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A cell size-dependent competition between geometry and polarity governs nuclear and spindle positioning in early embryos

Highlights

- Centrosomes progressively decenter toward the apex during early embryo development
- Centrosome decentration is mediated by apical microtubule pulling forces
- Apical decentering forces progressively outcompete centering ones
- Competition between centration and decentration is mediated by cell size reduction

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In brief

By mapping centrosome position and orientation during early embryo development, Nommick et al. uncover a progressive apical shift and continuous planar alignment of centrosomes. They propose a size-scaling model in which apical surface microtubule decentering forces take over bulk centering forces as cells become smaller during development.

Report

A cell size-dependent competition between geometry and polarity governs nuclear and spindle positioning in early embryos

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SUMMARY

Nuclei and mitotic spindles are actively positioned at defined locations within cells to regulate cell polarity, division, and multicellular morphogenesis.^{1–4} Forces generated by cytoskeleton networks regulate the positioning of these organelles and are commonly influenced by extrinsic cues, such as cell geometry or polarity.^{5–12} To date, however, most studies have investigated this problem in one given cell type, hampering our understanding of how mechanical systems that position nuclei and spindles may scale during multicellular development. We tracked the spatiotemporal behavior of centrosomes, nuclei, and spindles in early sea urchin embryos from the 1-cell to the ~1,000-cell blastula stage. We found that they are initially located at cell centers, but that they undergo a progressive decentration toward the embryo apical surface, as cells become smaller during development. This apical shift is mediated by microtubule (MT) pulling forces, which are influenced by both cell shapes and apical polarity domains. Using 3D mathematical models and embryo dissections, we propose that apical cortical polarity MT decentering forces progressively take over centering forces during development as a consequence of cell size reduction and resulting increase in surface-to-volume ratio. Our results support a self-organized scenario in which polarity cues progressively outcompete cell geometry to modulate the overall balance of MT forces and pattern nuclear and spindle positioning throughout early embryo development.

RESULTS AND DISCUSSION

Nuclei and mitotic spindles become decentered during development

Early embryogenesis is characterized by a series of reductive cell divisions that are essential to establish the foundational architecture of developing organisms. These division patterns are regulated by the position and orientation of interphase asters organized around nuclei, and are often readjusted in mitosis through additional spindle translational or rotational motion.^{9,10,13} We sought to characterize and understand how the position of interphase asters, nuclei, and spindles evolves throughout early development, using sea urchin *Paracentrotus lividus* embryos. Previous studies in diverse sea urchin species have shown that centrosomes are positioned at the geometrical cell center at early zygote and 2-cell stages, thanks to a force balance among interphase astral microtubules (MTs),⁹ but other reports have documented a marked apical location on nuclei at late ~1,000-cell blastula stage, reflecting the emergence of a force imbalance during development.¹⁴ We performed sequential immunostaining to visualize DNA, MTs, and F-actin at different developmental stages, from the 1-cell zygote stage to the ~1,000-cell blastula stage. Interestingly, this confirmed that interphase asters bound to nuclei, as well as spindles, are

located close to the cell center at early stages (2-cell to 4-cell), but that they progressively decenter toward the apical surface of the embryo at subsequent stages (8-cell to ~1,000-cell blastula stage). In addition, interphase aster pairs, as well as spindles, appeared to align along an axis mostly parallel to the embryo surface, indicating that divisions are oriented in a planar manner throughout early development (Figures 1A and 1B). We confirmed these observations using live imaging of embryos injected with a GFP-NLS construct to mark nuclei, or with mRNA encoding for TMBD-mStayGold and LifeAct-mScarlett3 to image MTs and F-actin, respectively, and visualize asters, spindles, and cell shapes (Figures S1A–S1C; Videos S1 and S2).

To quantify the position and orientation of interphase aster pairs and mitotic spindles, we segmented images from fixed embryos at all stages in 3D to extract the coordinates of centrosomes, geometrical cell centers, apical domains, and apico-basal (AB) cell radius (Figure 1C). As cell sizes rapidly decay during development, we normalized relevant distances to the AB cell radius. A size-normalized metric for centrosome positioning showed that interphase centrosomes at aster centers progressively shift toward the apical surface to reach a mean normalized decentering distance at the blastula stage of ~75% of the AB cell radius (Figures 1D and S1D). Similar decentration dynamics were obtained for interphase nuclei, but with

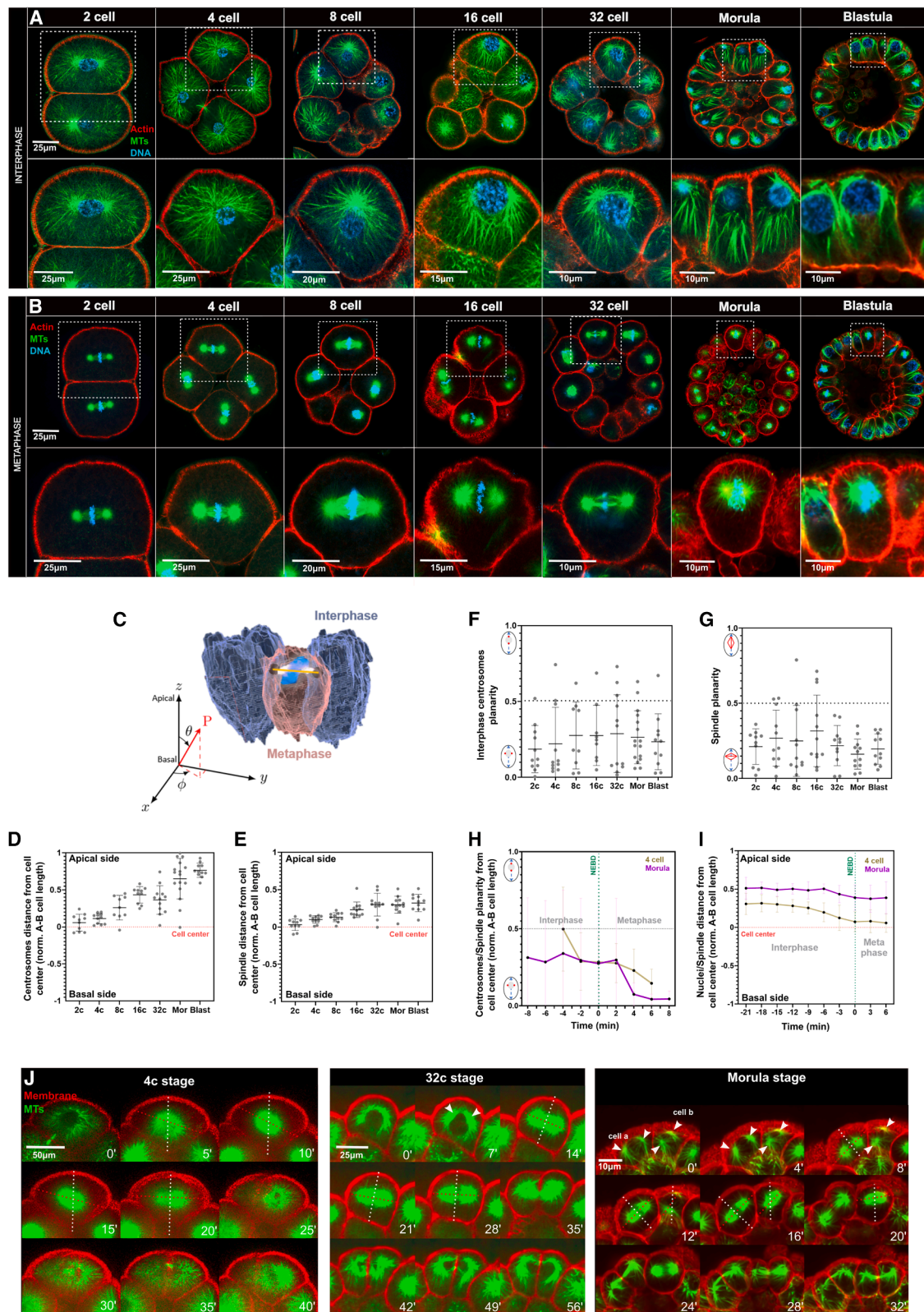


Figure 1. Patterns of nuclear and spindle positioning during early embryo development

(A and B) Confocal single-slice acquisitions of fixed sea urchin embryos at successive developmental stages in interphase (A) and metaphase (B). Embryos were stained for F-actin (red), microtubules (green), and DNA (blue). Insets highlight individual blastomeres.

