{cap}$ is the decentration force exerted by the cortical cap (Fig. 2 E). In the absence of a cap ($F_{cap} = 0$), the aster centered at a speed bounded by MT polymerization rate and stopped at the cell center ($x = 0$), with no decentring motion. When $F_{cap} > 0$, asters moved toward the center, but stopped before reaching the center at a final position given by $x = F_{cap}/2a$ and did not exhibit any decentering movement (Fig. 2 F).
Figure 1. Magnetic cortical pulling domains drive marked asymmetric divisions. (A) Magnetic beads injection, cap formation, and induced asymmetric division. (B–E) Time-lapse images of the cleavage of sea urchin zygotes. (B) The cap is formed with beads with strong MT minus end activity and maintained; (C) a noninjected control embryo; (D) the pulling cap is not maintained; and (E) a control using a cap formed with beads that do not interact with MTs. Yellow stars: female/zygote nucleus; red stars: blastomere nuclei after division. (F) Divided blastomere area ratio in conditions B to E (n = 10, n = 8, n = 11, and n = 10 cells). (G) Quantification of the angle (Δα) between the division axis and the axis from the cell center to the cap (n = 12, n = 17, and n = 10 cells). (H) Blastomere area ratio plotted as a function of the angle (β) formed by the edges of the pulling cap and the cell center. The dashed line is a visual guide. Results were compared by using a two-tailed Mann–Whitney test. ns, P > 0.05; ****, P < 0.0001. Error bars are SD. Bars, 20 μm.
Allowing the drag $\gamma$ to change over time affected the kinetics of centering, but not the final position, and did not account for the net change in aster directionality. Therefore, to account for the observed successive centering and decentering phases, we allowed forces to vary over time. Accordingly, increasing $F_{\text{cap}}$ or decreasing the centering constant $a$ after the aster had reached the cell center could account for both the successive centering and decentering phases and the reduction in aster speed during decenteration (Fig. 2 G and Fig. S3, A and B). This simple analysis suggests that, during asymmetric divisions, cortical pulling forces may increase in strength over time, or alternatively, that the centering forces become weaker.
Cortical pulling forces remain constant during and after aster centration

To address putative time-dependent variations of cortical forces, we set out to directly compute the net decentering force exerted by the domain during and after aster centration. For this, we progressively moved back the magnetic probe away from the egg surface (Fig. 3 A). When the endogenous force from MTs on the cap became larger than the decreasing magnetic force, the cap detached from the cortex and moved to the cell center (Fig. 3, B and C; and Video 8).

The distance between the magnetic probe and bead domain was converted into a force and served to extract a “take-off” force, corresponding to the moment when the pulling force on the domain overcomes the magnetic force (see Materials and methods). To compute asymmetric forces on the domain during centration, we used a set of caps located close to the site of sperm entry (Fig. S2 F). This analysis revealed similar mean “take-off” profiles during and after centration, with mean cortical forces of 293.9 and 287.4 pN, respectively (Fig. 3 D). Finally, in agreement with the influence of cap size on division asymmetry, larger caps appeared to exert more pulling forces (Fig. 1 H and Fig. 3 E). These results suggest that magnetic domains pull with near constant force and that changes in aster directionality and speed cannot be readily accounted for by a significant evolution of cortical forces.

Centering cytoplasmic pulling forces become weaker after centration

A second, less intuitive prediction of our 1D model is that a reduction in MT centering forces per unit length may account for changes in both aster directionality and aster speed. To test this possibility, we directly measured the time evolution of aster centering forces in vivo. We injected magnetic beads and let them aggregate to the aster center, and applied calibrated forces (Tanimoto et al., 2018). Aster responses at different time points were compared within the same cell and under the same magnetic force by applying consecutive pulls orthogonal to the centering path. During centration, this caused the aster to deviate from its centering path toward the magnet probe and to recoil back to its normal centered path upon force cessation. After centration, force exertion displaced asters and attached nuclei away from the center, which also recoiled back upon force cessation (Garzon-Coral et al., 2016; Tanimoto et al., 2018; Fig. 4, A and B; and Video 9).

