PLK1- and PLK4-Mediated Asymmetric Mitotic Centrosome Size and Positioning in the Early Zebrafish Embryo

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In Brief
During embryonic cell divisions, cell size changes rapidly. Rathbun et al. identify in zebrafish embryos that mitotic centrosomes scale with changes in cell size. In addition, an embryonic cell spindle has asymmetric in size mitotic centrosomes, where the largest mitotic centrosome is placed toward the embryo center in a PLK1/4-dependent manner.

Highlights
- Large mitotic centrosome identification (246.44 ± 11.93 μm²) in the zebrafish embryo
- Decreases in cell size scales closely with mitotic centrosome size
- Zebrafish mitotic centrosomes within a spindle are asymmetric in size
- PLK1 and PLK4 activity is required for asymmetric mitotic centrosome positioning

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SUMMARY

Factors that regulate mitotic spindle positioning remain unclear within the confines of extremely large embryonic cells, such as the early divisions of the vertebrate embryo, *Danio rerio* (zebrafish). We find that the mitotic centrosome, a structure that assembles the mitotic spindle [1], is notably large in the zebrafish embryonic cell (246.44 ± 11.93 µm² in a 126.86 ± 0.35 µm diameter cell) compared to a *C. elegans* embryo (5.78 ± 0.18 µm² in a 55.83 ± 1.04 µm diameter cell). During embryonic cell divisions, cell size changes rapidly in both *C. elegans* and zebrafish [2, 3], where mitotic centrosome area scales more closely with changes in cell size compared to changes in spindle length. Embryonic zebrafish spindles contain asymmetrically sized mitotic centrosomes (2.14 ± 0.13-fold difference between the two), with the larger mitotic centrosome placed toward the embryo center in a polo-like kinase (PLK) 1- and PLK4-dependent manner. We propose a model in which uniquely large zebrafish embryonic centrosomes direct spindle placement within disproportionately large cells.

RESULTS AND DISCUSSION

Spindle scaling occurs during early embryogenesis, as seen in *Caenorhabditis elegans* (*C. elegans*) and *Xenopus laevis*, where rapid cell divisions increase the number of cells without growth [4–9]. As a result, daughter cells become smaller with each round of division. One reason for spindle size to scale with cell size is that the spindle is limited by the abundance of cytoplasmic components [4, 7, 9]. Mechanisms proposed to assist in spindle shortening that may be separate from component limitation are changes in microtubule destabilizer availability, such as kinesin-13 [4], or in microtubule nucleators [10]. These scenarios highlight potential possibilities that assist in spindle scaling allowing for successful spindle placement. Our study finds an additional aspect of the spindle that adapts to cell size in the *Danio rerio* (zebrafish) embryo, the mitotic centrosomes.

The mitotic spindle is a macromolecular machine that is constructed in order to physically separate duplicated genetic material into two daughter cells during cell division. The mitotic centrosome assembles the microtubule-based spindle, and each mitotic centrosome consists of two centrioles surrounded by pericentriolar material (PCM) that contains microtubule nucleation sites [1]. Typically, astral microtubules emanate from the centrosome and project toward the cell cortex, where they anchor and facilitate pulling forces to position the spindle [1]. Since the mitotic spindle needs to contact the cell cortex on both sides of the cell in order to provide this pulling force, the relationship between spindle and cell size is crucial for spindle function. One proposed mechanism identified in *C. elegans* is that centrosome size may set the length of the mitotic spindle [11]. A separate mechanism proposed from work in *Xenopus* and zebrafish embryos suggests that large embryonic cells use acentrosomal microtubule nucleation sites so that astral microtubules can reach the cortex [12]. Both scenarios enable the mitotic spindle to span large cells and position astral microtubules closer to the cell cortex [11, 12]. Our studies identified that large dividing cells in the zebrafish embryo have notably large fragmented mitotic centrosomes that scale closely to cell size. We present a model where large fragmented spindle poles may also assist in scaling spindle length with cell size.