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normalized decentering distances shorter than for centrosomes, reflecting the more apical location of interphase centrosomes as compared with nuclei centers (Figures 1A, S1D, and S1E). This analysis also confirmed a progressive decentration of spindles, but these were located closer to the cell center as compared to interphase asters, reaching a normalized decentering distance of only $\sim 32\%$ of the AB cell radius by the blastula stage (Figure 1E). Accordingly, live imaging of embryos injected with mRNA encoding for TMBD-mStayGold and Membrane-mCherry confirmed a systematic recentering dynamic of centrosomes between interphase and metaphase (Figures 1I, 1J, and S1F).

We also used this 3D analysis in fixed cells to compute the dot product between interphase centrosomes or the spindle axis with the AB axis. Dot products were generally below 0.3–0.4, corresponding to angles above $\sim 60^\circ$ – 70° , confirming that centrosome axes lie mostly orthogonal to the AB axis, in the surface plane of the embryo. Dot products were generally smaller in metaphase, especially at late stages, suggesting slight realignments of centrosome/spindle axis in metaphase, which we also confirmed by live imaging (Figures 1F–1H, 1J, and S1G). Altogether, these results show that interphase asters, nuclei, and mitotic spindles follow a stereotyped positioning dynamics during early development, marked by a continuous planar alignment and a progressive apical decentration.

MTs promote the apical decentration of nuclei

To understand how centrosomes progressively decenter to the apical side of the embryo, we characterized cytoskeleton organization at early and late stages of development. In interphase, astral MTs filled the whole cell volume at all stages, indicating they may function to probe cell geometry and/or cortical polarity.^{9,15,16} In metaphase, astral MTs were significantly shorter, and appeared to be mostly uncoupled from the cell surface at early stages, reaching the lateral cortices only at stages beyond the 8- or 16-cell stage¹⁷ (Figures 1A and 1B). F-actin mostly decorated the cortex, but we noted the presence of F-actin cables ~ 2 – $3\ \mu\text{m}$ in length emanating from the apical cortex, forming a brush-like structure. These cables appeared to prolong microvilli at the apical surface of cells, and were generally longer in interphase as compared with metaphase (Figures 2A and S2A). Importantly, these cables were readily observed as early as the 2-cell stage, reflecting the existence of an internal AB polarity even at early developmental stages, as previously

proposed^{18,19} (Figure 2A). In addition to F-actin, we also imaged phospho-MyoII and observed that it accumulated at the apical cortex in interphase and metaphase at all stages.²⁰ This indicates that apical cortices may exhibit higher actomyosin contractile activity, as compared with basal ones, at all developmental stages (Figure 2B). Finally, we imaged organelle content in the cytoplasm with transmission electron microscopy, and found no evidence for organelle accumulation at the basal versus apical pole, indicating that cytoplasm content in organelles may not bias centrosome or nuclear positioning, as has been proposed for other embryos^{13,15} (Figures S2B and S2C).

The decentered apical location of centrosomes suggested the dominance of an apically directed force. To identify which cytoskeletal element may generate this asymmetric force, we treated late-stage embryos with low doses of specific cytoskeleton inhibitors. We labeled nuclei that are bound to centrosomes, and tracked their movement in the cell mid-plane, with live imaging following treatments with inhibitors. As some of these inhibitors also tended to affect cell shapes, we normalized nuclear position to AB cell length at each time point (Figures S2F and S2G). Depolymerizing F-actin with a low dose of Latrunculin B led to a net apically directed motion of nuclei, while affecting myosin activity with the ML7 inhibitor did not lead to any significant movement. This suggests that the apical F-actin brush and/or cortex may oppose an apically directed force to keep nuclei a few microns away from the apical cortex.^{21,22} In sharp contrast, treating cells with low doses of nocodazole to depolymerize MTs led to a rapid basally directed motion of nuclei, indicating that MTs are the primary force generators that decenter centrosomes toward the apex. Interestingly, nocodazole-induced nuclear movements were abolished by adding Latrunculin B or ML7, suggesting that basally directed movements of nuclei in the absence of MTs result from actomyosin contractile activity at the apex (Figures 2C–2E and S2D–S2I; Video S3). Accordingly, in nocodazole-treated embryos, we observed cytoplasm flows directed toward the basal poles, with speeds similar to those of descending nuclei (Figures 2F and 2G).^{21,22} One plausible interpretation of these flows is that MT depolymerization leads to an overactivation of apical actomyosin contractility that entrains the cytoplasm.²³ A second one is that MTs may solidify the cytoplasm, thereby suppressing actomyosin-induced flows in normal conditions.²⁴ Another possibility is that nuclei may be moved by MT- or actin-independent forces and drag the cytoplasm along.²⁵ Although these flows are suppressed by interphase MTs that

(C) Three-dimensional segmentation of three blastomeres (blue = interphase; red = metaphase). The vector P (in red) represents the orientation of the mitotic spindle axis in a three-dimensional reference frame associated with the apico-basal axis of the cell.

(D and E) Quantification of nuclear (D) and spindle (E) distances from the cell center (red dotted line), normalized to the apico-basal cell length across developmental stages ($n = 10$ cells/stage, 4–5 embryos/stage).

(F and G) Quantification of interphase centrosome-to-centrosome pairs (F) and spindle (G) orientation. Values approaching 1 indicate an orientation parallel to the apical-basal axis, and values close to 0 indicate a planar orientation orthogonal to the apico-basal axis ($n = 10$ cells/stage, 4–5 embryos/stage).

(H) Mean curve of time evolution of centrosome pair orientation from interphase to metaphase at 4-cell or morula stages, normalized to apico-basal cell length ($n = 4$ cells/stage, 2 embryos/stage) (NEBD, nuclear envelope breakdown).

(I) Mean curve of time evolution of nuclei and spindle positions from interphase to metaphase at 4-cell and morula stages, normalized to apico-basal cell length ($n = 4$ cells/stage, 2 embryos/stage).

(J) Time-lapse confocal projections of embryos injected with mRNA to express TMBD-mStayGold (MTs, green) and membrane-mCherry (Membrane, red). Left: transition from 4- to 8-cell stage. Middle: from 32-cell to morula. Right: from morula-to-morula stage. The red dotted line indicates spindle orientation, and the white dotted line the apico-basal cell axis. White arrows point to the position of centrosomes in interphase/prophase.

Scale bar lengths are indicated in the corresponding panels. Error bars represent $\pm$ SD.

See also Figure S1 and Videos S1 and S2.

