Revisiting the role of microtubules in *C. elegans* polarity

Fumio Motegi and Geraldine Seydoux

Department of Molecular Biology and Genetics, Howard Hughes Medical Institute, Center for Cell Dynamics, Johns Hopkins School of Medicine, Baltimore, MD 21205

Cells must break symmetry to acquire polarity. Microtubules have been implicated in the induction of asymmetry in several cell types, but their role in the *Caenorhabditis elegans* zygote, a classic polarity model, has remained uncertain. One study (see Tsai and Ahringer on p. 397 of this issue) brings new light to this problem by demonstrating that severe loss of microtubules impairs polarity onset in *C. elegans*.

Most cells become polarized during development to divide asymmetrically, to migrate, or to organize in tissues. Localized changes in the actin-rich cortex are essential to establish and maintain polarity in many cell types (Siegrist and Doe, 2007), but what initially triggers these changes is not always fully understood. Because polarization involves long-term reorganization throughout the cell, the initial cue must be accurate and self-reinforcing. Accumulating evidence suggest that microtubules often serve as an internal source of asymmetry (Siegrist and Doe, 2007). Now, a new study implicates microtubules in polarization of the *C. elegans* zygote (Tsai and Ahringer, 2007).

Microtubules are polar filaments with stable minus ends, which are typically pointed toward the centrosome near the nucleus, and unstable plus ends, which extend outward toward the cell cortex. This organization makes them ideally suited for the local delivery of regulators of cortical actomyosin. For example, in newly divided fission yeast, microtubules deliver Tea4p to the new cell tip. Tea4p interacts with the actin nucleator formin/For3p, stimulating local actin cable assembly and growth (Martin et al., 2005). Similarly, in migrating fibroblasts, growing microtubules at the leading edge activate the small GTPase Rac1. Activated Rac1 stimulates actin filament assembly and lamellipodial protrusions, which, in turn, accelerates microtubule growth (Wittmann and Waterman-Storer, 2001). In principle, positive feedback loops between the microtubule and actin systems could reinforce even small initial differences and lead to robust symmetry-breaking signals.

Correspondence to Fumio Motegi: fmotegi1@jhmi.edu; or Geraldine Seydoux: gseydoux@jhmi.edu

The *C. elegans* zygote may, at first glance, look like another example of microtubule-induced polarity, but the evidence so far has been contradictory (Siegrist and Doe, 2007). The zygote becomes polarized shortly after fertilization under the influence of the sperm and its associated centrosome (Goldstein and Hird, 1996; Sadler and Shakes, 2000). The sperm–centrosome complex remains near the cortex for several minutes after fertilization and, therefore, is in an ideal position to deliver a symmetry-breaking cue to the overlying actin cytoskeleton. The actin cytoskeleton initially is under dynamic tension throughout the cortex but becomes destabilized near the sperm–centrosome complex, coincident with the accumulation of pericentriolar material (PCM) and microtubule nucleation (Fig. 1; Munro et al., 2004). This local disruption leads to a flow of cortical actomyosin away from the sperm–centrosome complex, which transports polarity regulators PAR-3 (partitioning defective 3), PAR-6, and PKC-3 to the opposite pole (Munro et al., 2004). Reciprocal inhibitory interactions between PAR-6/PKC-3/PAR-3 in the anterior and PAR-1/PAR-2 in the posterior eventually lead to the formation of two nonoverlapping PAR domains (Kemphues, 2000; Cuenca et al., 2003).

How does the sperm–centrosome complex trigger polarity? Mutants that block PCM assembly (e.g., *spd-5* and *spd-2*) and laser ablation of the centrosome delay or prevent polarity initiation (O'Connell et al., 2000; Wallenfang and Seydoux, 2000; Hamill et al., 2002; Cowan and Hyman, 2004). Although all of the available evidence points to the centrosome as the source of polarity, the specific component involved has been difficult to pin down. Obvious candidates are the microtubules, which appear around the centrosome coincident with the onset of polarity. Indirect evidence for the involvement of microtubules first came from analyzing zygotes arrested in the first meiotic division (Wallenfang and Seydoux, 2000). These zygotes fail to nucleate microtubules around the centrosome and instead become polarized by the acentriolar meiotic spindle. The meiotic spindle typically is located at the other end of the zygote, causing an apparent reversed polarity (Fig. 1). This reversed polarity is sensitive to nocodazole, implicating microtubules. Direct evidence for a role for microtubules in "normal" polarity, however, has been difficult to obtain. Two studies using α- and β-tubulin RNAi and/or nocodazole treatment to disrupt microtubule assembly in wild-type zygotes failed to uncover polarity defects (Cowan and Hyman, 2004; Sonneville and Gonczy, 2004). In both studies, low levels of tubulin remained