Strikingly, the amplitude of the deviation away from the cell center was much larger after the aster had reached the center as compared to during centration, indicating that centered asters are easier to decenter (Fig. 4, A and C; and Video 9). To quantify those effects, we fitted the displacement time curves by using a viscoelastic Kelvin-Voigt model, in which an elastic spring and a dashpot act in parallel. We computed the aster centering spring
constant ($\kappa$, also called centering stiffness), which reflects the strength of length-dependent MT forces, and the aster drag ($\gamma$; Garzon-Coral et al., 2016; Tanimoto et al., 2018). Individual fitting of paired experimental curves performed in the same egg and under the same force showed that aster centering stiffness was systematically lower after centration, with a mean ratio before/after centration of $\sim 2.9$ (Fig. 4 D). At the population level, the mean spring constant during centration was $\kappa_1 = 61.48$ pN/µm and $\kappa_2 = 18.10$ pN/µm after centration (Fig. 4 F). Individual force measurements after centration yielded similar spring constant values, ruling out potential caveats of altering aster integrity after force application (Fig. 4 D). The decrease in aster centering stiffness $\kappa$ was represented by a linear decay over a 1-min period, at a fixed time after fertilization, but slower decays could also account for experimental behavior (Fig. S3, F and G). The model was compared with individual experiments, and accounted for the complex 3D trajectories of asters and yielded similar values for centered and final decentered aster positions, as well as centration-versus-decentration speeds (Fig. 5, A–E; and Video 10). Therefore, a model based on the quantified reduction of centering stiffness accounts for the inversion of aster directionality and net changes in speed and strongly supports a hitherto unappreciated mode of regulation of asymmetric division.

**A 3D force competition model for aster positioning**

To provide a faithful representation of the geometry of the system, we then developed a 3D model, in which we could input the respective 3D positions of the cap and site of sperm entry, for a direct comparison with individual experiments (see Materials and methods). Importantly, parameters were implemented using measured values for $\gamma$, $\kappa$, and $F_{\text{cap}}$, and not adjusted further. The decrease in aster centering stiffness $\kappa$ was represented by a linear decay over a 1-min period, at a fixed time after fertilization, but slower decays could also account for experimental behavior (Fig. S3, F and G). The model was compared with individual experiments, and accounted for the complex 3D trajectories of asters and yielded similar values for centered and final decentered aster positions, as well as centration-versus-decentration speeds (Fig. 5, A–E; and Video 10). Therefore, a model based on the quantified reduction of centering stiffness accounts for the inversion of aster directionality and net changes in speed and strongly supports a hitherto unappreciated mode of regulation of asymmetric division.

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**Figure 4. Aster centering stiffness decreases after centration.**

(A) Time-lapse of a centering aster subjected to two consecutive external magnetic forces. Top: Low-magnification views of the embryo and magnet tip. Bottom: Position of aster centers (nuclei) and beads aggregate. Orange line: the centration path (fertilization from the top); orange arrows: the direction of the magnetic forces. Bars, 20 µm. (B) Time-lapse projection of aster deviation from the centration path upon the first force application. (C) Kymograph of aster deviation from the cell center from the second force application. Black and white arrowheads: the initial centered position and the maximum deviation (plateau) during force application, respectively. Bars, 5 µm. (D) Quantification of paired aster centering spring constants (connected by gray dashed lines [n = 10]). The ’single-pull’ category corresponds to force measurement performed only once after aster centration (n = 15). (E) Quantification of paired aster drags. (F) Average displacement/force plotted as a function of time. Error bars are SEM. Dashed lines: Voigt model fit (see Materials and methods). Results were compared using a two-tailed Mann–Whitney test. ns, $P > 0.05$; **, $P < 0.01$. 