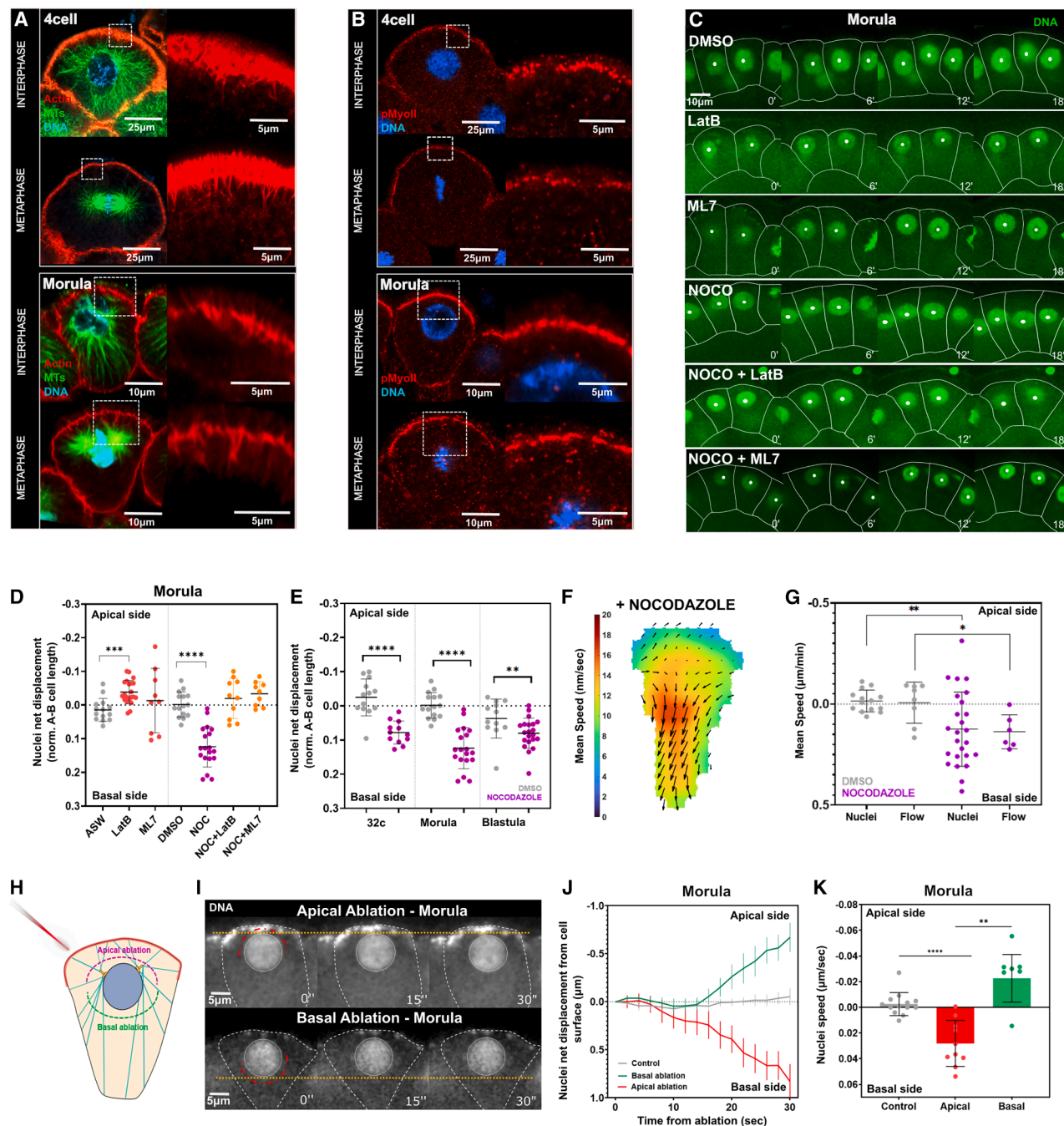


Figure 2. Microtubule pulling forces regulate centrosome apical decentration at late stages

(A) Confocal single slices of fixed sea urchin blastomeres at 4-cell and morula stages, in interphase and metaphase. Embryos were stained for F-actin (red), MTs (green), and DNA (blue). Insets highlight actin cables growing from the apical cell domain.

(B) Confocal single slices of fixed sea urchin blastomeres at 4-cell and morula stages, in interphase and metaphase. Embryos were stained for phospho-Myosin (red) and DNA (blue). Insets highlight apical domains enriched in phospho-myosin.

(C) Confocal time-lapse single slice of morula-stage embryos incubated in Hoechst to label DNA (green) and treated with the indicated chemicals. White lines delineate cell membranes, and white spots mark nuclear centers.

(D) Quantification of nuclear displacement (normalized to apico-basal cell length) after 15–30 min (from nuclei appearance until the end of interphase) in morula embryos, control and treated with indicated chemicals ($n > 10$ cells/condition, 3 embryos/condition) (ASW, artificial sea water).

(E) Quantification of nuclear net displacement (normalized to apico-basal cell length) after 15–30 min (from nuclei appearance until the end of interphase) in embryos treated with nocodazole or DMSO at the indicated developmental stages ($n > 10$ cells/stage, 3 embryos/stage).

(F) Cytoplasm flows, analyzed by tracking yolk granules in confocal imaging, within a single cell in an embryo treated with nocodazole at the morula stage. Vectors represent flow direction and amplitude.

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span the whole cell, they may be relevant to spindle recentering dynamics when astral MTs shorten (Figures 1I, 1J, and S1G). Together, these results suggest a predominant role for the MT cytoskeleton in decentering interphase centrosomes toward the apex, with some minor opposed resistance exerted by apical F-actin.

We next asked whether MTs may exert pushing or pulling forces to decenter nuclei and centrosomes toward the apical pole. In general, astral MTs appeared relatively straight in interphase asters, suggesting they may be subjected to tensile forces (Figures 1A and S1A). To more directly assay force direction, we performed UV-laser ablation of MTs.^{8,26,27} Ablating MTs on the apical side above the nucleus led to a basally directed movement of nuclei, while conversely, MT ablation from the basal side led to an apically directed movement (Figures 2H–2K and S2J). Although depending on laser wavelength and power, MT laser ablation assays may be prone to off-target effects,^{8,26,27} by altering other structures or networks in the cytoplasm, they suggest together with MT inhibition assays that MTs may primarily exert pulling forces to shift centrosome positions toward the surface of the embryo.

Cell geometry is not sufficient to account for centrosome position and orientation throughout embryo development

Cell geometry has been shown to serve as an important cue to position centrosomes in the cell center and orient them along the long cell shape axis, especially in the context of early embryo development. This is thought to be mediated by MTs that probe geometry through length-dependent forces.^{9,13,15,28–31} To explore the role of cell shape, we quantified cellular aspect ratios, defined as the AB cell length divided by the transverse length. This revealed that cells evolve from a flattened shape larger along the transverse axis at early stages, to a more rod-like shape now elongated along the AB axis at later stages (Figure 3A). To test whether these geometrical changes could account for modulations in centrosome positioning and orientation observed during development, we ran 3D simulations, using a previously established framework that we adapted to systematically model centrosome positions and orientations over multiple developmental stages.^{15,27,32} Using 3D segmentations of individual cell contours as input for cell geometries, we placed centrosome pairs at random positions and orientations and used a gradient descent simulation that computes force and torque exerted by astral MTs on centrosomes to identify

mechanical equilibria.^{15,27} This first model was based on pure geometrical considerations by assuming that astral MTs fill and probe the whole cell volume, by exerting pulling forces scaled to the cube of their length.^{9,15,29,33} In metaphase, these simulations revealed that geometry can serve as a good predictor of spindle position and orientation at early 2-cell or 4-cell stages, but that these geometrical predictions depart from experimental results at later stages, with spindles largely deviating from the cell geometrical center and also aligning along the shortest cell shape axis, in sharp contradiction to model predictions (Figures 3B–3D).

In addition, we observed some level of shape changes between interphase and mitosis, with cells undergoing partial rounding at late stages beyond the 32-cell stage (Figures 3E and 3F). This prompted us to run simulations based on interphase cell shapes at these late stages. Again, model predictions based on 3D interphase cell geometry failed to account for nuclear/centrosome apical locations and for their planar orientations (Figures 3G–3I). We conclude that cell geometry may constitute an important cue to control centrosome positioning at early developmental stages,^{9,13,15} but that it is insufficient to explain centrosome decentration and planar orientation at later stages.

A titrated competition between polarity and geometry predicts centrosome positioning throughout development

The progressive apical decentration of centrosomes suggested a relative increase in apically directed MT forces during early development, likely regulated by apical polarity domains.^{34–36} As mentioned above, our observation of a markedly different actin cortical network on the apical pole, as well as the apical enrichment in phospho-MyoII, indicates the emergence of AB polarity as early as the 2-cell stage.¹⁸ To more directly monitor upstream regulators of AB polarity, we stained embryos at all stages for phospho-PKC ζ (protein kinase C zeta). PKC ζ is an atypical isoform of protein kinase C, conserved across species, that plays a central role in the establishment and maintenance of AB polarity.^{19,37–39} This confirmed the presence of polarized membrane domains, as early as in the 2-cell stage, with a marked enrichment of the apical surface, which persisted throughout development. Apical enrichment in p-PKC ζ was constant around ~2-fold as compared with the basal one, ruling out any major developmental maturation of AB asymmetries in the relative apical versus basal amount of polarity effectors.

