Current Biology 22, 583–589, April 10, 2012 ©2012 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2012.01.064

Report

Centrosomes Can Initiate a Polarity Axis from Any Position within One-Cell *C. elegans* Embryos

Dominika Bienkowska[1] and Carrie R. Cowan[1,*]
[1]Research Institute of Molecular Pathology (IMP), Dr. Bohr Gasse 7, 1030 Vienna, Austria

Summary

The stereotyped asymmetry of one-cell *C. elegans* embryos has proven to be an important model for identifying molecular determinants of cell polarity [1]. How polarity is initiated is less well understood. Polarity establishment depends on centrosomes [2–4], which use two molecularly distinct pathways to break symmetry [5–8]. In both, the centrosome's position adjacent to the cell cortex is thought to determine where polarization starts. Defects in centrosome-cortex juxtaposition correlate with defects in polarity establishment in several mutants [4, 9, 10], suggesting that these processes may be linked, but there is no direct test of this. Here we assess how centrosome position relative to the cortex affects polarity establishment. We find that centrosomes can initiate polarity from any position within the embryo volume, but centrosome-cortex proximity decreases the time required to initiate polarity. Polarization itself brings about close centrosome-cortex proximity. Prior to polarization, cytoplasmic microtubules constrain centrosome movement near the cortex, expanding the controversial role of microtubules during polarity establishment [4–6, 11–13]. The ability of centrosomes to induce a single polarity axis from any position within the egg emphasizes the flexible, self-organizing properties of polarization in *C. elegans* embryos and contrasts the common view of *C. elegans* development as invariant.

Results and Discussion

In order to address centrosome position relative to the cortex during symmetry breaking, we analyzed centrosome movement in living one-cell *C. elegans* embryos prior to polarity establishment by time-lapse imaging and automated centrosome tracking. We used high temporal resolution—up to 3 frames per s—and carefully preserved embryo geometry. We applied sensitive criteria to assign symmetry breaking, defined by the earliest morphological indication of the cortical changes that underlie cell polarity in one-cell embryos: the progressive cessation of cortical contractions, the start of cortical and cytoplasmic flow, and, in some experiments, persistent reduction of nonmuscle myosin (NMY-2::GFP [8]) at the cortex (see Figure S1 available online). There are approximately 30 min between fertilization and polarity establishment; we followed centrosomes during the final 10 min. We assessed centrosome position with GFP::SPD-2 [14], a centrosome marker (Figure 1A); the edge of cytoplasmic GFP::SPD-2 signal was used to identify the cell periphery (Figure S1). In order to have a polarity-independent time standard, we assigned time0 to completion of female meiosis II (Supplemental Experimental Procedures). The time of symmetry breaking, timeSB, varies among embryos but occurs on average 150 s after time0 [15]. To avoid imprecise measurements in the z axis and the difficulty of assigning symmetry breaking in the cortical plane, we analyzed embryos in which centrosomes and symmetry breaking were visible in similar z axis positions, roughly the middle two-thirds of the embryo z axis in wild-type (WT) embryos. Further details can be found in the Supplemental Experimental Procedures.

Analyzing centrosome position at the time of polarity establishment (timeSB), we found that centrosomes initiated cortical polarization from a distance (Figures 1A and 1B; Movie S1). Centrosome-cortex distances at timeSB ranged from 2 to 7 μm in WT embryos (Figure 1C). After symmetry breaking, centrosomes moved processively toward the cortex (Figure 1B). In contrast to previous suggestions [4, 6, 9, 10], these results indicate that centrosome-cortex proximity is not a prerequisite for polarity establishment. Instead, we observed significant cortex-directed centrosome movement after symmetry breaking. At the same time, yolk granules, lipid droplets, and injected beads moved toward the site of symmetry breaking (Figure 2A; Movies S2 and S3), reflecting the bulk flow of cytoplasm [16]. Before symmetry breaking, we found no evidence of cortex-directed motion of any particles (Figure 2A; Figures S2C and S2D; Movies S2 and S3). Furthermore, centrosomes did not move to the cortex in embryos that had a disrupted actomyosin cortex and lacked cortical flow (latrunculinA-treated; Figure 2B; Figures S2A and S2B; Movie S4) or did not establish polarity [*spd-5(RNAi)* [2, 4]; Figures S3A and S3B]. Cytoplasmic flow, generated by polarity establishment, may move centrosomes to the cortex after symmetry breaking. The close approximation of centrosomes to the cortex appeared to be a consequence, rather than a cause, of polarity establishment.

To determine whether centrosomes could initiate polarity from any position relative to the cortex, we examined polarity establishment in embryos depleted of γ-tubulin [*γ-tubulin(RNAi)*], which we had previously found to increase centrosome-cortex distance at the time of symmetry breaking [4]. In γ-tubulin-depleted embryos, polarity establishment occurred regardless of centrosome position at timeSB, up to distances of 12 μm (Figures 3A and 3B; Movie S3), almost the maximal achievable distance in one-cell embryos. In both WT and *γ-tubulin(RNAi)* embryos, regardless of centrosome position, the site of symmetry breaking was approximately the closest point to the centrosomes on the cortex (Figures 3C and 3D). This was also true in embryos that were centrifuged to dramatically displace the centrosome from its original position in the embryo (D.B. and C.R.C., unpublished data). After symmetry breaking in *γ-tubulin(RNAi)* embryos, centrosomes moved directly to the cortex regardless of their initial distance (Figures 3A, 3C, and 3E; Figures 4A and 4B). In cases where the male and female pronuclei were near each other at the time of symmetry breaking, however, centrosomes did not reach the cortex, but polarity establishment proceeded normally. Thus, centrosomes could specify the site of polarity establishment from any position within the embryo.

