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Supplementary Information for “Stronger net posterior cortical forces and asymmetric microtubule arrays produce simultaneous centration and rotation of the pronuclear complex in the early C. elegans embryo”

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Results

LET-99 band size, MT nucleation angle, and dynein pulling force are key parameters for PNC rotation timing and positioning

We next wished to use an unbiased method to determine which model parameters were key determinants for replicating the experimentally observed centration and rotation behavior. To accomplish this, we sampled parameter space to generate 16,000 random parameter sets (see Materials and Methods) using the ranges for all 14 parameters denoted by an asterisk in Table 1. The properties of MT number ($N_{MT1}$) and nucleation angle ($arrayRange1$) for MTOC1 were varied while the corresponding properties for MTOC2 were kept fixed at the reference values (Table 1). Each parameter set was then used to simulate the model, and time course data was recorded from two independent runs.

We organized our 16,000 random parameter sets into two cases according to their ability to satisfy model criteria (Materials and Methods, Table 2): the general case, where parameters only had to satisfy the reasonable solutions, centration, and rotation criteria; and the strict case, where parameters had to satisfy the criteria of the general case as well as the $y$ excursion, timing, minimum MT density, and minimum LET-99 band size criteria. These criteria ensured that the model closely matched experimental data. Of the 16,000 randomly generated parameter sets, 3,107 were members of the general case, and 85 of those were also members of the strict case.

To determine in a broad sense which parameters may be more constrained in the strict case compared to the general case, we performed an unpaired two-sample t-test between the individual working parameters in the two cases. The following parameter distributions were statistically significantly different ($p < 0.05$) between the general and strict cases (Figure S4, A-F): the number of MTs nucleated by MTOC1 ($N_{MT1}$; $N_{MT2}$ was kept fixed at 1000), the contact time for pulling due to dynein ($C_{pull}$), MT growth velocity ($v_g$),
the pulling force due to dynein ($F_{\text{pull}}$), the angular range of MTs nucleated by MTOC1 ($\text{arrayRange}_{1}$), and the rotational drag coefficient ($\mu$). Interestingly, in the strict case, the only asymmetry we imposed on the MT arrays was that the density at MTOC1 had to be greater than or equal to the density at MTOC2. However, we found that requiring the relative timing of centration and rotation to match the experimental data yielded working sets that were constrained to take on values of $N_{\text{MT1}} > 1200$ and $\text{arrayRange}_{1} > 120$ (Figure S4, A and E). The size of the LET-99 band ($s$) was biased towards small values in both cases but more strongly in the strict case, although the distribution was not significantly different (Figure S4G). Further investigation suggested that small LET-99 bands may suppress large $y$ excursions during the centration and rotation process (Figure S3). Broadly, this analysis indicates that parameters related to MT dynamics, MT array asymmetry, and dynein activity were restricted when we required the model to satisfy the criteria of the strict case.

The analysis above focused on the consequences of the strict criteria on the distribution of permissible parameter values, so we next wished to determine if specific model outputs were sensitive to the choice or range of a specific parameter when all parameters were simultaneously varied. To determine whether any of the parameters were critical drivers of model behavior, we assessed the model outputs described in the Materials and Methods (Table 3): time to initiate rotation, time to center, time to finish rotation, and final $x$ position. Pearson correlation coefficients were calculated pairwise between each model output and each individual parameter. A correlation was considered significant if $|r| \geq 0.5$ with $p < 0.05$. For the 3,107 parameter sets in the general case, no significant correlations between model outputs and parameter values were found. For the 85 parameter sets in the strict case, we found six significant correlations between an output ($y$-axes, Figure S5) and a parameter ($x$-axes, Figure S5). These data suggest that MT array asymmetry mediated by the nucleation range of MTs ($\text{arrayRange}_{i}$), cortical asymmetry mediated by the size of the LET-99 band ($s$), and the force of MT/dynein interactions ($F_{\text{pull}}$) are critical drivers of biologically relevant behaviors in the model. Taken together, the results of this analysis when all parameters are varied simultaneously indicates that both MT array and cortical asymmetries are required in order to replicate the experimentally observed centration and rotation dynamics.
LET-99 band size and MT nucleation angle are critical for initiation and direction of rotation but only marginally affect final PNC position

Above we found that parameters associated with MT array and cortical asymmetries were constrained and correlated with specific model outputs when all parameters were varied simultaneously. Here we further investigated individual parameters identified by our analysis by varying LET-99 band size ($s$) or MT nucleation angle ($arrayRange_1$). The remainder of the parameters were fixed at their reference values (Table 1, Simulation Parameters). These simulations were run for 100 min. and only the final positions and angles were analyzed. Thus, the time course data for these simulations might not match experimental data.