(G) Nuclear and cytoplasm flow speeds in the indicated conditions in embryos at the morula stage ($n > 10$ cells for nuclei speeds, $n > 6$ cells for flow speeds, 2 embryos/condition).

(H) Scheme of a cell at the morula stage depicting regions in which MTs were ablated with a laser beam.

(I) Time-lapse confocal single slice of morula-stage embryos incubated in Hoechst to label DNA after MT laser ablation. White dashed lines delineate cell membranes, white lines delineate nuclei, and red dashed lines indicate ablation regions. Time is indicated in seconds.

(J) Nuclear net displacements after apical or basal MT laser ablation at the morula stage. Time 0 corresponds to the moment of ablation. The curves represent the mean of all nuclei displacements ($n > 10$ cells/condition, 3–4 embryos/condition).

(K) Nuclei speeds after laser ablation. Each dot represents the slope of the net displacement curves of single nuclei (Ct, control; Ap, apical ablation; Bs, basal ablation).

Scale bar lengths are indicated in the corresponding panels. Error bars represent $\pm$ SD. Data were compared using parametric two-tailed unpaired Student's t tests (between two groups following a normal distribution), or using the Mann-Whitney test (non-normal distribution). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

See also Figure S2 and Video S3.

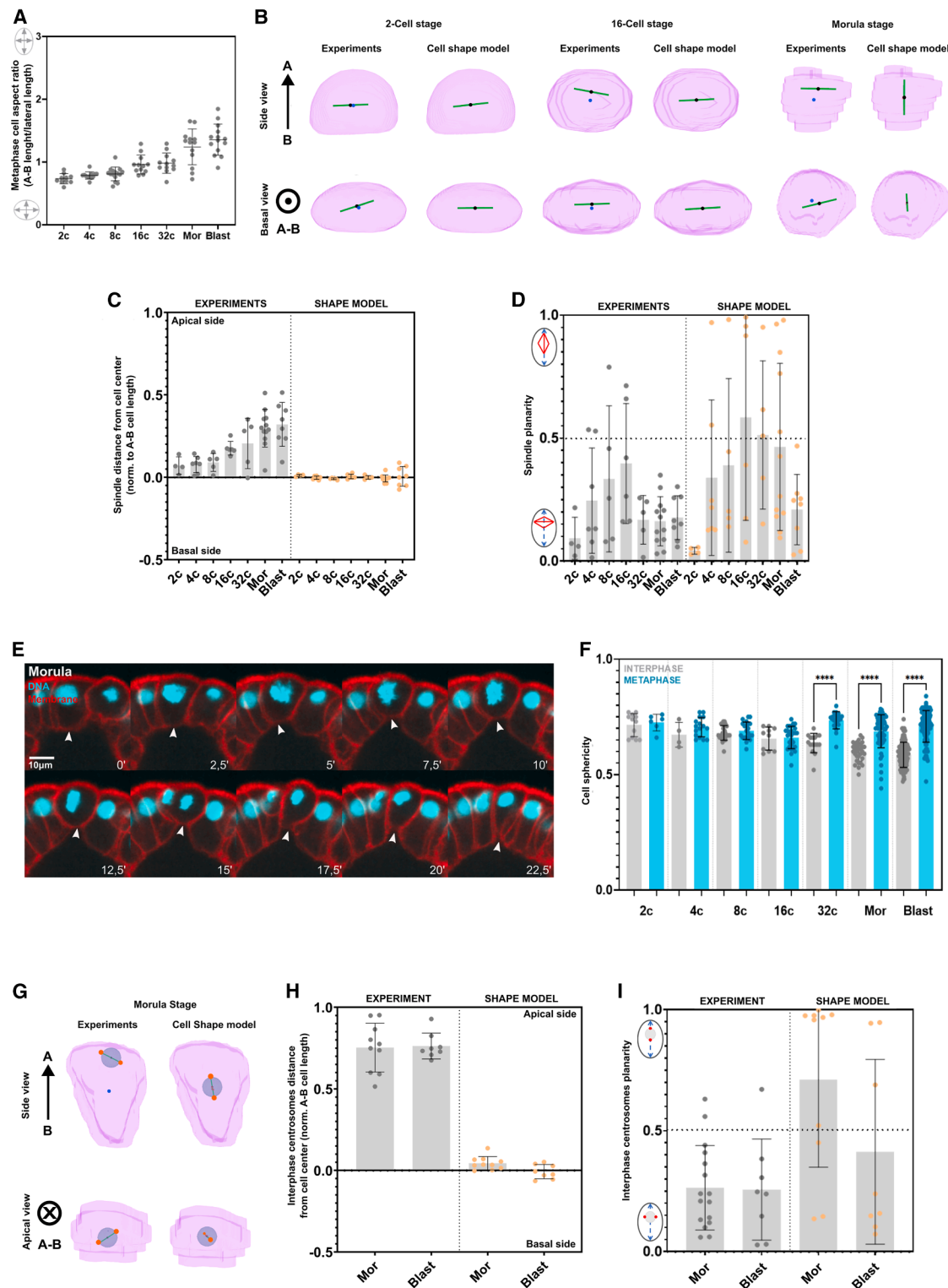


Figure 3. Cell geometry is not sufficient to explain centrosome positioning during early development

(A) Quantification of metaphase cell aspect ratio defined as the apico-basal cell length divided by lateral length ($n > 10$ cells/stage, 3–6 embryos/stage). (B) 3D model predictions of metaphase spindle orientation and position at different developmental stages. The green line marks the spindle axis, the blue dot the cell center of mass, and the black dot the spindle center. (C and D) Experimental results (gray dots) and 3D model predictions for the same cells (orange dots) of spindle distance from the cell center normalized to apico-basal cell length (C) and spindle planarity (D) at different developmental stages ($n > 4$ cells/stage, 2–3 embryos/stage).

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Importantly, these p-PKC ζ domains colocalized with apical zones enriched in actin cables, indicating that these apical protrusions indeed reflect the presence of upstream polarity effectors (Figures 4A, 4B, and S4A). Therefore, these results confirm the existence of bona fide AB polarity established from the beginning of embryo development.

In order to test the contribution of apical polar zones to centrosome positioning, we turned back to 3D simulations. We added to the 3D geometrical model a polar domain, with size and position inferred from 3D images of apical zones enriched in F-actin, and assumed that MTs contacting this domain exert an additional force as compared with other MTs in the cell (Figure 4C). As such, we first explored a model in which all MTs pull with a force that scales to the cube of their length to account for a geometrical effect based on probing cell volume,^{9,29} and in which MTs contacting the apical polar domain exert an additional force scaled to the square of MT length, to model a “surface-based” pulling, that was proposed on the premise that motors may be limiting for MT force exertion at cortical domains.^{15,40} The ratio between these two force contributions is embedded in the value of a single parameter, β .

We first used this model to test whether mere changes in cell geometry and polarity domain size and location during development were sufficient to recapitulate experimental behavior. For this, we used constant low or high values for β at different stages. This generally led to poor predictions of the model, with an underestimation of centrosome apical decentration or a premature one, respectively (Figures S3A–S3C). However, we posited that the reduction in cell size that marks early development could yield to an increase in surface-to-volume ratio, and thus a progressive dominance of surface-based polarity over bulk MT pulling. Therefore, to implement a size-dependent decrease in the ratio between geometrical-based centering forces and polarity-based decentering forces, we inversely scaled β to cell volume using a simple single law (Figures 4D and 4E). Remarkably, without adjusting any parameters between simulations, this model reproduced the complete trend of progressive decentration of interphase centrosomes and spindles, as well as their planar orientation at all stages (Figures 4F–4L, S3D, and S3E). We note that the model generally predicted a higher level of spindle planarity than in experiments, reflecting slight spindle dynamic realignments (Figures 1H, 4H, and S3E). To test the robustness of this size-scaling competition model, we also explored a model in which all MTs pull with forces scaled linearly to their length, with an additional constant force independent of their length when they contact the apical domain. This version of the model also reproduced the observed progressive decentration of centrosomes, but was less accurate in predicting spindle orientation (Figures S3F and S3G). This suggests that model

predictions are likely robust to details for MT force-scaling, provided that β is inversely scaled to cell size. Therefore, these modeling results suggest that a progressive competition between geometry and polarity, mediated by cell size reduction, may account for the full spatiotemporal pattern of centrosome positions during early development.