*Correspondence: cowan@imp.ac.at

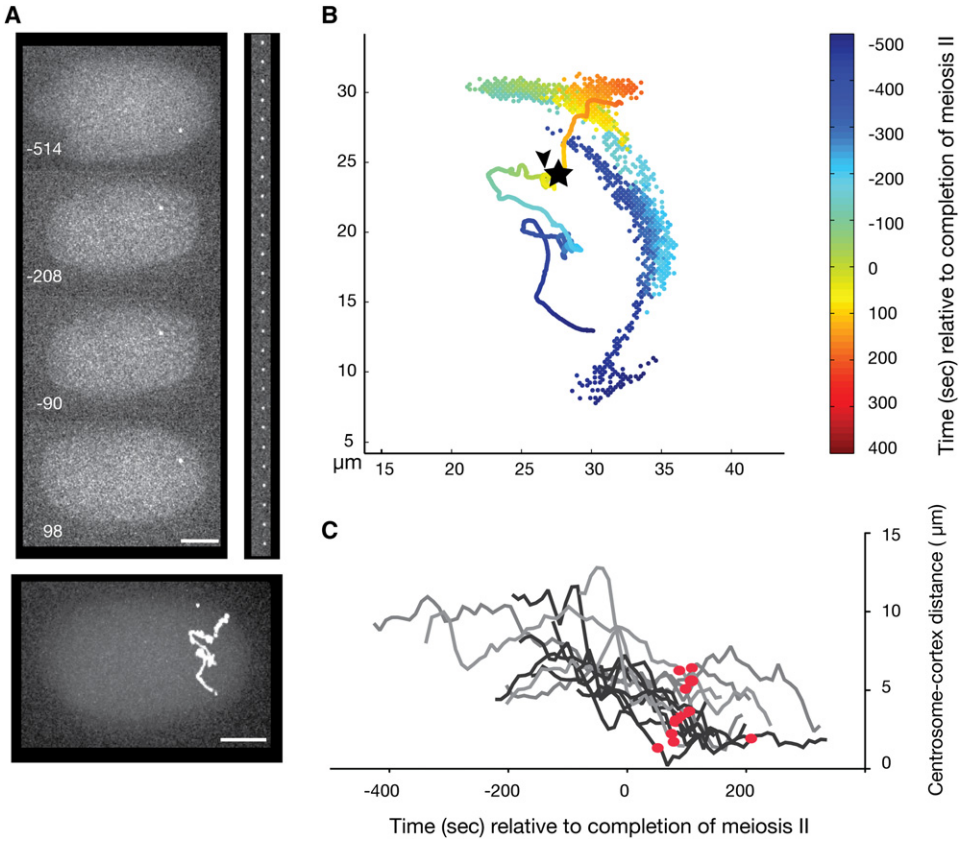

Figure 1. Centrosomes Initiate Polarity at a Distance from the Cortex

(A) Time-lapse images of GFP::SPD-2 labeled centrosome in one-cell *C. elegans* embryos before symmetry breaking. Left sequence shows embryo view; right sequence shows centrosome detail at actual temporal resolution (3 frames sec^{-1}). Time is indicated relative to completion of meiosis II (time0). Bottom panel shows projection of centrosome images from the time-lapse series shown. Scale bars represent 10 μm.

(B) Centrosome position (linear track) and closest cortical point (dots) extracted from time-lapse series in (A). Colors indicate time scale (blue, −500; red, 400). YX axes indicate absolute position. Centrosome position at time0 is indicated by an arrowhead. Centrosome position at timeSB is indicated by a star.

(C) Centrosome-cortex distance. Plots indicate the distance from centrosomes to the site of symmetry breaking on the cortex over time. Each line represents one centrosome. Centrosome-cortex distance at timeSB is indicated with a red dot.

Despite the flexibility in centrosome position at the time of polarity initiation, centrosomes were usually constrained to approximately 5 μm from the cortex at timeSB in WT embryos (Figure 1C; Figure 3B). To determine whether centrosome-cortex distance affected polarity establishment, we examined the time required for symmetry breaking in embryos exhibiting a range of centrosome-cortex distances. γ-tubulin depletion and physical embryo compression (Supplemental Experimental Procedures) both increased the distance of centrosomes to the cortex at timeSB (Figures 3B–3D), providing a large range of centrosome-cortex distances for analysis. We found a direct correlation between the distance of centrosomes to the cortex at timeSB and the time required for symmetry breaking (Figures 3E and 3F; Movie S3). Even centrosomes more than 10 μm from the cortex exhibited a proportionately long delay (Figure 3F), although distance measurements $\geq$8 μm may be inaccurate because of the ambiguous position of the cortex in the z axis. Centrosome-cortex proximity increased the efficiency of polarity establishment, decreasing the time required for symmetry breaking.