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Mitotic Centrosome Area Scales with Cell Length during Embryonic Cell Divisions in C. elegans and Zebrafish

Embryos from the invertebrate C. elegans and vertebrate Danio rerio (zebrafish) were used to identify whether mitotic centrosomes scale with cell size in a conserved way (Figures S1A–S1D). C. elegans embryos develop within an eggshell, where early divisions occur asynchronously [2] (Figures 1A, S1B, and S1C; Video S1). In contrast, early zebrafish embryos undergo rapid cleavage stage cell divisions (Figures 1B, S1B, and S1D; Video S1) where the first ten cell divisions occur synchronously before transitioning to an asynchronous wave [3]. During the first five cell divisions, blastomeres create a cellular monolayer on top of the yolk. Each division during this stage occurs perpendicular to the plane of the previous division, leading to the construction of a monolayer grid (Figure 1B) [3]. This is visualized through the use of a fluorescent microtubule transgenic zebrafish line (EMTB-3xGFP) [13], where the 16-cell stage embryo contains mitotic spindles oriented perpendicular to the previous division at the 8-cell stage (Figure 1B; Videos S2 and S3).

In early development, we measured the decrease in cell size that occurs following rapid rounds of division in zebrafish and C. elegans. Cell area and cell length (longest cell axis) were measured (1- to 6-cell stage embryo in C. elegans, 8- to 128-cell stage in zebrafish), as well as a later developmental stage (10-cell for C. elegans, 512-cell for zebrafish). Both organisms had a significant decrease in cell area and cell length during these divisions. In C. elegans embryos we found different trends when considering the decrease calculated in longest cell axis length (Figure 1C) compared to the decrease calculated in cell area (Figure S1E). The largest decrease in cell length occurred between the first and second divisions, whereas the decrease in cell area remained constant (Figures 1C and S1E). This difference is likely due to C. elegans embryo development occurring within the confines of an eggshell, where cells divide in various orientations and present with a range of cellular aspect ratios. In zebrafish, decreases in both cell area and length remained constant (Figures 1C and S1E), likely due to embryos lacking an eggshell and their rapid rate of synchronous cell divisions.

Spindle length, mitotic centrosome area, and cell length were measured in C. elegans embryos that stably expressed a centrosome marker (γ-tubulin::GFP), cell membrane marker (PH::mCherry), and/or a nuclear marker (H2B::mCherry) (Figures 1A, 1D, S1A, and S1B). In zebrafish, spindle length was measured using EMTB-3xGFP (Figures 1B, S1A, and S1B), and mitotic centrosome area was measured by immunostaining wild-type embryos for γ-tubulin (Figure 1E). In both C. elegans and zebrafish embryos, spindle length decreased as cell length decreased (Figure 1C). When considered as a ratio between spindle and cell length, mitotic spindles occupy a higher percentage of the cell length in later cell divisions compared to earlier divisions (Figure 1F). This increase in the relative size of the spindle within the cell in later divisions shortens the distance that astral microtubules need to span in order to contact the cell cortex, as seen by the significant decrease in the distance from mitotic centrosomes to cell membrane (Figures S1F and S1G). This is apparent in the 512-cell stage zebrafish embryo, where the spindle occupies almost 60% of the cell length (Figures S1F and S1H). Despite the stark size difference between cells in C. elegans and zebrafish embryos (Figure 1H), these data suggest a conserved trend of changes in cell and spindle dimensions during early cell divisions.

When measuring mitotic centrosome size in C. elegans and zebrafish embryos (Figures 1D and 1E), a significant decrease in mitotic centrosome area was identified from one round of division to the next (Figure 1F). Mitotic centrosomes within the 1-cell stage C. elegans embryo were significantly larger than the mitotic centrosomes of both subsequent daughter cells (1-cell stage at 5.78 ± 0.18 μm² to the 2- and 3-cell stage at 3.96 ± 0.19 μm² and 3.86 ± 0.12 μm², respectively). In zebrafish embryos, γ-tubulin-decorated metaphase mitotic centrosomes were large (246.44 ± 11.93 μm² at 8-cell stage) and significantly decreased in size in the 16-cell stage metaphase cell (173.21 ± 6.43 μm²) (Figures 1E and 1F). This decrease continued into later cell divisions at the 512-cell stage (1.83 ± 1.44 μm²; Figures 1F and S1H). To convey how changes in cell length, spindle length, and centrosome area relate to one another, measurements were normalized to the 1-cell stage in C. elegans and to the 8-cell stage in zebrafish. In both organisms, the change in cell length scaled closely with the change in mitotic centrosome area compared to spindle length (Figure 1G). Cell length and mitotic centrosome area decreased by 30%–40% over time. Spindle length decreased <20% in both organisms (Figures 1G and 1H). Taken together, these data suggest that decreases in cell size scale more closely with mitotic centrosome size than spindle length (Figure 1H).