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Conclusions
By developing a novel approach based on magnetic domains that can efficiently pull on MTs in a symmetrically dividing cell, we recapitulated most generic features of asymmetric divisions, including a dose-dependent effect of domain size and strength on daughter size asymmetry and a centering phase followed by a
decentering one. One key finding is that a reduction in aster centering stiffness (forces/per unit MT length) provides the trigger for the prevalence of asymmetric forces from the domain needed for asymmetric division (Fig. 5 F). Although our experimental data do not allow us to clearly resolve the decay time of the centering stiffness (Fig. S3, C and D), they suggest a relatively rapid decay over a traveling distance of ∼20–30% of the egg radius close to the cell center (Fig. S3, F and G).

An important question these findings pose is what molecular reorganization may drive a weakening in the cytoplasmic MT pulling system. One possibility is that dynein activity becomes globally reduced in the cytoplasm, through time-dependent exhaustion or cell cycle progression. This hypothesis does not readily agree with experimental findings, as bead aggregates moving to the aster center exhibit similar centripetal speeds during or after aster centration (Fig. S3 E). A second plausible hypothesis is that gradual changes in aster structure, through the promotion of MT branching and/or densification, for instance, alter cytoplasmic force transmission to the centrosome in a time-dependent manner (Dan and Inoué, 1987; Mitchison et al., 2012). Although we cannot firmly reject a contribution from this effect, we note that MT radial arrangements remain roughly similar during the few minutes corresponding to the decay in centering stiffness (Minc et al., 2011; Tanimoto et al., 2016). A last model, which we favor most, is that cargos and/or endomembranes pulled by dynein to support MT cytoplasmic pulling may accumulate at the aster center, which could cause a gradual depletion of available cytoplasmic anchors. Accordingly, structures such as the endoplasmic reticulum progressively concentrate around the sperm nucleus toward the end of aster centration, at timings that match our measured decay in centering stiffness (Terasaki and Jaffe, 1991; Reinsch and Gόnczy, 1998; Audhya et al., 2007).

Finally, we foresee that our model, based on a global rather than local regulation, could serve as a major mechanism regulating asymmetric centrosome positioning and divisions in many cell types. In C. elegans zygotes, asters also exhibit a net change in directionality, essentially recapitulated in experiments presented here in Fig. 2 A and Fig. S2 C (Kimura and Onami, 2007). Interestingly, time-resolved analyses of cortical forces in these cells suggest that they also act upon mitotic asters much before the onset of decentration (Labbé et al., 2004). In other cell types, the modulation of centering forces could also emerge from time-dependent changes in MT pushing forces (Tran et al., 2001; Tolic-Nerelykке et al., 2005; Garzon-Coral et al., 2016). Pushing, which promotes aster centering, could become less efficient as a result of aster size increase or changes in MT polymerization rate, all resulting from a global rather than local regulation at cortical domains (Letort et al., 2016; Howard and Garzon-Coral, 2017; Pitaval et al., 2017). Further quantitative mapping of the evolution of centering and decentering forces will be instrumental for understanding asymmetric divisions.

Materials and methods

Sea urchin maintenance and gametes collection

Purple sea urchins (P. lividus) were obtained from the Roscoff Marine station (France) and kept at 16°C in an aquarium for several weeks in artificial sea water (Reef Crystals; Instant Ocean). Gametes were collected by intracoelomic injection of 0.5 M KCl. Sperm was collected dry and kept at 4°C for 1 wk. Eggs were rinsed twice, kept at 16°C, and used on the day of collection. The jelly coat of unfertilized eggs was removed by passing them three times through an 80-µm Nitex mesh (Genesee Scientific) to facilitate egg adhesion on protamine-coated glass-bottom dishes (MatTek Corporation). Eggs were transferred on protamine-coated dishes for a maximum time of 15 min before injection, and were injected and fertilized under the microscope.

Magnetic particles preparation and injection

The spontaneous minus end–directed motion of specific superparamagnetic particles (NanoLink; Solulink) was used to both create cortical pulling caps and to apply forces on MT aster centers (Tanimoto et al., 2018). To prepare beads for injection, a solution of 10 µl of undiluted beads functionalized with streptavidin was first washed in 100 µl washing solution (1 M NaCl with 1% Tween-20) and sonicated for 5 min. The beads were then rinsed in 100 µl PBS, incubated in 100 µl 0.2 µg/ml Atto488-biotin (Sigma-Aldrich), rinsed again in 100 µl PBS, and finally resuspended in 20 µl PBS and kept on ice until use. Fluorescent and magnetic MyQuVigen Beads (Nvigen) served as control nonpulling beads, and were prepared according to the same protocol except for the incubation with fluorophores.