When varying the LET-99 band size, we found a threshold value below which the PNC would reliably rotate and align along the long axis of the cell (Figure S6A). When this threshold value was exceeded, the PNC often failed to rotate. For our reference parameter set (Table 1), this threshold value occurred at approximately $s = 13 \mu m$ (Figure S6D, vertical line). This threshold rotation failure occurred when the LET-99 band extended farther on the cortex than the maximum initial extent of the MT array specified by $arrayRange_2$ (Figure S6C, D).

In contrast, changes in the LET-99 band sizes had a gradual effect on the final $x$ position of the PNC, for values of $s > 6 \mu m$ (Figure S6B). By increasing the size of the LET-99 band, and thus increasing the portion of the cortex exerting only pushing forces, the PNC experienced more anteriorward force, causing displacement of the PNC towards the anterior pole. Final $x$ positions stayed within $5 \mu m$ of the center for band sizes that successfully rotated (Figure S6; see also Video 4 for the $s = 0$ case).

We next examined the effect of changing the angular extent of the MT array, ($arrayRange_1$), with corresponding changes in MT number ($N_{MT_1}$) to maintain the same density as the reference parameter set, while keeping $N_{MT_2}$ and $arrayRange_2$ at their reference values (Table 1). The angular extent of the MT array determined the direction of rotation, independent of MT density (Figure S7A). When $arrayRange_1$ was greater than $arrayRange_2$, the PNC rotated so that MTOC1 was the leading MTOC (finishing rotation with an angle of $0^\circ$), as in our previous simulations. In the reverse situation, with $arrayRange_2$ greater than $arrayRange_1$, MTOC2 became the leading MTOC (rotated to $180^\circ$), despite MTOC1 having a higher density of MTs. Changes in $arrayRange_1$ (and consequently in $N_{MT_1}$) had a small effect on the final $x$ position of the PNC with final positions clustering between 0 and $2 \mu m$ from the center (Figure S7B).
indicates the force balance responsible for centering the PNC as the MT numbers were changed was less sensitive to changes in `arrayRange1` compared to changes in the size of the LET-99 band (Figure S6B). Taken together, these data suggest that within a range of band and array sizes that produce rotation in the model, these parameters have a minor role in positioning the PNC during centration. However, LET-99 band size (`s`) and the angular extent of the MT array (`arrayRange1`) strongly influence the timing and direction of PNC rotation.