Centrosomes in Early Zebrafish Development

To characterize spindle and mitotic centrosome dynamics in the early embryo, we focused on the zebrafish embryo due to its large mitotic centrosomes. To do this, we employed iactin::EMTB-3xGFP [13] and iactin::centrin-GFP [14] embryos to mark microtubules and centrosomes. Volumetric projections of embryos from these transgenic lines were acquired over time (Figures 2A and 2B). The positioning of the mitotic spindles (Figure 2A) and mitotic centrosomes (Figure 2B; Video S3) are consistent with that modeled in Figure 2C. At prophase, the mitotic centrosomes are placed on either side of the nucleus (Figures 2D, 2E, and S2A) and begin to nucleate a robust microtubule-based spindle (Figure 2D). In metaphase to anaphase, mitotic centrosomes begin to enlarge, fragment, and disperse (Figures 2E, 2F, and S2A), where they then reform during telophase to prepare for cell-cycle re-entry (Figures 2E and S2A; Video S4). The γ-tubulin- and centrin-positive mitotic centrosomes (Figure 2G) also contain the mitotic kinase, polo-like kinase (PLK) 1, with microtubules extending from this locale (Figure 2H). Centrin normally marks centrioles [15–17], but in zebrafish embryonic cells centrin is enriched at the PCM, where it partially colocalizes with γ-tubulin (Figures 2G and S2A–S2C). The degree of colocalization increases when comparing 8-cell, 16-cell, and 512-cell stage embryos (Figure S2C), suggesting that centrin and γ-tubulin become more focused later in zebrafish development (Figure 1F).

Mitotic Centrosomes Are Asymmetric in Size during Embryonic Cell Divisions

An asymmetry in mitotic centrosome size was identified during embryonic divisions in C. elegans and zebrafish (Figures 3A–3F). In C. elegans, a slight asymmetry in metaphase mitotic
Figure 1. Mitotic Centrosome Area Scales with Cell Length during Embryonic Cell Divisions in C. elegans and Zebrafish

(A) Maximum confocal projection of a C. elegans embryo at 1- and 3-cell stages. DNA and cell membrane (H2B::mCherry and PH::mCherry, inverted grayscale) and microtubules (α-tubulin::GFP, magenta) shown. Model depicting position of metaphase spindle within embryo. Scale bar, 10 μm.

(B) Three-dimensional rendering of zebrafish embryo at the 8- and 16-cell stage. Microtubule marker (EMTB-3xGFP) in grayscale. Model depicting position of spindle within embryo. Scale bar, 250 μm.

(C) Bar graphs depicting spindle length (orange) and cell length along spindle axes (gray) during C. elegans (left, n > 14 embryos) and zebrafish development (right, n > 3 embryos). Mean ± SEM shown. One-way ANOVA, ****p < 0.0001 for spindle and cell length in both C. elegans and zebrafish. Inset graph depicts magnified spindle length data.

(D and E) Representative images of metaphase cell at the 1-cell (left) and 3-cell stage (right) in a C. elegans embryo (D), and at the 8-cell (top) and 16-cell stage (bottom) in a zebrafish embryo (E). DNA and γ-tubulin in white; DNA marked by blue arrowhead. Magnified mitotic centrosomes in insets on right. Scale bar, 15 μm.

(F) Violin plot depicting two-dimensional centrosome area (μm²) at the 1-, 2-, 3-, and 6-cell stage in C. elegans (left, n > 38 centrosomes), and at the 8-, 16-, and 512-cell stage in zebrafish (right, n > 28 centrosomes). One-way ANOVA, ****p < 0.0001 for both C. elegans and zebrafish. Inset depicts magnified 512-cell stage data.

(G) Violin plot depicting cell length (n > 14), spindle length (n > 14), and mitotic centrosome area for C. elegans at the 1- and 2-cell stage (n > 42 centrosomes, left), and zebrafish at the 8- and 16-cell stage (n > 147 centrosomes, right). Values normalized to mean of earliest developmental stage (1-cell for C. elegans, 8-cell for zebrafish), dashed line at value of 1. See (C) and (F) for raw values prior to normalization.