Cell size is a key regulator of centrosome and nuclear position

One direct prediction from the model is that the competition between geometry and polarity may be titrated by cell size, but an important alternative is that it may be regulated by a progressive maturation of apical polarity domains. To directly test this, we performed microdissections of unfertilized eggs and fertilized these cut eggs to produce smaller embryos with smaller cells and tracked nuclear positioning (Figures 4J and 4K). Quantification of cell volumes from immunostaining images confirmed a reduction of ~ 1.5 -fold on average, in cut embryos as compared with control embryos from the same batch at the same developmental stage. Remarkably, this size reduction caused a marked precocious apical shift in nuclear positioning, with nuclei at the 4- or 8- cell stage in cut embryos being as close to the apical cortex as those of 16- or 32-cell stages in control non-cut embryos (Figures 4L and S4C). We noted, however, that this impact of cell size reduction saturated at later stages, presumably because interphase asters and nuclei reach a minimal distance to the apex set by the presence of apical F-actin brushes (Figure 2D). Finally, plotting nuclear apical shifts as a function of cell volumes for control and cut embryos revealed a clear negative dose-dependence correlation (correlation coefficient: -0.71), well aligned with model predictions, supporting the impact of cell size on MT mechanical force balance that positions centrosomes and nuclei (Figure 4M).

The mechanical elements that position and orient centrosomes are fundamental regulators of embryo development and tissue morphogenesis, and have been investigated in numerous single-cell types.^{7–9,41} Here, we systematically mapped centrosome, nuclear, and spindle positions throughout the course of multicellular embryo development. We found that centrosomes follow stereotypical graded positional mechanisms, with a progressive apical shift and a near constant planar orientation throughout early development. Models and experiments suggest that a single rule based on the competition between the influence of AB polarity domains and cell shape on astral MT forces, titrated by cell volume reduction, is sufficient to account for these patterns (Figure 4N). However, the precision of our models and experimental measurements cannot preclude that other types of scaling laws based on,

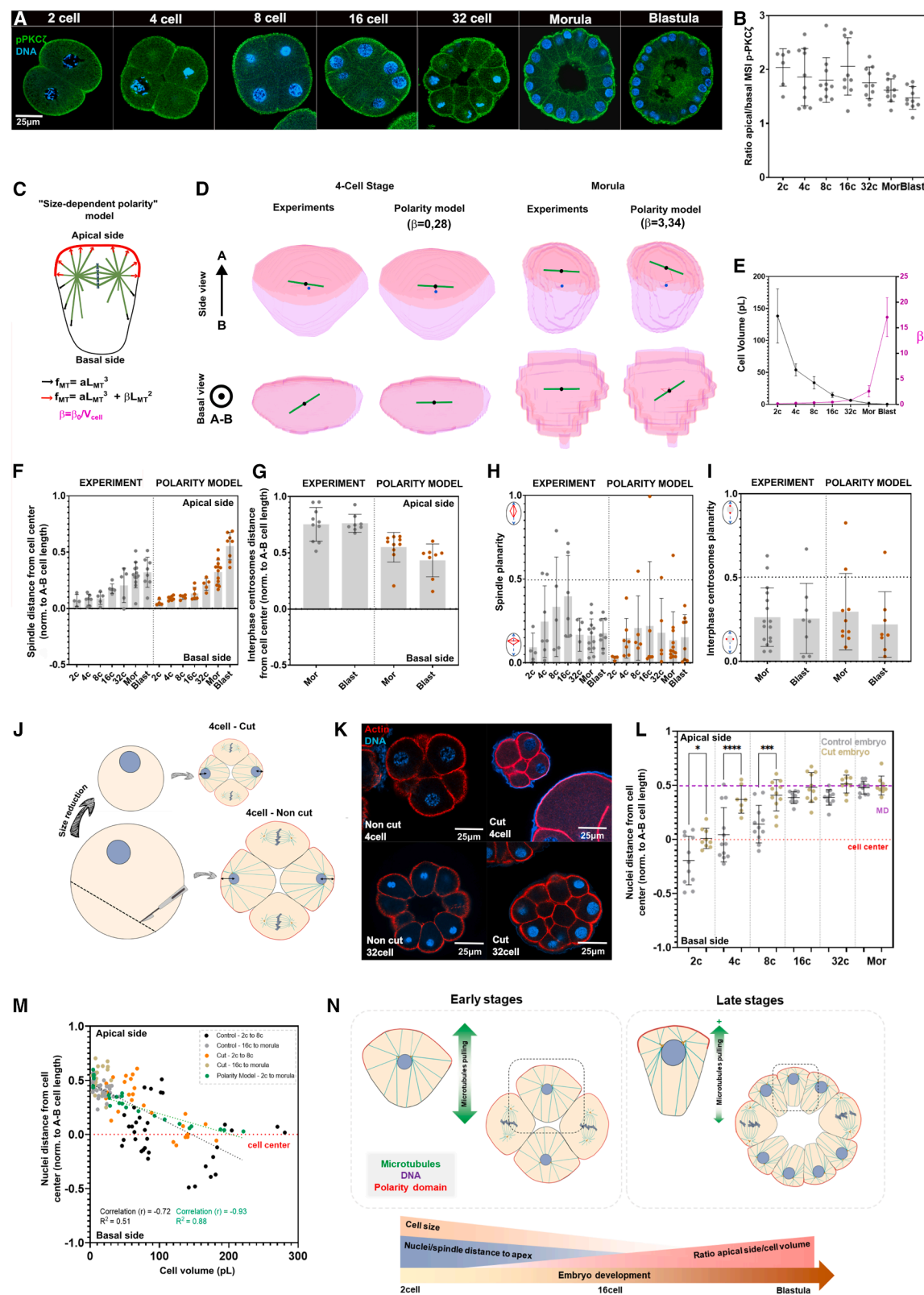
(E) Confocal time-lapse projections of morula-stage embryo expressing H2B (DNA, cyan) and membrane-mScarlet3 (red). Arrowheads indicate the basal cell domain that rounds up during mitosis.

(F) Quantification of cell sphericity across different stages in interphase (gray) and metaphase (blue) ($n = 6$ –60 cells/stage, 3–6 embryos/stage).

(G) 3D model predictions of interphase centrosome pairs orientation and position. Orange dots mark centrosomes; the gray circle, the nucleus; the blue dot, the cell center of mass; and the black dot, the nucleus center.

(H and I) Experimental results (gray dots) and 3D model predictions for the same cells of interphase centrosome pairs position (H) and planarity (I) at morula and blastula stage ($n = 8$ –10 cells/stage, 2–3 embryos/stage).

Scale bar lengths are indicated in the corresponding panels. Data were compared using parametric two-tailed unpaired Student's t tests (between two groups following a normal distribution), or using the Mann-Whitney test (non-normal distribution). Error bars represent $\pm$ SD. **** $p < 0.0001$.