Unfertilized eggs were placed on a protamine-coated glass-bottom dish. The bead solution was injected using a microinjection system (FemtoJet 4; Eppendorf) and a micromanipulator (Injectman 4; Eppendorf). Injection pipettes were prepared from siliconized (Sigmacote; Sigma-Aldrich) borosilicate glass capillaries (1 mm diameter). Glass capillaries were pulled with a needle puller (P-1000; Sutter Instruments) and ground with a 30° angle on a diamond grinder (EG-40; Narishige) to obtain a 5–10-µm aperture. Injection pipettes were back-loaded with 2 µl bead solution before each experiment and were not reused.

Magnetic tweezers

The magnetic probe was built from three rod-shaped, strong neodymium magnets (diameter, 4 mm; height, 10 mm; S-04-10-AN; Supermagnet) prolonged by a sharpened steel piece with a tip radius of ∼50 µm to create a magnetic gradient. The surface of the steel tip was electrocoated with gold to prevent oxidation. Magnetic probes were calibrated by tracking the velocity of single magnetic beads in a medium of known viscosity, as a function of the distance to the magnet, and by using the Stokes relationship to compute the net magnetic force. The same experiment performed on bead aggregates of various sizes at a given distance allowed us to establish force–size relationships. For this part, the drag of bead aggregates was assayed by measuring sedimentation speed in a medium of known viscosity, and found to be well represented by the drag of a spherical particle with a radius equal to the geometrical mean of the aggregate size (Tanimoto et al., 2018). The magnetic tweezers position was controlled with the injection micromanipulator (Injectman 4; Eppendorf). To assemble the pulling cortical cap, we injected the beads and aggregated them at the cell cortex ∼10–20 min before fertilization by positioning the magnetic tip at 50–100 µm from the egg.
and maintaining it during all the experiment. Step-like motion during time-lapse acquisition, used for measuring cortical forces, was achieved with a home-built Micro-Manager (Open Imaging) script piloting the microscope stage (MS-4400; ASI) to move the sample relative to the fixed magnetic tweezers, with step sizes of 5 µm spaced by 30-s intervals.

**Microscopy and image analysis**

Injections, magnetic tweezer manipulations, and time-lapse acquisitions were performed on a wide-field fluorescence microscope (TI-Eclipse; Nikon) equipped with a complementary metal oxide–semiconductor camera (Orca-Flash4.0LT; Hamamatsu). Samples were imaged using a 40× dry objective (NA, 0.75; Apo; Nikon) and a 1.5× magnifier (final pixel size, 0.217 µm). The microscope was operated with Micro-Manager (Open Imaging). Live imaging was carried out in artificial sea water at a stabilized room temperature (18–20°C). Immunostained samples were imaged with a confocal microscope (LSM780; Zeiss) with a 63× water immersion objective (NA, 1.4; C-Apo; Zeiss). Images were processed with Fiji (ImageJ; National Institutes of Health) and assembled in Photoshop (Adobe). Noise was reduced by using a median filter (1-pixel width) for the DNA (Hoechst) channel. Aster and bead trajectory tracking was performed with a custom code written in MatLab (MathWorks). Centration and decentration speed measurements were computed from a linear fit on 70% of the centration trajectory around the midpoint of the centration trajectory and during the 5 min after centration, respectively, by using a representative subset of 18 individual trajectories. The centration position was defined as the position with the shortest distance to the center. The decentration position was obtained by fitting a decreasing exponential to the trajectory after centration. To represent the effect of magnetic forces on the position of aster centers during and after centration (Fig. 4, B and C), we used a simple time-lapse projection that highlights the deviation from the centering path during centration (Fig. 4 B) and a kymograph to visualize the deviation of an already centered aster along the pulling axis (Fig. 4 C).