© The Rockefeller University Press $30.00
The Journal of Cell Biology, Vol. 179, No. 3, November 5, 2007 367–369
http://www.jcb.org/cgi/doi/10.1083/jcb.200710062

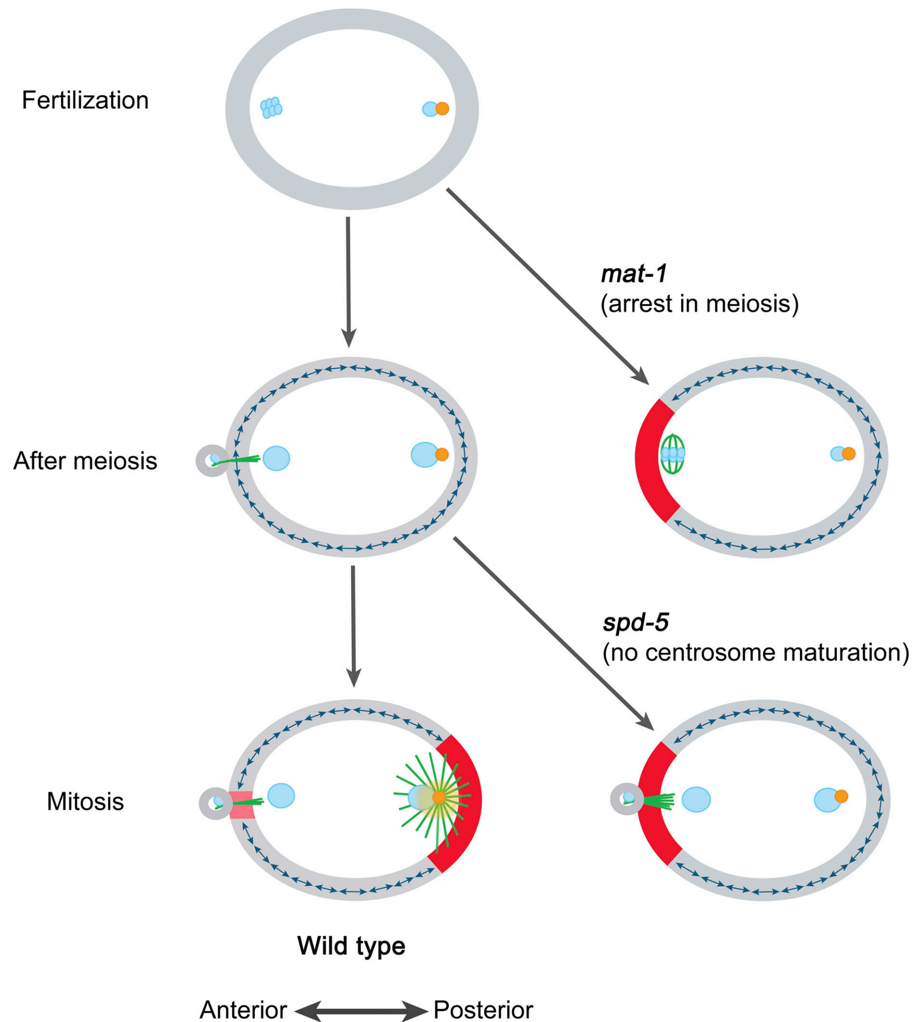

Figure 1. **Working model for the initiation of cortical polarity in *C. elegans* zygotes.** Wild type: fertilization results in a zygote with a sperm pronucleus (light blue; right) at one end of the zygote and a maternal pronucleus (light blue; left) undergoing meiosis at the other end. After meiosis (polar bodies are extruded outside the zygote [small gray circle]), the actomyosin-rich cortex undergoes dynamic contractions (arrows). Upon entry into mitosis, microtubules (green) and PCM (light orange) are assembled around the sperm-donated centrosome (orange) at one end of the zygote. Microtubules and/or PCM-associated proteins stimulate local disassembly of the actomyosin network (gray) and cortical flows that clear anterior PARs, allowing PAR-2 (red) to associate with the cortex. At the other end of the zygote, a hypothetical meiotic spindle remnant also nucleates microtubules, but without the help of a centrosome. This weaker microtubule nucleation site induces a transient PAR-2 domain (light red), which quickly is overtaken by the cortical flows coming from the opposite end. *mat*-1 mutant: *mat-1* mutants are arrested during the first meiotic division and never assemble microtubules or PCM around the sperm centrosome. Microtubules from the acentriolar meiotic spindle induce an unopposed reversed PAR-2 domain. *spd*-5 mutant: *spd-5* mutants progress through meiosis normally but fail to nucleate microtubules and PCM around the sperm centrosome in mitosis. Microtubules from the hypothetical meiotic spindle remnant induce an unopposed reverse PAR-2 domain.

around the centrosome. However, these levels appeared lower than those observed in *spd-2* mutants that fail to initiate polarity, suggesting that microtubule nucleation and polarity establishment are not correlated.

This issue has been taken up again in a new study by Tsai and Ahringer (2007), who used RNAi to achieve severe depletion of α/β tubulin by RNAi in gravid hermaphrodites. Sustained depletion of tubulin eventually leads to sterility, so the authors analyzed the last embryos produced before the onset of sterility. Zygotes that experienced the most severe loss of α/β tubulin assembled microtubules around the sperm centrosome later than in wild type. In these zygotes, PAR-2 localization to the cortex was also delayed, suggesting a correlation between microtubules and polarity. Although nonspecific effects caused by the severe tubulin