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e.g., cell length or cell surface, could also presumably reproduce these patterns. Therefore, an important output of this study is to put forward a “cell size scaling” model for MT force balance and consequent nuclear and spindle positioning. Size scaling is fundamental for early embryo development.^{42,43} It ensures that organelles such as nuclei or spindles will assume adequate dimensions to fulfill cellular functions, or that dynamic processes such as cytokinesis or chromosome segregation are executed within the same timing through embryo development.^{44–49} We propose that size scaling of MT forces for centrosome positioning may be essential to ensure that spindles end up apically located in forming columnar morula/blastula cells, to promote faithful bipolar spindle formation, chromosome segregation, or monolayered blastoderm tissue architecture.^{27,50} Future work addressing in quantitative terms how cytoskeletal forces intersect with cell size will bring important insights into mechanisms regulating organelle positioning and multi-cellular morphogenesis.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Nicolas Minc (nicolas.minc@ijm.fr).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- All data needed to evaluate the conclusions in the paper are present in the paper and/or the [supplemental information](#).
- This paper does not report any original code.
- Any additional information required to reanalyze the data reported in this work is available from the [lead contact](#) upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, N.M. and A.N.; methodology, N.M., R.L.B., C.M.-D., J.S., M.B., and A.N.; writing – original draft, N.M. and A.N.; draft editing, N.M., R.L.B., C.M.-D., J.S., and A.N.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Figure 4. Size-dependent competition between apical polarity and cell geometry accounts for centrosome positioning dynamics during development

- (A) Confocal projections of fixed embryos at successive developmental stages stained for the phosphorylated form of PKC ζ (p-PKC ζ , green) and DNA (blue). The signal has been adjusted differently in each panel to facilitate visualization.
- (B) Quantification of the ratio between apical and basal p-PKC ζ mean signal intensity (MSI) ($n = 10$ cells/stage, 2–3 embryos/stage).
- (C) Scheme of the size-dependent polarity model for centrosome positioning: All astral MTs pull with a force proportional to the cube of their length, $f_{MT} = aL_{MT}^3$, with a , a fixed constant, but MTs that contact the apical domain pull with an additional force so that $f_{MT} = aL_{MT}^3 + \beta L_{MT}^2$. To account for volume reduction of blastomeres during development, β is inversely scaled to cell volume.
- (D) 3D model predictions of metaphase spindle orientation and position at different stages. The green line marks the spindle axis, the blue dot marks the cell center of mass, and the black dot marks the spindle center; the pink area delineates the apical polar domain.
- (E) Evolution of measured cell volume during development, and consequent impact on values of the parameter β as a function of developmental stages.
- (F) Experimental results (gray dots) and 3D model predictions for the same cells (orange dots) of spindle distance from the cell center normalized to apico-basal cell length ($n = 4–10$ cells/stage, 2–3 embryos/stage).
- (G) Experimental results (gray dots) and 3D model predictions for the same cells (orange dots) of interphase centrosome distance from the cell center normalized to apico-basal cell length, at morula and blastula stages ($n = 8–12$ cells/stage, 2–3 embryos/stage).
- (H) Experimental results (gray dots) and 3D model predictions for the same cells (orange dots) of spindle planarity ($n = 4–10$ cells/stage, 2–3 embryos/stage).
- (I) Experimental results (gray dots) and 3D model predictions for the same cells (orange dots) of interphase centrosome pairs planarity ($n = 8–12$ cells/stage, 2–3 embryos/stage).
- (J) Scheme representing microdissection experiments in unfertilized sea urchin eggs. Eggs were mechanically cut with a micro-needle and then fertilized. Nuclei position was tracked at different stages and compared with non-cut embryos from the same batch.
- (K) Confocal acquisitions of fixed intact (“non-cut”) and cut embryos at the 4- and 32-cell stages. Embryos were stained with phalloidin (F-actin, red) and Hoechst (DNA, blue).
- (L) Nuclear distance from the cell center normalized to the apico-basal cell length in cut versus control embryos at different developmental stages ($n = 3–6$ embryos/stage, 3–6 embryos/stage) (MD, minimal distance).
- (M) Experimental and theoretical nuclear distance from the cell center normalized to the apico-basal cell length plotted as a function of cell volume for individual blastomeres in the indicated stages and conditions. The dotted lines are linear regressions (black line = LR of all cells from control and cut embryos together, green line = LR of cells from the polarity model). Linear regression data were fitted using a simple linear regression model.
- (N) Proposed “scaling” model for centrosome positioning in early embryos. As cells reduce in volume during development, the amplitude of MT centering forces reduces, allowing apical surface decentering forces to take over and progressively bring centrosomes, nuclei, and spindles closer to the embryo surface. Scale bar lengths are indicated in the corresponding panels. Error bars represent $\pm$ SD. Data were compared using parametric two-tailed unpaired Student’s t tests (between two groups following a normal distribution), or using the Mann-Whitney test (non-normal distribution). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

See also [Figures S3 and S4](#).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
YL1/2 rat monoclonal	Abcam	Cat# ab6160; RRID: AB_305328
DM1A mouse monoclonal	Abcam	Cat# ab7291; RRID: AB_2241126
p-PKC ζ (Thr 410) rabbit polyclonal	Santa Cruz Biotechnology	Cat# sc-12894-R; RRID: AB_2237780
Phospho-myosin light Chain2 (Ser 19) Rabbit polyclonal	Cell Signalling Technology	Cat# 3671; RRID: AB_330248
Nano mouse Alexa Fluor 488 IgG1	Thermo Fisher Scientific	Cat# SMS1AF488-1-100
Goat anti-rabbit IgG DyLight 488	Invitrogen	Cat# 35552
Donkey anti-rat IgG secondary antibody, DyLight 650	Invitrogen	Cat# SA5-10029
Bacterial and virus strains		
DH5 α competent cells	Thermo Fisher Scientific	EC0112
Chemicals, peptides, and recombinant proteins		
DMSO	Euromedex	UD8050
Triton X-100	Sigma-Aldrich	T8787
HEPES	Sigma-Aldrich	H3375
EGTA	Sigma-Aldrich	E3889
Triton X-100	Sigma-Aldrich	T8787
Formaldehyde 16% (w/v), methanol free	Thermo Fisher Scientific	28908
NaBH ₄	Sigma-Aldrich	D282
PABA	Sigma-Aldrich	A9878
MgSO ₄	Sigma-Aldrich	M7506
Nile blue A	Sigma-Aldrich	N0766
Glutaraldehyde Grade I, 25%	Sigma-Aldrich	G5882
Glycerol	Sigma-Aldrich	G5516
Protamine sulfate	Sigma-Aldrich	P4020
Tween 20	Sigma-Aldrich	P1379
CF 555/ maleimide	Sigma-Aldrich	SCJ4600021
Hoechst	Sigma-Aldrich	33342
Rhodamine phalloidin	Invitrogen	R415
Nocodazole	Sigma-Aldrich	M1404
ML-7	Sigma-Aldrich	I2764
Latrunculin B	Sigma-Aldrich	428020
Experimental models: Organisms/strains		
Sea urchins (<i>Paracentrotus lividus</i>)	Roscoff Marine Station (France)	N/A
Plasmids		
H2B-GFP	Lab Stocks	pAN01
H2B-RFP	Lab Stocks	pAN02
GST-GFP-NLS	Lab Stocks	pJS15
GST-mCherry-NLS	Lab Stocks	pJS16
EMTB-3GFP	A gift from Alex McDougall (LBDV, Villefranche)	pAN04
TMBD-mStaygold	This Study	pJS37
Membrane-mCherry	Lab Stocks	pAN07
Membrane-mScarlet	Lab Stocks	pJS36

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
LifeAct-mScarlet	This Study	pJS40
Software and algorithms		
Image analysis software	FIJI	https://imagej.net/software/fiji/downloads
Statistical analysis	GraphPad Prism 10	https://www.graphpad.com/
MATLAB	MathWorks	mathworks.com/products/matlab.html ; RRID: SCR_001622
3D models for centrosome positioning	Ershov and Minc ⁴⁴ and Pierre et al. ¹⁴	http://www.minclab.fr/research/
Cell segmentation	IMARIS 9.2	https://imaris.oxinst.com/
Cell segmentation	DM3D Fiji Plugin	https://franciscrickinstitute.github.io/dm3d-pages/tutorial.html
Other		
Artificial seawater (Reef Crystal)	Instant Ocean	RC15-10
Sigmacote	Sigma-Aldrich	SL2

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Sea urchin gametes

Purple sea urchins (*Paracentrotus lividus*) were obtained from Roscoff Marine station (France) and maintained at 16°C in aquariums of artificial sea water (ASW; Reef Crystals, Instant Ocean). Female and male gametes were collected by opening animals, or by intracoelomic injection of 2mL 0.5 M KCl. Eggs were collected and washed in ASW.F (artificial sea water filtered). Sperm was collected with similar methods, and kept at 4°C and used within 2–4 days after collection. When eggs were injected, they were first filtered 3 times through a 70-μm Nitex (VWR) mesh to remove the vitelline membrane and facilitate adhesion to the protamine-coated dish. Unfertilized eggs were incubated at 16°C in ASW.F until use the same day of collection. If the final purpose was to fix embryos for immunostaining, they were fertilized in ASW.F + PABA 0.274mg/mL for 2 min to remove the fertilization membrane and facilitate antibody (Ab) penetration. Then, they were filtered through an 80-μm Nitex (VWR), and incubated in ASW.F at 16°C to develop until the desired stage prior to fixation.