**Drug treatments**

Drugs were prepared as 100× stock aliquots in DMSO. Nocodazole (Sigma-Aldrich) was used at a final concentration of 20 µM. Cilobrevin D (EMD Millipore) was used at a final concentration of 50 µM. Drugs were rapidly added (within less than a minute) when aster centration was completed and maintained until the end of the experiment.

**Immunostaining**

Immunostaining was performed by using procedures similar to those described previously (Foe and von Dassow, 2008; Minc et al., 2011). Embryos were fixed in the same glass-bottom dish, after filming centration or decentration, for 70 min in 100 mM Hepes, pH 6.9, 50 mM EGTA, 10 mM MgSO4, 2% formaldehyde, 0.2% glutaraldehyde, 0.2% Triton X-100, and 800 mM glucose. To limit autofluorescence, samples were rinsed in PBS and placed in 0.1% NaBH4 in PBS made fresh 30 min before use. Samples were then rinsed in PBS and PBT (PBS 0.1% Triton X-100) and incubated for 24 to 48 h in mouse anti-tubulin (DM1A; Sigma-Aldrich) primary antibody at 1:5,000 in PBT. After three washes of 1 h in PBT, samples were incubated for 12 h in goat anti-mouse secondary antibody coupled with Dylight 550 (Thermo Fisher Scientific) at 1:1,000 in PBT. Samples were then washed three times for 1 h in PBT, transferred in 50% glycerol PBS, and finally transferred into mounting medium (90% glycerol and 0.5% N-propyl gallate PBS).

**Cortical force measurement**

To compute the cortical force exerted by the cap, we fitted the binned distance–force curves (Fig. 3 D) with a simple model, assuming that the cap takes off at a critical force (when the magnet is far enough) and then moves at constant speed toward the center. A more complete model including a small decreasing force that highlights the deviation from the centering path during centration (Fig. 4 B) and a kymograph to visualize the deviation of an already centered aster along the pulling axis (Fig. 4 C). To represent the effect of magnetic forces on the position of aster centers during and after centration (Fig. 4, B and C), we used a simple time-lapse projection that highlights the deviation from the centering path during centration (Fig. 4 B) and a kymograph to visualize the deviation of an already centered aster along the pulling axis (Fig. 4 C).

**Computing aster drag and spring constant**

To compute aster drags and centering spring constants from individual experiments (Fig. 4, D and E), we fitted each displacement curve by using a Kelvin–Voigt model, in custom-made MATLAB scripts, as previously described (Garzon-Coral et al., 2016),

\[
\frac{\kappa}{F} = \frac{1}{\beta} \left[ 1 - \exp \left( -\frac{t}{\beta} \right) \right],
\]

where \( \kappa \) is the aster centering stiffness (or centering spring constant) and \( \gamma \) the drag coefficient. In experiments, the duration of force application was adjusted to allow the aster to reach the plateau when magnetic forces equilibrate MT centering forces (Garzon-Coral et al., 2016; Tanimoto et al., 2018). Mean values reported in the text correspond to fits of binned data (Fig. 4 F). We note that this method allowed us to reliably compare drag values before and after centration, but most likely correspond to a lower bound of the actual values of the drag (Garzon-Coral et al., 2016; Tanimoto et al., 2018).

**Theoretical models**

**1D model**

In the 1D model, the aster was represented by one frontal MT segment (directed toward the center) of length \( L_a \) and one rear MT segment (directed toward the nearest cortex) of length \( L_e \) (Fig. 2 E; Tanimoto et al., 2016). In sea urchin zygo...
In which \( u(\theta, \phi) \) is the unit vector oriented with angles \( \theta \) and \( \phi \)

gangular distribution of MTs, \( \rho(\theta, \phi) = N/4\pi \) (with \( N \) the number of

in which \( F_{\text{cap}} \) is the decentration force applied by the cortical do-

and \( v_p = 8.25 \mu m/\text{min} \) (fitted and adjusted, and consistent with

in the 1D model, with additional parameters \( \kappa_2 = 18.01 \text{ pN}/\text{µm} \)

Eqs. 4, 5, and 6 were integrated by using a Euler scheme, implemented through a MatLab script. For this process, we discretized \( L(\theta, \phi) \) using a uniform range of \( \theta \) and \( \phi \), spherical coordinates with the aster center as the origin, and verified that this distribution was homogeneous and did not induce a systematic bias.