Recent models suggest that direct centrosome-cortex contact may facilitate localization of the posterior domain component PAR-2 to the cortex [6]. We wanted to determine whether centrosome-cortex distance affected the time required for cortical PAR-2 accumulation in addition to the initial effect on symmetry breaking. In WT embryos, symmetry breaking could be detected several minutes before PAR-2 localization at the cortex is apparent (data not shown). Because of high variability in mCH::PAR-2 expression, it was difficult to unambiguously assign the initiation of cortical PAR-2 domain formation. We therefore quantified the time between symmetry breaking and a defined level of cortical mCH::PAR-2 accumulation (Supplemental Experimental Procedures) in embryos with varying centrosome-cortex distances [*γ-tubulin(RNAi)*]. We found only a minor increase in the time required for cortical PAR-2 domain establishment after symmetry breaking (Figure 3G). Thus, once symmetry was broken, centrosome-cortex distance had little influence on the establishment of cortical polarity.

How is centrosome position constrained toward the cortex before polarity establishment? Before time0 in WT embryos, centrosomes traveled large distances—more than 50 μm, the length of an embryo (Figures 1A and 1B; Movie S1)—but with no obvious directionality. Centrosome trajectories consisted of processive runs interspersed with dwells (Figure 1B), resembling micro- and macroscopic search strategies that rely on random walks [17]. Centrosome step sizes appeared to fall in a half-normal distribution, consistent with a random

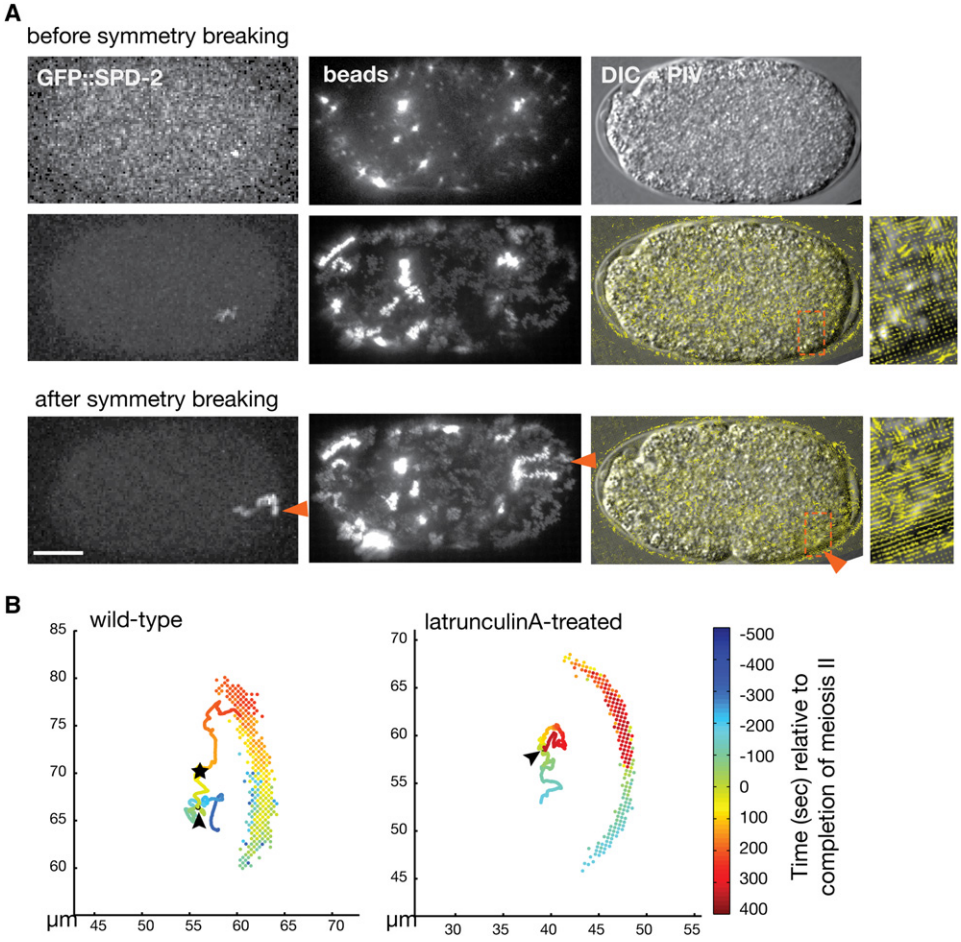

Figure 2. Polarity Establishment Moves Centrosomes to the Cortex

(A) Single z images (top) and projections (middle, bottom) of centrosomes (GFP::SPD-2) and injected fluorescent beads from time-lapse images before and after symmetry breaking. Projections encompass approximately 150 s. PIV analysis of endogenous yolk granules and lipid droplets from time-lapse images before (top) and after (bottom) symmetry breaking. In the PIV images, yellow arrows indicate processive motion; high arrow density suggests a coordinated flow field. Symmetry breaking sites are indicated by orange arrowheads. The orange boxed regions are magnified to the right.