(H) Scaled model depicting cell (green), spindle (orange), and mitotic centrosome (purple) during the 1- and 2-cell stage in C. elegans embryos, and the 8- and 16-cell stage in zebrafish embryos. Percentages listed at the 2-cell and 16-cell stage refer to the percent decrease in value compared to the previous developmental stage. Scale bar, 20 μm.

For violin plots: plot boundaries depict minimum and maximum, 25th and 75th quartiles represented by thin black line, median represented by thick black line. For all graphs: detailed statistical analysis in Table S1. See also Figure S1 and Video S1.
centrosomes existed at the 1-, 3-, and 6-cell stages (Figures 3A and 3B). Specifically, at the 3-cell stage embryo a 1.16 ± 0.04 ratio was calculated when comparing largest metaphase centrosome to smallest (Figures 3A and 3E). This asymmetry was also present at prometaphase and anaphase (Figure S3A). Strikingly, zebrafish early embryos immunostained for γ-tubulin (Figures 3C, 3D, and S3B) or stably expressing centrin-GFP (Figures S3C and S3D) displayed an asymmetry in spindle pole size at metaphase, with the largest metaphase mitotic centrosome pointing toward the midline of the embryo cell grid 88.5% ± 2.7% of the time (n = 36 embryos at the 8- and 16-cell stage; refer to dashed orange line marking midline, Figure 3C). This asymmetry in mitotic centrosome size was consistent in cells placed next to the midline or farther away from the midline in zebrafish (Figure 3C). Like with C. elegans, this asymmetry between poles was also present at prometaphase and metaphase (Figure S3B).

Additionally, we found that the asymmetry in mitotic centrosome size was maintained at a later embryonic stage (512-cell; Figure 3D). When calculating a ratio of largest to smallest mitotic centrosomes, zebrafish mitotic centrosomes exhibit a more pronounced asymmetry at the 8- (2.21 ± 0.12), 16- (2.13 ± 0.08), and 512-cell (1.64 ± 0.05) stages compared to a more modest asymmetry at the 1- (1.14 ± 0.03), 3- (1.16 ± 0.04), and 6-cell (1.34 ± 0.06) stages of C. elegans embryo development (Figure 3E).

While centrin-GFP-decorated mitotic centrosomes were asymmetric in size, there was no difference in the mean fluorescent intensity between the two mitotic centrosomes (Figure S3D; refer to Figure 2G). This was surprising because centrin is asymmetric in concentration between the two mitotic centrosomes in mammalian tissue culture due to the nature of centriole duplication where the spindle pole with the oldest centriole contains the most centrin [15, 18]. In addition, the fluorescent intensity of centrin-GFP at mitotic centrosomes in zebrafish embryos decreased by half when comparing the 8- to 16-cell embryonic stage, suggesting that centrin-GFP was obtained through maternal stores that were halved following each round of division (Figure S3D). Taken together, these data suggest that even though one mitotic centrosome is larger, the two mitotic centrosomes equally distribute centrin. In conclusion, zebrafish mitotic centrosomes present with an asymmetry in centrosome size starting at prophase/prometaphase with a bias for the largest mitotic centrosome positioned toward the midline at the 8- and 16-cell stage (Figure 3F). However, the size asymmetry is not transient, as it persists later on in embryo development even when centrosomes are decreasing in size (Figures 3D and 3E).