METHOD DETAILS

Immunostaining

Embryos were fixed either directly on protamine-coated dishes or in bulk in falcon tubes in FA buffer (100mM HEPES-pH 6.9, 50 mM EGTA, 10mM MgSO₄, 0.32% Formaldehyde, 0.05% Glutaraldehyde, 0.2% Triton X-100, 400mM Dextrose) for 70min at room temperature (RT). Then, they were washed 3X in PBT and stored at 4°C or used directly for immunostaining. First, to remove auto-fluorescence, they were incubated 30 min in PBS 1x + 0.1%NaBH₄ and washed 2X in PBS for 10min. Then, embryos were blocked in PBT + 0.1%BSA + 5% Goat Serum for 30min at RT. The blocking solution was replaced by the primary antibody (Ab) diluted in PBT + BSA 0.1% and embryos were incubated ON at 4°C. The next day, they were washed 3X in PBT for 30min and incubated in the solution containing the secondary Ab, and Hoechst (1/200) to label DNA diluted in PBT for 3h at RT in the dark. Finally, they were washed 1X in PBT 30min, 1X in PBS and finally in PBS 1X + 50% Glycerol and let to sediment for 30 min until mounting. Embryos were mounted on a glass dish (50mm Dish, No. 1.5 coverslip, 14mm Glass DiaMTser, MatTek Corporation) in glycerol-based Mounting Medium, and covered with a 18x18mm coverslip sealed with nail polish. All antibodies used and their concentrations are listed in the [key resources table](#).

Protein synthesis

Recombinant GST-GFP-NLS and GST-mCherry-NLS were expressed and purified as previously described.^{46,51} Protein stock concentrations were generally around ~1–10 mg/ml.

mRNA synthesis

Capped-mRNA were synthesized with the mMessage mMachine kit (ThermoFisher) and purified with the MEGAClearPURIF kit (ThermoFisher). To increase mRNA stability and translation efficiency a Poly(A) sequence was added with the Poly(A) Tailing KIT (Ambion). All constructs are listed in the [key resources table](#).

Egg injection

Injection experiments were performed on an inverted epifluorescence microscope (TI-Eclipse, Nikon) combined with a complementary metal–oxide–semiconductor (CMOS) camera (Hamamatsu), using 20x and 10x dry objectives (Apo, NA 0.75, Nikon). The microscope is operated with Micro-Manager (Open Imaging), at a stabilized room temperature (18–20°C).

Injectations were done with capillaries TW100F-4 (borosilicate glass capillaries 1mm diameter) (World Precision Instruments, WPI), previously pulled (P-1000, Sutter Instrument) and tip-ground with the MicroGrinder (EG-402 Narishige) at an angle of 40°. Eggs were adhered to glass dishes (μ -Dish 35mm 1.5 low, IBIDI) coated with 1% protamine and placed on the inverted epifluorescent microscope, equipped with a micromanipulator (Injectman 4, Eppendorf) and a micro-injection system (FeMTsoJet 4, Eppendorf). After injections, eggs were fertilized with 1 drop of diluted sperm, and imaged for *in vivo* experiments, fixed, or incubated at 16°–20°C until the desired stage.

Before each experiment, the -80°C RNA stock aliquot was centrifuged 5min at 19000rpm at 4°C/RT. Then, it was dissolved at the desired concentration in RNase-free-H₂O.

Chemical inhibitors

The concentrations and incubation times used for different cytoskeleton inhibitors were: Nocodazole 20 μ M for 5min, ML7 100 μ M for 15min, Latrunculin B 0.5 μ M for 15min. Drugs were diluted to their final concentration from a 100X stock in DMSO. For immunostainings, embryos were developed in ASW.F, and transferred in a tube containing 5mL ASW.F supplemented with inhibitors, and let to incubate for varying time depending on the inhibitor, prior to fixation. For live imaging, embryos were developed on a dish in ASW.F until the desired stage. Then, the drug was diluted in 500 μ L of ASW.F and added to the dish. All drug concentrations and incubation times were calibrated in order to minimize toxicity and embryo mortality. The control conditions used to compare drug effects have been ASW.F or ASW.F + DMSO, depending on the medium in which the original drug stock was dissolved.

Egg bisection

Non-fertilized eggs were adhered to glass dishes (μ -Dish 35mm 1.5 low, IBIDI) coated with 1% protamine and incubated in ASW.F + Hoechst (1/10000). A micro dissecting knife (Item No. 10055-12 FST) was used for cutting the embryos under a binocular microscope (Nikon SMZ1270). Cut eggs were then fertilized by adding one drop of diluted sperm and incubated at 16°C until the desired stage of imaging or fixation.

Imaging

Live-imaging and immunostaining of fixed embryos were performed on three different microscopes: (1) A spinning-disk confocal microscope (TI-Eclipse, Nikon) equipped with a Yokogawa CSU-X1FW spinning head, and an EM-CCD camera (Photometrics, PRISM BSI) and a 40xWater, 40xOil or 60xOil objectives; (2) A spinning-disk confocal microscope (TI-Eclipse, Nikon) equipped with a Yokogawa CSU-W1 spinning head, and an EM-CCD camera (Photometrics, KINETIX 22) and a 60xWater objective; (3) A confocal microscope Zeiss LSM980, coupled to the Airyscan 2 module equipped with 63xOil and 40xWater objectives. Imaging was performed at room temperature (between 18–25°C).

Electron Microscopy

Sea urchin embryos, at the morula stage, were processed by high-pressure freezing and freeze substitution. The samples were placed in a 200 μ m deep flat specimen carrier, 1.5mm dia. The sample was immediately high-pressure frozen (EMPact2, Leica). Frozen carriers were transferred to a cryotube containing 1 mL of cryo-substitution mixture, consisting of 2% osmium, 0.1% glutaraldehyde (GA) and 0.1 % uranyl acetate (UA) in acetone. Since GA and UA were diluted from an aqueous solution, the final mix included 2.9 % H₂O. Freeze-substitution was performed in an AFS2 (Leica), at -90 °C for 50 hours, then the temperature was gradually increased to -30°C, this temperature was maintained for 24 hours. Finally, the temperature was gradually raised to +15 °C. The freeze-substitution mixture was removed and specimens were washed in two 15 min acetone baths and infiltrated with epoxy resin (Agar, low viscosity resin) in baths of increasing concentration of resin, up to 100%. Polymerization was done at 60°C for 24h. 70-nm-ultrathin sections were cut using an EM UC6 ultramicrotome (Leica) and collected on slot grids. The sections were post-stained in 2% aqueous uranyl acetate and lead citrate (Reynold's solution) and observed at 120 kV with a Tecnai12 transmission electron microscope (ThermoFisher Scientific) equipped with a 4K OneView camera (Gatan).

Laser Ablation

Single Z-plane laser ablation experiments were performed using a 355 nm UV laser integrated into a spinning disk confocal microscope (iLas Pulse system, MetaMorph software). Laser calibration was carried out every 30 minutes to correct for laser alignment drift over time and temperature. Embryos were adhered on glass dishes (μ -Dish 35mm 1.5 low, IBIDI) coated with 1% protamine with ASW.F + Hoechst (1/10000) under a 40XOil objective. Laser parameters were optimized for the experiment: 10 repetitions, 100% laser power, thickness 2. Time-lapse acquisitions were done every 2 seconds after ablation.