(B) Centrosome position (linear track) and nearest cortical point (dots) extracted from time-lapse images of GFP::SPD-2 WT and latrunculin A-treated embryos. Colors indicate time scale (blue, −500; red, 400). YX axes indicate absolute position. Centrosome position at time0 is indicated by an arrowhead. In WT, centrosome position at symmetry breaking is indicated by a star. Polarity establishment does not occur in latrunculin A-treated embryos.

walk, although skewed at step sizes approaching zero (Figure S1C). We found no correlation between run orientation or velocity and distance to the cortex (data not shown), and we detected no net displacement relative to the cortex (Figure S1D), further indications that centrosomes move randomly. Thus, cortical bias did not arise from discrete features of centrosome steps, suggesting that the bias could instead reflect a large-scale property of centrosome movement.

Cytoplasmic microtubules in early *C. elegans* embryos are reported to have a moderate cortical enrichment [18], suggesting a potential source of cortical bias. γ-tubulin depletion appeared to reduce the cortical bias of centrosome position before symmetry breaking (Figures 3A and 3B; Figures 4A–4C), although centrosomes moved with step sizes similar to WT embryos (Figure S3D). We analyzed cytoplasmic microtubule distribution in γ-tubulin-depleted embryos and found fewer cytoplasmic microtubules that were generally longer than in WT embryos (Figure 4D), often projecting into the embryo interior. The increased microtubule length in *γ-tubulin(RNAi)* embryos may reflect an increase in free tubulin due to a reduced microtubule number [19]. γ-tubulin-depleted embryos show disruptions in both cytoplasmic microtubule organization and cortically biased centrosome movement. In embryos that lacked centrosomal microtubules but retained a cytoplasmic microtubule network [*spd-5(RNAi)*], centrosome movement was normal prior to time0 (Figures S3A and S3B; Movie S4), with step sizes similar to WT (Figure 3D). Centrosomes showed almost no movement before symmetry breaking in embryos without microtubules (nocodazole treated; Figures S3A, S3B, and S3D), but centrosomes moved to the cortex after symmetry breaking, likely by cytoplasmic flow (Figure S3B). Thus, the cytoplasmic microtubule network—independent of centrosomal microtubules—appears to move centrosomes and impart cortical bias prior to symmetry breaking.

To further understand how centrosome movement might be constrained toward the cortex, we analyzed molecules with established roles in noncentrosomal microtubule organization for effects on centrosome-cortex proximity. The microtubule