PLK1 and PLK4 Activity Is Required for Asymmetric Mitotic Centrosome Positioning

As cells progress through the cell cycle, they normally require PLK4 to duplicate their centrosome and PLK1 for robust PCM assembly during bipolar spindle construction [19]. The assembly of PCM components that interact with γ-tubulin, such as pericentrin and CEP215, is facilitated by the phosphorylation activity of PLK1 [19, 20]. With PLK4 inhibition, centriole duplication is disrupted, causing spindles to assemble through acenotropic organization of PCM [21–23]. However, the role of PLK1 and/or PLK4 at mitotic centrosomes in the early zebrafish embryo is unknown. Transcripts for PLK1 and PLK4 have been detected as early as the 1-cell stage in zebrafish embryos [24], and we have successfully noted PLK1 at mitotic centrosomes (Figure 2H), indicating that both are likely maternally supplied prior to zygotic genome activation. Due to this, we tested the hypothesis that PLK1 and/or PLK4 regulate γ-tubulin organization at mitotic centrosomes in zebrafish embryos. The PLK1 and PLK4 small molecule inhibitors, BI2536 [15, 25–27] and centrinone [28], were injected into 1-cell stage embryos. Control embryos were injected with DMSO at the 1-cell stage and analyzed at the 16-cell stage. In 87.14% ± 4.16% of DMSO-injected embryos, the larger mitotic centrosome was positioned toward the midline whereas the smaller was positioned away (Figures 4A and 4B). This directional positioning of the larger mitotic centrosome toward the midline was significantly decreased when embryos were injected with BI2536 (44.78% ± 7.18% with 1 μM) or centrinone (60.67% ± 6.87% with 1 μM; Figures 4A and 4B), along with an associated decrease in the ratio of mitotic centrosome size difference within a spindle (Figures 4A, 4C, and S4A–S4C). However, BI2536 and centrinone treatment caused an overall increase in centrosome area (Figures 4A and 4D). These studies suggest that PLK1/4 regulate mitotic centrosome structure and the placement of the larger mitotic centrosome within a spindle toward the embryo’s midline (Figure 4E).

We were surprised that PLK1 inhibition caused an increase in the area occupied by γ-tubulin in mitotic centrosomes due to its known role in recruiting the pericentrin-CEP215 complex that

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**Figure 2. Centrosomes in Early Zebrafish Development**

(A and B) Three-dimensional rendering of mitotic spindle positioning during early embryonic divisions in a zebrafish embryo using EMTB-3xGFP (microtubules, A) and centrin-GFP (centrosome, B). Microtubules shown in depth-coded z stack such that z-slices closest to the embryo yolk are colored red and z-slices farthest from the yolk are colored blue (A). Centrin-GFP (inverted grayscale, B) shown at the 8- and 16-cell stage. Cell highlighted by dashed box magnified in (E). Scale bar, 100 μm.

(C) Model depicting the placement of mitotic spindles within embryonic zebrafish cells from the 1-cell stage to the 16-cell stage. Cells are viewed from top of cell mass with yolk placed below (XY view). Mitotic centrosomes (purple) and metaphase plate (blue) shown.

(D and E) Stills from time lapse of a cell division in EMTB-3xGFP transgenic embryo (microtubules, inverted grayscale, D) and a centrin-GFP embryo (centrosome, Fire LUT, E, inset from B). Mitotic stages denoted. Insets below centrin-GFP time lapse depict magnified poles denoted by white (top) and cyan (bottom) astersisks.

(F) Violin plot depicting normalized mitotic centrosome area at 16-cell stage during prophase/prometaphase, metaphase, and anaphase. Values normalized to the mean mitotic centrosome area at prophase/prometaphase. One-way ANOVA, *p < 0.0310. n > 88 mitotic centrosomes measured. Plot boundaries depict minimum and maximum, 25th and 75th quartiles represented by thin black line, median represented by thick black line. Detailed statistical analysis in Table S2.

(G) Maximum confocal projection of a single mitotic spindle with γ-tubulin (magenta), centrin-GFP (cyan), and DNA (DAPI, blue) shown. Insets below depict mitotic centrosome denoted by yellow box. Scale bar, 10 μm.

(H) Maximum confocal projection of a single mitotic spindle with PLK1 (magenta), DNA (DAPI, blue), and microtubules (white) shown. Insets below depict mitotic centrosome denoted by yellow box. Scale bar, 10 μm.

Detailed statistical analysis in Table S1. See also Figure S2 and Videos S2, S3, and S4.
anchors the γ-TURC at the centrosome [19, 29]. To confirm this, we employed a second PLK1 inhibitor, GSK461364, that inhibits early embryonic development due to cytokinesis failure (Figures S4D and S4E). With injection of both BI2536 and GSK461364, an increase in the area occupied by γ-tubulin in metaphase centrosomes within a 16-cell stage embryo occurred (Figures 4A, 4D, S4F, and S4G). In C. elegans, the removal of PLK1-added phosphates from substrates is a key molecular determinant of PCM disassembly [7, 31, 32]. Thus, when inhibiting PLK1 a common result is reduced γ-tubulin signal at mitotic centrosomes [19]. In zebrafish, we argue for a similar mechanism, but instead of causing reduced signal of γ-tubulin,
Figure 4. PLK1 and PLK4 Activity Is Required for Asymmetric Mitotic Centrosome Positioning

(A) Representative images of 16-cell stage embryos during metaphase under conditions of DMSO (left), 1 μM BI2536 (center), or 1 μM centrinone treatment (right). Single cells denoted in embryo image magnified in inset. γ-tubulin (magenta/inverted grayscale) and DNA (DAPI, blue) shown. Model depicting mitotic centrosome positioning in embryo shown on left (cyan/correct and gold/incorrect positioning depicted with arrows). Larger and smaller mitotic centrosomes not drawn to scale in model. Scale bar, 10 μm.