The integral in Eq. 4 was accordingly replaced by a discrete sum of the \( n = 28,654 \) discrete elements,

\[
F_c = k \sum_{i=1}^{n} L_i u_i
\]

in which \( L_i \) and \( u_i \) are the length and orientation of MTs for the orientation \( i \).

The force per MT unit length \( a \) was computed from the experimental measurement of the aster centering spring constant \( \kappa \). A Taylor expansion of Eq. 4 around \( x = 0 \) leads to

\[
a = \frac{3}{N} \kappa,
\]

in which \( \kappa \) is the centering spring constant measured experimentally.

Once a MT contacted the cap, the full pulling force from the cap was added to that force balance and assumed to be \( F_{\text{cap}} = F_{\text{cap}} u_i \), in which \( u_i \) is the unit vector directed from the aster center to the cap position. As a consequence, although the cap had a fixed size, this size had little influence on the model prediction. To incorporate the decrease of \( \kappa \) after centration, we used a linear decrease between two time points \( t_1 \) and \( t_2 \) from a spring constant \( \kappa_1 \) to a value \( \kappa_2 \). Positions and speeds extracted from model results were computed in the same manner as for the experiments. The parameter values used were the same as in the 1D model, with additional parameters \( \kappa_2 = 18.01 \text{ pN}/\text{µm} \) (measurement), \( \text{cap size} = 5 \mu m \) (close to measurement values), \( t_1 = 10 \text{ min} \), and \( t_2 = 11 \text{ min} \) (best-fitting values; see influence in Fig. S3, F and G).

**Online supplemental material**

Fig. S1 includes the characterization of bead aggregates motion; an example of cap formation and relocation; an example of the long-term effect of a small pulling cap during embryonic cleav-
gages; details of the MT network under cortical pulling; and control experiments demonstrating the requirement of MTs and dynein for cortical pulling. Fig. S2 shows other examples of aster centration/decentration trajectories; the details of MT networks during centration under cortical pulling; and a characterization of the relationship between aster size and cortical pulling. Fig. S3 includes the effect of increasing cap force and decreasing centering constant on aster position in the 1D model; the evolution of measured spring constants and released cap velocities before and after centration; and the simulated aster positions from different profiles of centering force reduction. Video 1 is an example of cortical cap assembly and relocation. Video 2 shows an asymmetric division induced by an artificial cortical pulling domain (Fig. 1 B). Video 3 is a control embryo with a “nonmaintained” cap (Fig. 1 D). Video 4 is a control embryo containing a “nonpulling” cap (Fig. 1 E).

Video 5 shows decentration failures upon dynein inhibition and MT depolymerization (Fig. S1 I). Video 6 shows aster centration and decentration under cortical pulling (Fig. 2 A and Fig. S2, A–C). Video 7 shows examples of “free-cap” motions when fertilization

**3D model**

For the 3D model, the aster was represented with a constant an-
gular distribution of MTs, \( \rho(\theta, \phi) = N/4\pi \) (with \( N \) the number of MTs), of length \( L(\theta, \phi) \) (Minc et al., 2011; Tanimoto et al., 2016).

The net centration force on the aster thus reads:

\[
F_c = k \sum_{i=1}^{n} L_i u_i
\]

in which \( u(\theta, \phi) \) is the unit vector oriented with angles \( \theta \) and \( \phi \) and \( a \) is the pulling force per unit length of an MT computed from the values of \( \kappa \) measured in experiments (see below).

The dynamic equations for MT lengths and aster position \( r \) are similar to those in the 1D model,

\[
\gamma r = F_r + F_{\text{cap}}
\]

\[
L(\theta, \phi) = v_p,
\]

with MT length limitation by the cortex implying \( R > |r + u(\theta, \phi)L(\theta, \phi)| \).