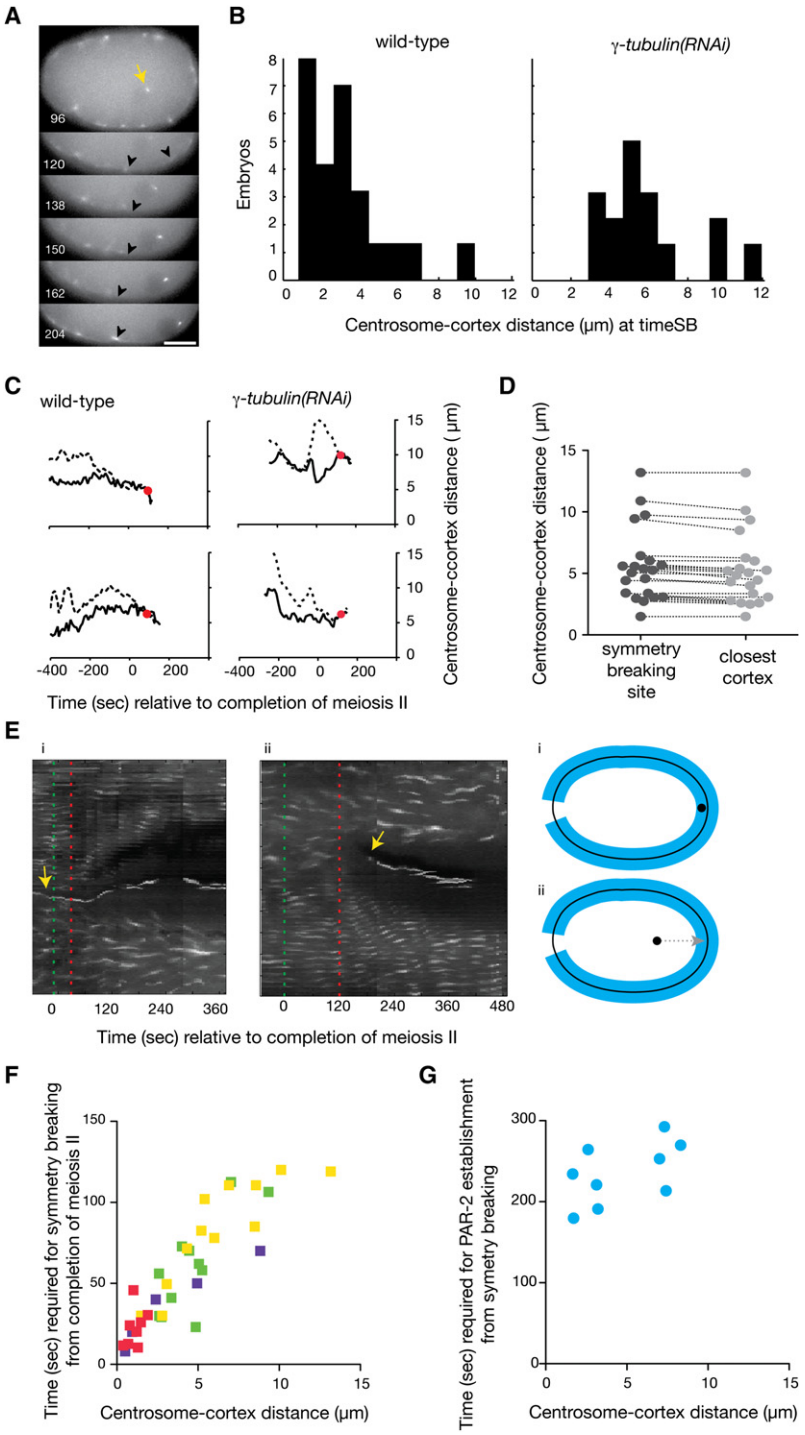

Figure 3. Centrosomes Determine the Site of Polarity Establishment from Any Position within the Embryo but Are Less Efficient When Far from the Cortex

(A) Time-lapse images of centrosomes (GFP::SPD-2) and cortical myosin (NMY-2::GFP) in a γ-tubulin-depleted embryo during symmetry breaking. Time elapsed is indicated relative to completion of meiosis II (time0). Yellow arrow, centrosome; black arrowheads, boundary of cortical myosin indicating symmetry breaking and posterior domain establishment. Scale bars represent 10 μm.

(B) Distribution of centrosome-cortex distance at symmetry breaking in WT and γ-tubulin-depleted embryos. All embryos established polarity.

(C) Centrosome distance to symmetry breaking site (dotted line) and closest cortex (solid line). Two individual centrosomes from representative WT and *γ-tubulin(RNAi)* embryos are shown. Distance at timeSB is indicated with a red dot.

(D) Distance from centrosomes to symmetry breaking site and to the nearest cortical point determined at timeSB. The data includes WT and *γ-tubulin(RNAi)* embryos with and without physical compression.

(E) Kymograph of the cortex in GFP::SPD-2; NMY-2::GFP embryos depleted of γ-tubulin. The loss of myosin foci from the cortex indicates symmetry breaking and posterior domain establishment. Vertical green lines, time0; vertical red lines, timeSB; yellow arrows, centrosomes. Diagrams show kymograph method: black dots, centrosomes; black outline, embryo cortex; blue line, kymograph region. Centrosomes are only detectable in the kymograph when they are directly at the cortex.

(Ei) Centrosomes were 2.1 μm from the cortex at timeSB.

(Eii) Centrosomes were 10.1 μm from the cortex at timeSB.

(F) Centrosome-cortex distance and the time required for symmetry breaking. Centrosome distance to the closest point on the cortex was measured in GFP::SPD-2; NMY-2::GFP embryos at timeSB. Time required for symmetry breaking is the interval between time0 and timeSB. Red: WT, no compression; purple: WT, compressed; green: *γ-tubulin(RNAi)*, no compression; yellow: *γ-tubulin(RNAi)*, compressed.

(G) Centrosome-cortex distance and the time required for PAR-2 establishment. Centrosome distance to the closest point on the cortex was measured in GFP::SPD-2; mCH::PAR-2 embryos at timeSB. Time required for PAR-2 establishment is the interval between symmetry breaking and when cortical PAR-2 intensity reaches 0.5 relative to the minimum standardized intensity (0.0) (see Supplemental Experimental Procedures).

motor dynein and the microtubule depolymerizer kinesin-13 have both been shown to be important for microtubule organization in acentrosomal spindles [20, 21]. Dynein is responsible for microtubule interactions with the cortex, including generation of pulling forces [22] and microtubule sliding [23]. In *C. elegans* embryos, both dynein and conventional kinesins have been shown to move the acentrosomal meiotic spindle toward the cortex [24]. The small GTPase Ran is required for nucleating and stabilizing microtubules independent of centrosomes [25, 26]. We tracked centrosome movement prior to symmetry breaking in embryos depleted of kinesin-13 (*klp-7*), dynein (*dhc-1*), conventional kinesin-1 (*klc-1*, *unc-116*), and Ran (*ran-1*). Only partial depletion of Ran led to a robust defect in centrosome constraint prior to symmetry breaking (Figure 4B); polarity establishment itself appeared normal. In *ran-1(RNAi)* embryos, centrosomes were 5.8 ± 1.4 μm from the cortex at the time of symmetry breaking, similar to γ-tubulin-depleted embryos (4.6 ± 2.1 μm) and substantially further than in WT embryos (1.9 ± 2.1 μm). Cytoplasmic microtubules in *ran-1(RNAi)* embryos were less abundant but more pronounced in the embryo interior compared to WT (Figure 4D), further supporting a role for cytoplasmic microtubules in biasing centrosome position near the cortex before symmetry breaking.

Taken together, our data support the following model of centrosome positioning during polarity establishment.