(B) Bar graph depicting percentage of spindles with largest centrosome pointed toward midline under conditions of DMSO (gray), BI2536 (100 nM or 1 μM, blue), or centrinone (100 nM or 1 μM, gold) exposure. One-way ANOVA with Dunnet’s multiple comparison to DMSO control, *p < 0.05, ****p < 0.0001; n.s., not significant.

(C and D) Violin plot depicting the ratio of mitotic centrosome areas binned by size (larger-to-smaller centrosome ratio, C) or centrosome areas unbinned (D) under conditions of DMSO (gray), BI2536 (100 nM or 1 μM, blue), or centrinone (100 nM or 1 μM, gold) exposure. Metaphase centrosome areas measured from γ-tubulin signal from fixed zebrafish embryos at the 16-cell stage. One-way ANOVA with Dunnett’s multiple comparison test performed with DMSO control, *p < 0.05, **p < 0.01, ****p < 0.0001.

(E) Model depicting the positioning of the asymmetric mitotic centrosomes in relation to the embryonic midline during the 16-cell stages under conditions of DMSO (gray), BI2536, or centrinone (purple) exposure. Metaphase centrosomes (purple), metaphase plate (blue), and embryonic midline (orange dashed line) depicted.

(F) Bar graphs representing percentage of normal embryos (gray), dead (black), or with abnormal phenotypes (orange) at 2, 4, 9, and 120 h post-fertilization (hpf) in uninjected embryos (n = 271 embryos) and after DMSO vehicle control injection (n = 131 embryos), 1 μM BI2536 (n = 200 embryos), or 1 μM centrinone (n = 247 embryos) injections.

For all violin plots: plot boundaries depict minimum and maximum, 25th and 75th quartiles represented by thin black line, median represented by thick black line. For all graphs: detailed statistical analysis in Table S4. See also Figure S4.
increased PCM fragmentation occurs, causing an enlarged centrosome (Figures 4D, S4F, and S4G). We attribute this discrepancy to potential differences in centrosome structure when comparing the early zebrafish centrosomes to those of C. elegans, where a disorganization in PCM caused by PLK1 inhibition may manifest in differing phenotypes.

In order to determine the importance of PLK1/4-dependent asymmetric mitotic centrosome size placement in early zebrafish divisions, we raised embryos after injection with a vehicle control (DMSO), 1 μM BI2536, or 1 μM centrinone and compared to an uninjected control group (Figures 4F, S4H, and S4I). Injections were performed during the first cell cycle (1-cell stage) and embryos monitored every half hour. PLK1 or PLK4 inhibition resulted in a lower survival rate over the first 5 days post-fertilization when compared to control conditions (Figures 4F, S4D, and S4H). With both PLK1 inhibition and PLK4 inhibition embryonic cells can still divide, although embryonic cells present with defects resulting from cytokinetic failure [33] (Figures S4D and S4H). At 5 days post-fertilization, heart edema, embryo elongation defects, yolk elongation defects, and small eyes were noted in a small fraction of BI2536-injected embryos (Figures 4F and S4I). Given that the injections of BI2536 or centrinone likely diffuse out when the chorion starts to become more permeable, earliest cell divisions are liable to be most impacted, suggesting that PLK1- and PLK4-dependent asymmetric mitotic centrosome placement influences later embryonic development.

## STAR METHODS

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

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## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2020.08.074.