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occurs close to or far from the cap. Video 8 is a magnetic force reduction experiment during aster centration under cortical pulling (Fig. 3B). Video 9 is an aster pulling experiment during and after centration (Fig. 4A). Video 10 shows a 3D simulation of aster centration and decentering under cortical pulling (Fig. 5A).

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Author contributions: J. Sallé and J. Xie performed the experiments. D. Ershov developed the image analysis scripts. M. Lacassine and S. Dmitrieff designed and performed the models. J. Sallé, S. Dmitrieff, and N. Minc designed the research and wrote the manuscript.

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References


Supplemental material

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Figure S1. Cortical pulling from magnetic beads domains depends on MTs and dynein. Related to Fig. 1. (A) Time-lapse sequence showing the centripetal motion of a free aggregate during aster centration. Orange star: the aster center; arrowhead: the position of the aggregate. Bar, 20 µm. (B) Quantification of the distance between a free cap and aster center during centration. Three representative cases are displayed. (C) Quantification of free aggregate and female pronucleus velocity during aster centration (n = 78 and n = 33, respectively). Error bars correspond to SDs. (D) Time-lapse sequence showing cortical cap assembly and relocation in an unfertilized egg. Top: Low-magnification view of the egg and the different magnetic probe positions. Bottom: Details of cap assembly and relocation around the same cell (arrowhead). (E) Time-lapse sequence of small cap maintained during subsequent embryonic divisions. Note how the cap can drive asymmetric division in the small four-cell blastomere but not in bigger one- and two-cell-stage blastomeres. 8 and 16 cell stages are schematized to illustrate the cap-induced asymmetric divisions (yellow blastomeres) that occur independently of endogenous micromere generation. (F) Time-lapse sequence of a decentering aster under cortical pulling. Yellow star: aster center/zygote nucleus. Right: Confocal image of the same embryo after in situ fixation. Red: DNA; cyan: MTs; green: beads. Bars, 20 µm. (G) Quantification of MT density during aster decentration in the region facing the pulling cap (orange dotted box in F) and in the region facing the cell cortex (white dotted box in F; n = 23). Error bars correspond to SD. (H) Confocal image of a decentered aster in late interphase. Colors as in F. Bars, 10 µm. (I–K) Details of the MTs network surrounding a maintained pulling cap (I and J) and a free cap moving toward the aster center (K). Note that I corresponds to a magnification of the region surrounding the cap in H. Colors as in F. Bar, 10 µm. (L and M) Inhibition of MT and dynein prevent aster decentration from the cortical domain. The chemical inhibitors were added after centration. Top: Control DMSO; middle: 50 µM Ciliobrevin D (CiliD); bottom: 20 µM Nocodazole (Noco). (M) Quantification of the impact of chemical inhibitors on aster decentration by the domain. Error bars correspond to SD. Final aster positions were compared by using a two-tailed Mann–Whitney test. P = 0.0095 for CiliD and 0.0286 for Nocodazole.
Figure S2. **Aster centration and decentration under cortical pulling.** (A–C) Time-lapse sequences showing successive aster centration and decentration under cortical pulling forces when fertilization occurs: at the opposite of the cap (A), ~90° relative to the cap (B), and close to the cap (C). Arrowheads: the position of the nuclei at the center of asters; red stars: the blastomere nuclei after division. Contrast is adjusted to compensate for an increase in DNA labeling (Hoechst). (D) Time-lapse sequence of a centering under cortical pulling. Arrow: the site of sperm entry, yellow star: aster center/zygote nucleus. Right: Confocal image of the same embryo after in situ fixation. Red: DNA; cyan: MTs; green: beads. Bars, 20 µm. (E and F) Time-lapse sequences illustrating the link between aster growth and the start of aggregate centripetal motion during late (E) and early (F) centration. Orange arrowheads: sperm entry point; orange circle and star: the estimated aster size and aster center, respectively. Red arrows: the beginning of aggregate centripetal motion. Bars, 20 µm. (G) Correlation between aster radius and the shortest distance at which free aggregates are attracted toward aster center (white arrows in E and F).
Figure S3. **Influence of aster centering stiffness dynamics on centration and decentration.** (A and B) Aster position dynamic in the 1D model when the cap pulling force, $F_{\text{cap}}$, is increased in a dose-dependent manner after centration (A) or when aster centering force coefficient, $a$, is decreased in a dose-dependent manner after centration (B). (C) Measured spring constants during centration (in green) and after centration (in red) plotted as a function of aster position relative to the cell center. Negative values on the x axis correspond to a slight overshoot in the centered position. (D) Average spring constant values measured early after centration (25–45 min after fertilization [post-fert], light red, $n = 10$) and late after centration (>45 min post-fert, dark red, $n = 16$). The same datasets are also displayed in C. (E) Quantification of the velocity of a released bead cap during and after centration ($n = 78$ and $n = 24$, respectively). In D and E, error bars correspond to SD, and results were compared by using the two-tailed Mann–Whitney test. ns, $P > 0.05$. (F and G) Effect of aster centering stiffness dynamics in 3D simulation. Different transition kinetics between centration and postcentration stiffness were tested (F) and their impact on aster trajectories assessed (G).
Video 1. **Pulling cap assembly and relocation.** Unfertilized egg after magnetic bead injection. Embryos were visualized using differential interference contrast; beads are in green. The magnetic probe is used to assemble and relocate a cortical pulling cap. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 30 s for 17 min.