**Figures**

Figure S1: LET-99::YFP localizes to a cortical band centered at 60% EL. (A) Cortical-plane micrograph of LET-99::YFP during the first mitosis in the *C. elegans* embryo. The cortical intensity peaks in the band (arrowheads). The white dashed line shows the size of the egg in the mid-plane for reference. Scale bar: 5 µm. (B) Average fluorescence intensity (black line) from cortical micrographs of 6 embryos (gray lines; see Materials and Methods). The blue bar represents the band position used in our model.
Figure S2: The EBP-2::GFP intensity at the leading and lagging MTOCs is equivalent. (A) Schematic showing measurements of MTOC- and MT array-associated EBP-2::GFP intensities. Black dashed box shows the measurement area. Pink dashed box shows the nuclear background intensity measurement area (subtracted as background). Blue outlined circle represents the MTOC, and gray circles represent the pronuclei. Included area for Figure 5C is shown using diagonal lines in the top measurement area, whereas included area for panel B is shown using diagonal lines in the bottom measurement area. (B) EBP-2::GFP intensity from leading and lagging MTOCs normalized to the time-averaged anterior value for each embryo (see Materials and Methods). These data are not significantly different.
Figure S3: Frequency of working sets, with respect to maximum MTOC excursion in the $y$ direction and cortical LET-99 band size ($s$) when all parameters were varied simultaneously. (Top) The general case (satisfying reasonable solutions, centration and rotation requirements). (Bottom) The strict case (satisfying all conditions specified in the main text and Materials and Methods). The horizontal white line denotes the minimum LET-99 band size: in the strict case, all acceptable values of this parameter are greater than that value. The vertical white line denotes the maximum permitted $y$ excursion in the strict case. Note the difference in maximum frequency between the two plots given in the colored bar to the right. Small $y$ excursions correspond to small LET-99 band sizes in both cases, suggesting that small LET-99 band sizes may act to suppress $y$ excursions, or conversely that large LET-99 bands may promote $y$ excursions.
Figure S4: Parameters related to MT dynamics, MT array asymmetry and dynein activity are restricted
when required to satisfy all model-based requirements. (A-F) Histograms of working parameters satisfying
the strict case requirements for the model behavior (Table 2) —reasonable solution, centration, rotation, \( y \)
excursion, timing, minimum MT density, and minimum LET-99 band size (gray bars)—that are statistically
different from parameter distributions that only satisfy the first three, general case, requirements (black
bars). Parameter ranges are binned into 10 equal groups for each parameter. (G) While not statistically
significant, the distribution of the parameter denoting size of the LET-99 band is clustered around small
values suggesting small LET-99 bands are needed to replicate pronuclear centration and rotation.
Figure S5: Significant correlations reveal key parameters driving model outputs. (A-C) Graphs of dynein pulling force ($F_{\text{pull}}$) vs. the correlated model outputs. (D-E) Graphs of the angle of MT nucleation at MTOC1 ($arrayRange_1$) vs. the correlated model outputs. (F) Graph of the parameter denoting the size of the LET-99 band, $s$, vs. the correlated model output.
Figure S6: The cortical LET-99 band has a threshold effect on final angle but a gradual effect on final x position. (A) Graph of final rotation angles from long simulations for LET-99 band arc lengths $s = 12$, $s = 13$, and $s = 14$, showing that $s = 13$ is the threshold for normal rotation. (B) Graph of final x positions of the PNC for long simulations with various LET-99 band arc lengths showing the gradual leftward shift as the band size increases above $s = 6$. (C) Schematic of the relationship between band size ($s = 12$ is shown) and $arrayRange_i$ (size from reference parameter set is shown). Between $s = 12$ and $s = 13$, the band exceeds the array at the anterior side of the MTOC2 array. Bar = 5 μm. (D) Graph of the relationship between band size and $arrayRange_i$ with the threshold for rotation failure marked by a solid vertical line.
Figure S7: The larger MT array determines the direction of rotation. (A) Graph of final rotation angles from long simulations for given arrayRange1 and $N_{MT1}$ values, with the arrays on MTOC2 unchanged from Table 1. (B) Graph of final $x$ positions for long simulations with the same parameter modifications as in (A).
**Video Legends**

In all of the videos, MTOC1 and its associated MTs are red, MTOC2 and its associated MTs are blue, and the region occupied by the pronuclei is green, although the pronuclei are not an explicit part of the model. The total time is 31 minutes, corresponding to the amount of time needed for our complete model to finish centering and rotating the PNC (Video 3).

Video 1: Simulation of PNC centration and rotation with symmetric MT arrays, using values for MTOC2 for both, no cortical LET-99 band (s = 0), and all other parameters from Table 1. This video corresponds to Figure 3, A-C and Figure 4, A and B.

Video 2: Simulation of PNC centration and rotation with symmetric MT arrays, using values for MTOC2 for both, and all other parameters from Table 1. This video corresponds to Figure 3, D-F and Figure 4, C and D.

Video 3: Simulation of PNC centration and rotation using all reference parameters from Table 1, including asymmetric MT arrays and a cortical LET-99 band. This video corresponds to Figure 6.

Video 4: Simulation of PNC centration and rotation with no cortical LET-99 band (s = 0), and all other parameters from Table 1. This video corresponds to Figure S6, s = 0.