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## AUTHOR CONTRIBUTIONS

L.I.R. designed, performed, and analyzed experiments, and wrote the manuscript; H.H. oversaw the project and edited and wrote the manuscript; J.N.B. edited the manuscript and advised on C. elegans studies; J.D.A. edited the manuscript and provided zebrafish expertise; and A.A.A., J.M., X.B., and N.A.H. performed experiments and associated analyses and edited the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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**STAR METHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Gamma-tubulin       | SigmaAldrich | Cat#T5192; RRID: AB_261690 |
| PLK1                | Cell Signaling Technology | Cat#4513S; RRID: AB_2167409 |
| α-tubulin with FITC conjugate | Krackeler Scientific | Cat# 45-T6793 |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| DAPI                | SigmaAldrich | Cat#D9542-10MG |
| NuclBlue            | ThermoFisher | Cat#R37606 |
| Agarose             | Thermo-Fisher | Cat#16520100 |
| Bi2536              | Selleck Chemicals | Cat#S1109 |
| BSA                 | Fisher Scientific | Cat# BP1600-100 |
| Centrinone B        | R&D Systems | Cat#5690 |
| Dimethylsulfoxide   | Fisher Scientific | Cat#BP231100 |
| GSK461364           | Sigma-Aldrich | Cat#45-SML1912-5MG |
| Paraformaldehyde    | Fisher Scientific | Cat#AA433689M |
| PBS                 | Fisher Scientific | Cat# 10010023 |
| Prolong Diamond     | Fisher Scientific | Cat#P36971 |
| Triton x-100        | Fisher Scientific | Cat#BP151500 |
| Tween 20            | Thermo-Fisher | Cat# BP337500 |
| **Experimental Models: Organisms/Strains** |        |            |
| Zebrafish           | Zebrafish International Resource Center (ZIRC) | TAB (wild-type) |
| Zebrafish           | Gift from Solnica-Krezel Lab, generated by Harris Lab | Tg(–Sactb2:cetrn4-GFP) |
| C. elegans          | Megason Lab | [9actin::EMTB-3xGFP aka. Tg(actb2:Hsa.MAP7-EGFP)] |
| C. elegans          | Bembenek Lab | JAB23: unc-119(+); [wels21[pJA138 (pie-1::mCherry::tub)]] |
| C. elegans          | Bembenek Lab | JAB24: zen-4(or153ts); Zen-4:GFP rescue construct complex wels21 [pJA138 (pie-1::mCherry::tub::pie-1)] |
| C. elegans          | Bembenek Lab | JAB52: unc-119(ed3) iii; dds6[bg-1::GFP + unc- 119(+)] v; ruls32[ppie-1::GFP::His-58; unc- 119(ed3) iii; wels21[pJA138 (pie-1::mCherry::tub)]] |
| C. elegans          | Bembenek Lab | JAB141: ojls2[alpha-tubulin::GFP]; tfts37 [PPie-1::mCHERRY::his-58] |
| C. elegans          | Bembenek Lab | JAB142: ojls2[alpha-tubulin::GFP]; tfts37 [PPie-1::mCHERRY::his-58]; tfts44 [PPie-1::mCherry::PH PLC1delta1] |

**Software and Algorithms**

| Software and Algorithms | [34] | https://imagej.net/Fiji |
| Beamline Information | IMARIS Bitplane | Oxford Instruments | https://imaris.oxinst.com |
| Software and Algorithms | Prism8 | GraphPad | https://www.graphpad.com/scientific-software/prism/ |
| Software and Algorithms | LAS-X software | Leica Microsystems | https://www.leica-microsystems.com/products/microscope-software/p/leica-las-x-ls/ |

**RESOURCE AVAILABILITY**

**Lead Contact**
For further information or to request resources/reagents, contact the Lead Contact, Heidi Hehnly (hhehnly@syr.edu).

**Materials Availability**
No new materials were generated for this study.
Data and Code Availability
All datasets analyzed for this study are displayed.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Zebrafish
Zebrafish lines were maintained using standard procedures approved by the Syracuse University IACUC committee (protocol #18-006). Embryos were raised at 28.5 °C and staged as described in [3]. Wildtype zebrafish lines as well as transgenic lines were used for live imaging and immunohistochemistry. See Key Resources Table for list of zebrafish transgenic lines used.

C. elegans
Transgenic C. elegans lines were maintained at 20 °C. For all experiments, the animals were imaged and characterized by the Bembenek lab immediately after fertilization. See Key Resources Table for list of transgenic C. elegans lines used.