depletion could not be excluded, the accumulation of two centrosomal markers appeared unaffected. Tsai and Ahringer also found that a mutant that fails in PCM assembly (*spd-5*) often develops a reversed PAR-2 domain during mitosis, indicating that a centrosome-independent mechanism of polarity exists even in embryos that are not blocked in meiosis (Fig. 1). The *spd-5* reversed polarity was highly sensitive to tubulin depletion and appeared later than wild type, suggesting a microtubule-dependent cue that is less efficient when uncoupled from the centrosome. Interestingly, in wild-type embryos, PAR-2 occasionally appears transiently at both poles, but the PAR-2 domain nearest the centrosome always eventually wins out (Boyd et al., 1996; Cuenca et al., 2003). Together, these observations suggest that the symmetry-breaking cue involves microtubules as well as a centrosome-dependent mechanism to increase robustness.

How could microtubules affect polarity? Actomyosin contractions in the cortex require the small GTPase RHO-1, its activator the RhoGEF (guanine nucleotide exchange factor) ECT-2, and its negative regulator the RhoGAP (GTPase-activating protein) CYK-4 (Jenkins et al., 2006; Motegi and Sugimoto, 2006; Schonegg and Hyman, 2006). Local inactivation of RHO-1 by the down-regulation of ECT-2 or up-regulation of CYK-4 in principle could weaken the actomyosin network, causing an asymmetric contraction and cortical flows away from the centrosome. In fact, ECT-2 and CYK-4 have been shown to be excluded and enriched, respectively, from the cortex overlying the centrosome (Jenkins et al., 2006; Motegi and Sugimoto, 2006). Whether microtubules contribute to these distributions remains to be determined. RhoGEFs and RhoGAPs have been reported to interact with proteins that associate with microtubule plus ends in several organisms (Siegrist and Doe, 2007).

In *C. elegans*, CYK-4 is known to bind the kinesin-like protein ZEN-4 (Mishima et al., 2002). In *Drosophila melanogaster* neuroblasts, the kinesin Khc-73 is required to link the mitotic spindle axis to cortical polarity (Siegrist and Doe, 2005). Clearly, it will be important to investigate whether microtubule-associated proteins affect polarity in *C. elegans*. The role of the centrosome also remains to be investigated. One possibility is that the centrosome increases the robustness of the microtubule-dependent cue simply by stimulating microtubule polymerization. Alternatively, the centrosome may act independently of microtubules, as suggested by the lack of correlation between polarity and microtubule nucleation in *spd-2* mutants (Cowan and Hyman, 2004).