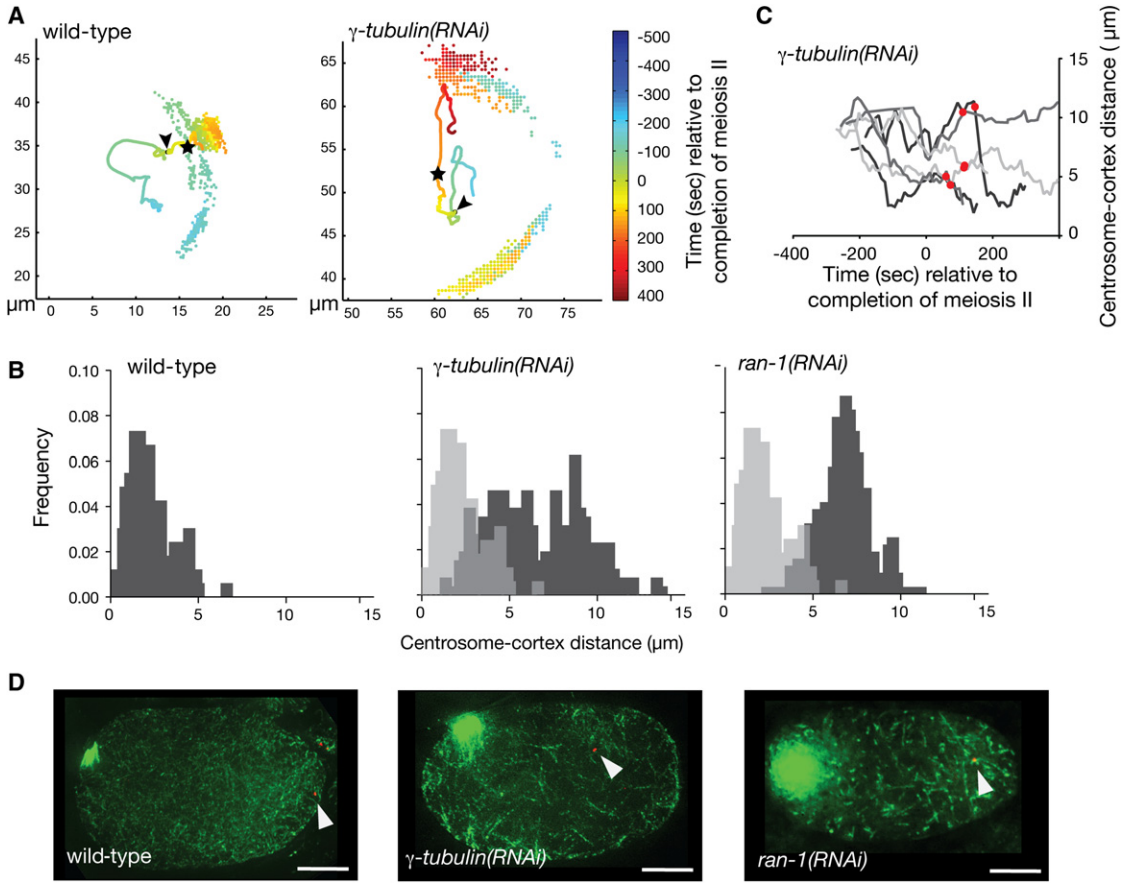

Figure 4. Cytoplasmic Microtubules Bias Centrosome Movement toward the Cortex before Symmetry Breaking

(A) Centrosome position (linear track) and nearest cortical point (dots) extracted from time-lapse images of WT and γ-*tubulin(RNAi)* embryos. Colors indicate time scale (blue, −500; red, 400). Centrosome position at time0 is indicated by an arrowhead. Centrosome position at timeSB is indicated by a star.

(B) Frequency histogram of centrosome-cortex distances before symmetry breaking in WT (n = 7), γ-*tubulin(RNAi)* (n = 10), and *ran-1(RNAi)* (n = 8) embryos (dark gray bars). In γ-*tubulin(RNAi)* and *ran-1(RNAi)*, the WT distribution is shown for comparison (light gray bars).

(C) Centrosome-cortex distance. The distance from centrosomes to the site of symmetry breaking on the cortex over time. Each line represents one centrosome. Centrosome-cortex distance at timeSB is indicated with a red dot.

(D) Microtubules in WT, γ-tubulin-depleted, and Ran-depleted embryos before polarity establishment. Immunofluorescent images show tubulin (green) and the centriolar protein SAS-4 (red, indicated by white arrowheads). Scale bar represents 10 μm.

Between fertilization and entry into the first mitotic cell cycle (time0), the network of cytoplasmic microtubules facilitates centrosome movement in a random walk. A bias toward the cortex arises from cytoplasmic microtubules, controlled by γ-tubulin and Ran. Regardless of centrosome position, centrosomes signal to the closest cortex to break symmetry and initiate polarity. The start of polarization generates large-scale cytoplasmic flow toward the site of symmetry breaking that moves centrosomes into close proximity to the cortex. Our finding that centrosomes initiate cortical polarity establishment from a distance implicates a signal that can travel away from centrosomes. Centrosome bias toward the cortex could increase the efficiency of symmetry breaking by decreasing the distance over which the signal would have to be transferred. Importantly, close centrosome-cortex association was not a requirement for symmetry breaking. Symmetry breaking drives movement of the centrosome to the cortex, allowing self-organizing polarization process to emerge from a largely stochastic initial event.