METHOD DETAILS

Imaging
For zebrafish, a Leica SP8 (Leica, Bannockburn, IL) laser scanning confocal microscope (LSCM) was used throughout manuscript. A HC PL APO 20x/0.75 IMM CORR CS2 objective, HC PL APO 40x/1.10 W CORR CS2 0.65 water immersion objective, and an HCX Plan Apochromat 63x/1.40-0.06 NA OIL objective were used. Images were acquired using LAS-X software. Images taken with the SP8 LSCM were obtained through lightning, a built-in deconvolution algorithm. A Leica DMi8 (Leica, Bannockburn, IL) with a X-light v2 confocal unit spinning disk was also used, equipped with an 89 North – LDI laser and a Photometrics Prime-95B camera. Optics used were either 10x/0.32 NA air objective, HC PL APO 63X/1.40 NA oil CS2, HC PL APO 40X/1.10 NA WCS2 CORR, a 40X/1.15 N.A. 19 Lambda S LWD, or 100Å~/1.4 N.A. HC Pl Apo oil emersion objective. A Leica M165 FC stereomicroscope equipped with DFC9000 GT sCMOS camera was used for phenotypic analysis of embryos.

For live cell imaging of C. elegans embryos, a spinning disk confocal system was used. The system is equipped with a Nikon Eclipse and is an inverted microscope with a 60X 1.40NA objective, a CSU-22 spinning disc system and a Photometrics EM-CCD camera from Visitech International. Images were obtained every 2 min with a 1-micron z stack step size.

Pharmacological treatments
Zebrafish embryos were injected with either DMSO (0.1%-1%), BI2536 or centrinone (final concentration 100nM or 1 μM), or GSK461364 (10 μM) post-fertilization at the 1- to 2-cell stage. Embryos are incubated at 30 °C until they reach the developmental stage of interest, at which time they are fixed with 4% paraformaldehyde in PBS followed by immunostaining.

Zebrafish immunohistochemistry
Zebrafish embryos were fixed using 4% PFA containing 0.5% Triton X-100 overnight at 4 °C. Embryos were dechorionated and incubated in PBST (phosphate buffered saline + 0.1% Tween) for 30 min, blocked in Fish Wash Buffer (PBS + 1% BSA + 1% DMSO + 0.1% Triton X-100) for 30 min followed by primary antibody incubation (antibodies diluted 1:200 in Fish Wash Buffer) overnight at 4 °C or 3 h at room temperature. Embryos are washed five times in Fish Wash Buffer and incubated in secondary antibodies (diluted 1:200 in Fish Wash Buffer) for 3 h at room temperature. After five more washes, embryos were incubated with 4’,6-diamidino-2-phenylindole (NucBlue Fixed Cell ReadyProbes Reagent) for 30 min. For imaging, embryos were either halved and mounted on slides using Prolong Diamond (Thermo Fisher Scientific cat. # P36971) or whole-mounted in 2% agar (Thermo-Fisher cat. # 16520100).

Phenotypic characterization
Wildtype zebrafish embryos were injected as described in the pharmacological section. The embryos were maintained at 30 °C and assessed for abnormality in development and number of deaths every 30 min for 9-10 h post injection then once 24 h post injection. At 5 days post fertilization, the phenotypes of injected embryos were characterized and the number of embryos with developmental defects were recorded.

To generate death curves for the pharmacological treatments, the number of embryos treated with each drug were standardized to the starting number of embryos and were displayed as ratios over time.

QUANTIFICATION AND STATISTICAL ANALYSIS

Image and Data Analysis
Images were processed using both FIJI/ImageJ software [34] and Adobe Photoshop. All graphs and statistical analysis were produced using Graphpad Prism software. 3-D images, videos, surface rendering, and co-localization analysis (Pearson’s coefficient) were performed using Bitplane IMARIS software Surface, Smoothing, Masking, and Thresholding functions were all used.
To calculate two-dimensional area, a boundary was drawn around the structure of interest (cell, spindle pole, etc.) in ImageJ/FIJI and the area within this shape was calculated. To calculate spindle length, cell length, aspect ratio, etc., a line was drawn in ImageJ/FIJI from one end of the structure of interest to the other. This length was then measured and recorded.

**Statistical analysis**

Unpaired, two-tailed Student’s t tests and one-way ANOVA analyses were performed using GraphPad Prism software. **** depicts a p value < 0.0001, *** p value < 0.001, **p value < 0.01, *p value < 0.05. See Tables S1–S4 for detailed information regarding statistics. All experiments were completed with at least three independent replicates.