3D simulations

The simulation package used for 3D models was developed in Matlab and was adapted from Pierre et al. and Ershov et al.^{15,32} This package includes a module to extract and reconstitute 3D shapes, centrosome positions and polarity domain center and size from segmented experimental imaging stacks. For all simulations, inputs taken from individual experimental 3D stacks were thus: 3D cell contours, the distance between centrosomes, and the center and size of apical polar domains. Once a cell with its shape and polarity is defined as an input, the model places the centrosome pair in a random position and orientation, and traces MTs from centrosomes to measure their length. MTs density in asters is set as one MT per 10° angle, but is a parameter that can be tuned and generally has

little influence on simulation predictions.¹⁵ The length of each MTs serves to compute a force f_{MT} , based on a predefined hypothesis for how MTs may pull in the cell, using the generic formula:

$$f_{MT} = aL_{MT}^{\alpha} + Q(Pol) * \beta L_{MT}^{\gamma} \quad (\text{Equation 1})$$

in which α and γ are exponents for length scaling hypotheses; $Q(Pol)$ is 1 when MTs contact the apical polarity domain, and 0 otherwise; a a fixed constant and β a pre-factor that ponders polarity- over geometry-based forces. These forces are integrated over the whole cell to compute both a global force and torque exerted by both MT asters on the centrosome pair. To search for minima, the simulation was based on a random gradient descent strategy, which follows the minimization of torques and forces. This was achieved by randomly modulating one of the 5 spatial parameters (3 for the position and 2 for the orientation angles of the centrosome pair), and recalculating the length of all MTs and thus the force and torque exert at each the subsequent step of the simulation. The simulations followed the direction of force/torque minimization and stopped at a position/orientation once the iteration returns to this equilibrium after 500 number of iterations. The minimal total length of the simulations was set to 3000 iterations. To prevent the simulated centrosome positions and orientations to be trapped in minimal equilibria or the simulation to be deterministic, two parameters are used to define (i) a jump probability to move to a new set of coordinates even if it is less favourable than the previous one, and (ii) an inertia probability to vary the same spatial parameter as in the previous step if during this step centrosomes were moving along the gradient descent. Finally, to ensure that simulations output did not result from a bias in the initial conditions, the simulations were run typically 3-4 times from another random starting position/orientation. Finally, once a position was found, the model recalculated the torque landscapes as a function of the 2 possible angles to refine the energy landscape. Importantly, in these simulations total forces and torques are renormalized, making the model completely independent of absolute cell size, but very sensitive to anisotropies in cell shapes and/or polarity.^{15,32}

Two general classes of simulations were performed in this work. The first one, the “geometrical model” assumes that all MTs pull with a force that scales to the cube of their length, so that $\alpha=3$ and $Q=0$ for all MTs (Figure 3). Importantly, in the context of this model, the value of α has little influence on the predicted position or orientation of centrosome pairs, as long as it is not set to 0.¹⁵ The second one, the “polarity model” assumes that all MTs pull with a force that scales to the cube of their length ($\alpha=3$) and that MTs that contact the apical polar domain, selected using the function $Q(Pol)$, exert an additional pulling force scaled to the square of the length of MTs ($\gamma=2$), with a parameter β that represents the competition between geometry and polarity (Figures 4 and S3). Another version of this polarity model was also tested in Figures S3F and S3G, in which $\alpha=1$ and $\gamma=0$. The parameter β was either set at different low (0.15) or high (15) values for different developmental stages for the simulations presented in Figures S3A–S3C; or inversely scaled to cell volume, so that: $\beta = \frac{\beta_0}{V}$. The value of β_0 was calculated based on a dichotomic approach from an initial set of simulations considering the smallest blastula stage cell, intermediate 16-Cell stage and 2-Cell stage, aiming to minimize the predicted position of centrosome to that obtained in experiments. Once calculated the same β_0 was used for all the simulations presented in the MS. For metaphase simulations $\beta_0 = 4.16$ and for interphase simulations $\beta_0 = 22.12$. We note that a model in which β would be scaled to the inverse of AB cell length or cell surface, would probably also reproduce the progressive decentration of centrosomes.

QUANTIFICATION AND STATISTICAL ANALYSIS

Cell sphericity

To segment cells in 3D and quantify cell sphericity, Z-stacks confocal acquisitions were segmented using the semi-automatic tool “membrane-based cell segmentation” of the IMARIS 9.2 software, based on membrane signals (Membrane-RFP). A background noise subtraction filter was applied to all acquisition. Errors were corrected manually and cells were classified as interphasic or mitotic based on MTs (using EMTB-GFP) and Hoechst signals.

3D tracking of centrosome orientation and position, geometrical cell center and Apico Basal axis

For all the 3D analysis in this MS, the 3D coordinates of interphase or metaphase centrosomes were obtained manually with Fiji, from fixed or live Z-confocal-stacks MT signal, assuming that centrosomes are localized at the centre of interphase MT asters or spindle poles (Figure S1D). The coordinates of the center of the apical domain, and the size of this domain were manually computed based on the signal of F-actin at the apical membrane, with Fiji, from fixed or live Z-confocal-stacks of F-actin signal. 3D cell contour masks to monitor cell geometries were obtained with the Fiji-plugin “DM3D-Deforming Mesh”, from fixed Z-confocal-stacks of F-actin signals. These masks were refined using sub-slicing in Fiji and served as an entry for a home-made Matlab code (Mathwork).³² This code served to reconstitute the 3D geometry of cells, and compute the geometrical center, the length of the apico-basal axis measured along the axis from the Apical domain center and the cell center; end thus to extract normalized centrosome positions or orientations with respect to the AB axis (computed as a dot product between the centrosome centrosome axis vector and the AB vector) as presented in Figure 1C for instance. The 3D geometry and the coordinate of the Apical domain center and its size, served as entry for the different 3D simulations discussed below. In experiments, in which 3D analysis was not possible because of the rapid time course of acquisitions (Figures 2D, 2E, S2F, and S2G), cells were imaged in their mid-plane; and the geometrical cell center was computed as the middle of the AB axis, and the AB axis length as the distance from the center of the apical part to the middle of the basal part. We estimate these 2D estimation to cause deviations of less than ~20% in terms of relative centrosome positioning or orientation.

Cell Volumes

Cell volumes at all stages were calculated by considering cells as either prolate or oblate spheroids, based on their aspect ratio in the mi-plane of imaging and measurement of long and short axis in this plane.⁴⁶

Nuclei position

Nuclei were tracked from 2D confocal timelapses of cells imaged in the mid-plane, based on different nuclei markers (GFP-NLS, H2B-GFP, H2B-RFP or Hoechst). To obtain the *in vivo* nuclei distance from the cell surface we drew with Fiji a vector from the nuclei center to the center of the apical cell membrane. The nuclei-apical distance was measured at each time point to avoid errors due to cell shape changes and membrane contractions along the cell cycle. The net nuclear displacement in response to drug treatment or laser ablation was calculated as the difference between the nuclear-apical membrane distance at time 0s and that at the onset of metaphase. All distances were normalized to the AB cell length at each time points.

Cytoplasm flows

To track cytoplasm flows, we labelled yolk granules which are dense in early embryos. For this, embryos were incubated in Nile blue at a 1/1000 dilution in ASW.F from fertilization, and imaged at the morula/blastula stage with spinning disk microscopy, following treatments with either DMSO or Nocodazole. Movies were analysed using the particle image velocimetry PIVlab tool in Matlab (Mathworks). The exterior of the cell was masked to be excluded from the analysis. Contrast limited adaptive histogram equalization and two-dimensional Wiener filter with accordingly windows of 20 and 3 pixels widths were applied on the images in the pre-processing steps for noise reduction. Image sequences were investigated in the Fourier space by four interrogation windows with 64, 32, 16, and 8 pixels widths and 50% overlapped area. The distribution of velocity components of vectors for each set was visually inspected and restricted to remove outliers in the post-processing stage. The output vector fields after smoothing were used for further analysis and for plotting flow maps and streamlines in Matlab.

Statistical analysis

For all experiments, the statistical analysis was performed using GraphPad Prism (GraphPad Software, Boston, Massachusetts USA). First, the normality of the data distribution (Gaussian distribution) was tested using a D'Agostino and Pearson test (sample >20) or Shapiro-Wilk test (sample < 20). When the data followed a normal distribution, we then compared them using parametric two-tailed unpaired Student's t-tests. When the data did not follow a normal distribution, we compared them using Mann-Whitney. $P < 0.0001$ (****), $P < 0.001$ (***), $P < 0.01$ (**); $P < 0.05$ (*) and $P \geq 0.05$ was considered not significant (ns). The number of embryos and experiments performed for all analysis are listed in the Figure legends. Linear regression data were fitted using a simple linear regression model.