Cytoplasmic microtubules increase the efficiency of symmetry breaking by constraining centrosome movement near the cortex. Centrosome motion reflected the organization of the cytoplasmic microtubule network. In WT embryos, centrosomes moved in short runs interspersed with dwells and frequent changes in direction, and cytoplasmic microtubules formed a randomly oriented, dense network of short filaments. In γ-*tubulin(RNAi)* embryos, we observed more processive—longer, straighter—centrosome trajectories and longer, fewer cytoplasmic microtubules. In *ran-1(RNAi)* embryos, centrosomes were found far from the cortex, and dense cytoplasmic microtubules were prominent in the embryo interior. These parallels suggest a possible direct relationship between microtubule organization and centrosome motility, for instance, through transport of centrosomes along microtubules or by microtubules pushing centrosomes. Cytoplasmic microtubules were dynamic, growing and sliding along the cortex: the rate of plus-end movement, estimated from the plus-end tracking protein EB1 (EB1::GFP; [27]), was similar to the average velocity of centrosome movement (0.4 μm sec^{-1}) (D.B. and C.R.C., unpublished data). Growing microtubules might push centrosomes—or the centrosome-pronucleus complex—until random encounters with different microtubules would cause a change in direction. The cortical constraint of centrosome motion could arise through a higher

density of microtubules near the cortex, for instance through a spatial bias in nucleation.

Several lines of evidence indicate that microtubules are not essential for polarity establishment in WT *C. elegans* embryos [4, 12]. Two recent studies, however, propose that centrosomal microtubules participate in establishment of the PAR-2 cortical domain when normal polarization pathways are compromised, either by reduced actomyosin contractility [5, 6] or imbalanced anterior and posterior PAR protein levels [13]. The involvement of such microtubule-dependent polarization in WT embryos has been suggested on the basis of delayed polarity establishment in embryos lacking microtubules [6]. Based on our findings, first, that centrosomes are constrained near the cortex by cytoplasmic microtubules, and second, that centrosome-cortex distance affects the time required for symmetry breaking, the observed delays in polarization following microtubule disruption [6] might be caused by increased centrosome-cortex distance rather than a lack of PAR-2-microtubule interaction as proposed. We found no additive delay in polarity establishment in embryos that lack of centrosomal microtubules: the centrosome-cortex distance versus symmetry breaking efficiency relationship was the same in embryos with and without centrosomal microtubules [WT and *γ-tubulin(RNAi)*, respectively; Figure 3F; Figure S3C]. The importance of centrosomal microtubule-dependent polarization in WT embryos remains unclear.

Symmetry breaking in *C. elegans* embryos appears to reflect the inherent asymmetry of centrosome position. The cortical bias supplied by cytoplasmic microtubules ensures that centrosomes do not enter the middle of the egg and instead retain an eccentric position. This random event in an otherwise highly stereotyped process is perhaps not surprising, because two features of polarity establishment in *C. elegans* embryos make the precise site of symmetry breaking unimportant. First, the polarity axis does not need to respond to the external environment, unlike chemotaxing cells or polarized epithelia. Second, polarity establishment is largely self-organizing. Symmetry breaking first generates cytoplasmic flows, which bring the centrosomes into close association with the cortex to allow for PAR polarity establishment. The polarization process also generates a rotation of the embryo within the eggshell to align the polarity axis with the long axis of the egg, in a process known as posteriorization [10, 28]. This realignment of the polarity axis to eggshell geometry serves to coordinate geometry-dependent processes and polarity-dependent processes, such as during positioning of the mitotic spindle [29]. Our data indicate that symmetry breaking was more efficient when centrosomes were close to the cortex, which might become critical in embryos that are challenged by environmental variability or other external stresses.

Supplemental Information

Supplemental Information includes three figures, Supplemental Experimental Procedures, and four movies and can be found with this article online at doi:10.1016/j.cub.2012.01.064.

Acknowledgments

The authors thank M. Mayer and S. Grill (MPI-CBG, Dresden, Germany) for the PIV code and members of the Grill, Julicher, and Hyman laboratories for discussions at early stages of the project. We thank Juergen Knoblich, Stefan Westermann, Nate Goehring, and Alex Dammermann for comments on the manuscript, members of the Cowan and Dammermann laboratories for comments and discussion, and H. Wada for technical assistance. D.B. thanks the faculty of the Marine Biological Laboratory Physiology Course 2010, particularly R. Wollman, A. Besser, W. Marshall, and C. Huang for introduction to image processing and analysis. Some worm strains used in this study were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health. Research at the IMP is partially funded by Boehringer Ingelheim.

Received: November 25, 2011
Revised: January 5, 2012
Accepted: January 30, 2012
Published online: March 15, 2012