The results of Tsai and Ahringer (2007) provide a new impetus for investigating the role of microtubule and centrosome-associated proteins in *C. elegans* polarity. Their results also are an important reminder of the challenges associated with investigating the role of essential cytoskeletal components, which are difficult to deplete while avoiding catastrophic effects. Hopefully, identification of the molecules that make up the symmetry-breaking cues will clarify the role of microtubules in *C. elegans* zygotes and perhaps uncover new unifying principles for how cells break symmetry.

Submitted: 9 October 2007
Accepted: 16 October 2007

References

Boyd, L., S. Guo, D. Levitan, D.T. Stinchcomb, and K.J. Kemphues. 1996. PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in *C. elegans* embryos. *Development*. 122:3075–3084.

Cowan, C.R., and A.A. Hyman. 2004. Centrosomes direct cell polarity independently of microtubule assembly in *C. elegans* embryos. *Nature*. 431:92–96.

Cuenca, A.A., A. Schetter, D. Aceto, K. Kemphues, and G. Seydoux. 2003. Polarization of the *C. elegans* zygote proceeds via distinct establishment and maintenance phases. *Development*. 130:1255–1265.

Goldstein, B., and S.N. Hird. 1996. Specification of the anteroposterior axis in *Caenorhabditis elegans. Development*. 122:1467–1474.

Hamill, D.R., A.F. Severson, J.C. Carter, and B. Bowerman. 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.

Jenkins, N., J.R. Saam, and S.E. Mango. 2006. CYK-4/GAP provides a localized cue to initiate anteroposterior polarity upon fertilization. *Science*. 313:1298–1301.

Kemphues, K. 2000. PARsing embryonic polarity. *Cell*. 101:345–348.

Martin, S.G., W.H. McDonald, J.R. Yates III, and F. Chang. 2005. Tea4p links microtubule plus ends with the formin for3p in the establishment of cell polarity. *Dev. Cell*. 8:479–491.

Mishima, M., S. Kaitna, and M. Glotzer. 2002. Central spindle assembly and cytokinesis require a kinesin-like protein/RhoGAP complex with microtubule bundling activity. *Dev. Cell*. 2:41–54.

Motegi, F., and A. Sugimoto. 2006. Sequential functioning of the ECT-2 RhoGEF, RHO-1 and CDC-42 establishes cell polarity in *Caenorhabditis elegans* embryos. *Nat. Cell Biol*. 8:978–985.

Munro, E., J. Nance, and J.R. Priess. 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.

O'Connell, K.F., K.N. Maxwell, and J.G. White. 2000. The *spd-2* gene is required for polarization of the anteroposterior axis and formation of the sperm asters in the *Caenorhabditis elegans* zygote. *Dev. Biol.* 222:55–70.

Sadler, P.L., and D.C. Shakes. 2000. Anucleate *Caenorhabditis elegans* sperm can crawl, fertilize oocytes and direct anterior-posterior polarization of the 1-cell embryo. *Development*. 127:355–366.

Schonegg, S., and A.A. Hyman. 2006. CDC-42 and RHO-1 coordinate acto-myosin contractility and PAR protein localization during polarity establishment in *C. elegans* embryos. *Development*. 133:3507–3516.

Siegrist, S.E., and C.Q. Doe. 2005. Microtubule-induced Pins/Galphai cortical polarity in *Drosophila* neuroblasts. *Cell*. 123:1323–1335.

Siegrist, S.E., and C.Q. Doe. 2007. Microtubule-induced cortical cell polarity. *Genes Dev.* 21:483–496.

Sonneville, R., and P. Gonczy. 2004. zyg-11 and cul-2 regulate progression through meiosis II and polarity establishment in *C. elegans. Development*. 131:3527–3543.

Tsai, M.-C., and J. Ahringer. 2007. Microtubules are involved in anterior-posterior axis formation in *C. elegans* embryos. *J. Cell Biol*. 179:397–402.

Wallenfang, M.R., and G. Seydoux. 2000. Polarization of the anterior-posterior axis of *C. elegans* is a microtubule-directed process. *Nature*. 408:89–92.

Wittmann, T., and C.M. Waterman-Storer. 2001. Cell motility: can Rho GTPases and microtubules point the way? *J. Cell Sci.* 114:3795–3803.