Video 2. **Asymmetric division induced by artificial cortical pulling.** A cortical pulling cap is maintained during the first cleavage in embryos by using a magnetic probe. Embryos were visualized using differential interference contrast; the beads forming the pulling cap are in green. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 10 s for 98 min.

Video 3. **“Free cap” control embryo.** First cleavage visualized in an embryo containing a free pulling cap (no magnet). Embryos were visualized using differential interference contrast; the beads are in green. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 20 s for 63 min.

Video 4. **“Non-pulling cap” control embryo.** First cleavage visualized in an embryo containing a nonpulling cap maintained at the cortex. Embryos were visualized using differential interference contrast; the beads forming the cap are in green. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 30 s for 62 min.

Video 5. **Decentration after dynein motors inhibition or MT depolymerization.** Nucleus decentration after drug addition (white square at the bottom left corner of each video). From top to bottom: DMSO; 50 µM Ciliobrevin D; 20 µM Nocodazole. Embryos were visualized using differential interference contrast; the beads forming the cap are in green. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 30 s for 15 min.
Video 6. **Aster centration and decentration under cortical pulling.** Aster center was visualized using male pronucleus and zygote nucleus DNA (after pronuclei fusion) in white. Beads forming the pulling cap are in green. The four panels represent different angles between the pulling cap and the sperm entry point: (i) ∼120°, (ii) ∼80°, (iii) ∼40°, and (iv) ∼5°. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 30 s for up to 61 min.

Video 7. **“Free cap” during centration.** Centration was visualized using differential interference contrast, and beads forming the free pulling cap (no magnet) are in green. In the top video, fertilization occurs close to the cap, while in the bottom video, fertilization occurs on the opposite cortex. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 30 s for 20 min.

Video 8. **Magnetic force reduction during aster centration under cortical pulling.** Centration was visualized using differential interference contrast; beads forming the pulling cap are in green. Top: An embryo containing a pulling cap moving away from the magnetic probe. Bottom: A registered and magnified view of the same embryo. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 30 s for 14 min.

Video 9. **Aster pulling during and after centration.** Two short magnetic pulls were applied, perpendicular to the centration axis (horizontal) during and after centration. The left panel (differential interference contrast) shows a low-magnification view of the embryo and magnet. The right panel shows the same embryo containing a beads aggregate bound to the aster center. DNA is in white, and beads are in green. Images were obtained by wide-field time-lapse microscopy. Frames were taken every 20 s for 44 min.

Video 10. **3D simulation of aster centration and decentration under cortical pulling.** MTs are in green, aster trajectory is in blue, and the pulling cap is represented as a red hemisphere. Frames were taken every 20 s for 50 min.