References

1. Gönczy, P., and Rose, L.S. (2005). Asymmetric cell division and axis formation in the embryo. WormBook, 1–20.
2. Hamill, D.R., Severson, A.F., Carter, J.C., and Bowerman, B. (2002). Centrosome maturation and mitotic spindle assembly in C. elegans require SPD-5, a protein with multiple coiled-coil domains. Dev. Cell *3*, 673–684.
3. O'Connell, K., and Maxwell, K. (2000). The spd-2 gene is required for polarization of the anteroposterior axis and formation of the sperm asters in the Caenorhabditis elegans zygote. Developmental Biology *222*, 55–70.
4. Cowan, C.R., and Hyman, A.A. (2004). Centrosomes direct cell polarity independently of microtubule assembly in C. elegans embryos. Nature *431*, 92–96.
5. Zonies, S., Motegi, F., Hao, Y., and Seydoux, G. (2010). Symmetry breaking and polarization of the C. elegans zygote by the polarity protein PAR-2. Development *137*, 1669–1677.
6. Motegi, F., Zonies, S., Hao, Y., Cuenca, A.A., Griffin, E., and Seydoux, G. (2011). Microtubules induce self-organization of polarized PAR domains in Caenorhabditis elegans zygotes. Nat. Cell Biol. *13*, 1–9.
7. Jenkins, N., Saam, J.R., and Mango, S.E. (2006). CYK-4/GAP provides a localized cue to initiate anteroposterior polarity upon fertilization. Science *313*, 1298–1301.
8. Munro, E., Nance, J., and Priess, J.R. (2004). Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early C. elegans embryo. Dev. Cell *7*, 413–424.
9. Rappleye, C.A., Tagawa, A., Le Bot, N., Ahringer, J., and Aroian, R.V. (2003). Involvement of fatty acid pathways and cortical interaction of the pronuclear complex in Caenorhabditis elegans embryonic polarity. BMC Dev. Biol. *3*, 8.
10. Rappleye, C.A., Tagawa, A., Lyczak, R., Bowerman, B., and Aroian, R.V. (2002). The anaphase-promoting complex and separin are required for embryonic anterior-posterior axis formation. Dev. Cell *2*, 195–206.
11. Wallenfang, M.R., and Seydoux, G. (2000). Polarization of the anterior-posterior axis of C. elegans is a microtubule-directed process. Nature *408*, 89–92.
12. Sonneville, R., and Gönczy, P. (2004). Zyg-11 and cul-2 regulate progression through meiosis II and polarity establishment in C. elegans. Development *131*, 3527–3543.
13. Tsai, M.C., and Ahringer, J. (2007). Microtubules are involved in anterior-posterior axis formation in C. elegans embryos. J. Cell Biol. *179*, 397–402.
14. Pelletier, L., Ozlü, N., Hannak, E., Cowan, C., Habermann, B., Ruer, M., Müller-Reichert, T., and Hyman, A.A. (2004). The Caenorhabditis elegans centrosomal protein SPD-2 is required for both pericentriolar material recruitment and centriole duplication. Curr. Biol. *14*, 863–873.
15. Cowan, C.R., and Hyman, A.A. (2006). Cyclin E-Cdk2 temporally regulates centrosome assembly and establishment of polarity in Caenorhabditis elegans embryos. Nat. Cell Biol. *8*, 1441–1447.
16. Hird, S.N., and White, J.G. (1993). Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. J. Cell Biol. *121*, 1343–1355.
17. Codling, E.A., Plank, M.J., and Benhamou, S. (2008). Random walk models in biology. J. R. Soc. Interface *5*, 813–834.
18. McNally, K., Martin, J., and Ellefson, M. (2010). Kinesin-dependent transport results in polarized migration of the nucleus in oocytes and inward movement of yolk granules in meiotic embryos. Developmental Biology *339*, 126–140.
19. Lüders, J., and Stearns, T. (2007). Microtubule-organizing centres: a re-evaluation. Nat. Rev. Mol. Cell Biol. *8*, 161–167.

20. Walczak, C.E., Vernos, I., Mitchison, T.J., Karsenti, E., and Heald, R. (1998). A model for the proposed roles of different microtubule-based motor proteins in establishing spindle bipolarity. Curr. Biol. *8*, 903–913.
21. Zhang, X., Ems-McClung, S.C., and Walczak, C.E. (2008). Aurora A phosphorylates MCAK to control ran-dependent spindle bipolarity. Mol. Biol. Cell *19*, 2752–2765.
22. Nguyen-Ngoc, T., Afshar, K., and Gönczy, P. (2007). Coupling of cortical dynein and G alpha proteins mediates spindle positioning in Caenorhabditis elegans. Nat. Cell Biol. *9*, 1294–1302.
23. Gusnowski, E.M., and Srayko, M. (2011). Visualization of dynein-dependent microtubule gliding at the cell cortex: implications for spindle positioning. J. Cell Biol. *194*, 377–386.
24. Ellefson, M.L., and McNally, F.J. (2009). Kinesin-1 and cytoplasmic dynein act sequentially to move the meiotic spindle to the oocyte cortex in Caenorhabditis elegans. Mol. Biol. Cell *20*, 2722–2730.
25. Wilde, A., and Zheng, Y. (1999). Stimulation of microtubule aster formation and spindle assembly by the small GTPase Ran. Science *284*, 1359–1362.
26. Ohba, T., Nakamura, M., Nishitani, H., and Nishimoto, T. (1999). Self-organization of microtubule asters induced in Xenopus egg extracts by GTP-bound Ran. Science *284*, 1356–1358.
27. Srayko, M., Kaya, A., Stamford, J., and Hyman, A.A. (2005). Identification and characterization of factors required for microtubule growth and nucleation in the early C. elegans embryo. Dev. Cell *9*, 223–236.
28. Goldstein, B., and Hird, S.N. (1996). Specification of the anteroposterior axis in Caenorhabditis elegans. Development *122*, 1467–1474.
29. Tsou, M.-F.B., Ku, W., Hayashi, A., and Rose, L.S. (2003). PAR-dependent and geometry-dependent mechanisms of spindle positioning. J. Cell Biol. *160*, 845–855.