Surfing the Actomyosin Wave: Polarization of the *C. elegans* Zygote

Many cell types rely on asymmetrically localized PAR proteins to become polarized. New evidence reveals that cortical flows powered by actomyosin contractions can mobilize PAR complexes to create distinct cortical domains.

Cells become polarized for a variety of reasons: to move, to form distinct membrane domains, to divide asymmetrically, or to organize within a tissue. To become polarized, a cell must segregate some of its components away from others. One of the challenges of discovering what initiates asymmetries in cells has been developing methods to observe cells directly during polarization. In this issue of *Developmental Cell*, Munro and colleagues meet this challenge by filming live *C. elegans* zygotes as they first become polarized. Their observations suggest a simple mechanism for initiating polarity using a contractile actin cytoskeleton.

C. elegans eggs are ovoid cells that become polarized along their long axis shortly after fertilization. The cue that initiates polarization is not yet known, but appears to be linked to the centrosome, or microtubule organizing center (MTOC), associated with the sperm pronucleus (see Schneider and Bowerman, 2003, for review). The sperm pronucleus/MTOC complex first appears near the cortex 30 min after fertilization, in a position that correlates with the future posterior end of the embryo. Upon arrival of the sperm pronucleus/MTOC complex, the polarity regulators PAR-3, PAR-6, and PKC-3 (anterior PARs) become enriched in the anterior half of the cortex away from the MTOC, and PAR-1 and PAR-2 (posterior PARs) become enriched in the posterior. Genetic analyses had shown that anterior and posterior PARs compete with each other for cortical localization and that the MTOC functions primarily by displacing the anterior PARs from the nearby cortex, allowing the posterior PARs to accumulate there (Schneider and Bowerman, 2003).

How are the anterior PARs removed from the posterior cortex? Several lines of evidence have implicated the actin cytoskeleton. First, examination of zygotes by light microscopy revealed that yolk granules nearest the cortex flow away from the MTOC, while granules deeper in the cytoplasm flow toward the MTOC, suggesting dramatic cytoskeletal rearrangements during polarization (Goldstein and Hird, 1996; Hird and White, 1993). Second, disruption of the actomyosin network, with drugs or by RNAi, prevents both cortical flows and PAR asymmetry (Schneider and Bowerman, 2003). Finally, live examination of PAR-2 tagged with green fluorescent protein (GFP) showed that the boundary between anterior and posterior domains coincides with the retreating edge of "old cortex" during flow (Cheeks et al., 2004). Could anterior PARs be mobilized by cortical flow? To address this possibility, Munro et al. tagged the nonmuscle myosin II heavy chain NMY-2 with GFP and tracked its dynamics in the cortex of live zygotes using time-lapse confocal microscopy. The NMY-2:GFP movies reveal a dynamic myosin network made up of dense foci interconnected by filaments. Before polarization, the network appears uniformly tensioned, with many transient contractions squeezing and relaxing the cortex locally as foci form and dissipate randomly throughout the cortex (Figure 1). Upon appearance of the sperm pronucleus, new NMY-2:GFP foci stop forming on the cortex nearest the sperm, and existing foci move away in a wave that eventually caps the anterior half of the zygote. Neighboring foci move toward each other during capping, consistent with a global contraction of the network. Confocal microscopy also revealed PAR-6:GFP puncta in the cortex streaming away from the sperm MTOC. Kymograph analyses correlating the movement of PAR-6:GFP puncta and NMY-2:GFP foci with nearby yolk granules confirmed that these structures move together and at identical speeds (Munro et al., 2004; Cheeks et al., 2004). Partial depletion of the myosin regulatory light chain MLC-4 reduces the speed of PAR-6:GFP puncta, NMY-2:GFP foci, and yolk granules to the same extent, consistent with all these structures being swept away by the same myosin-driven contraction.

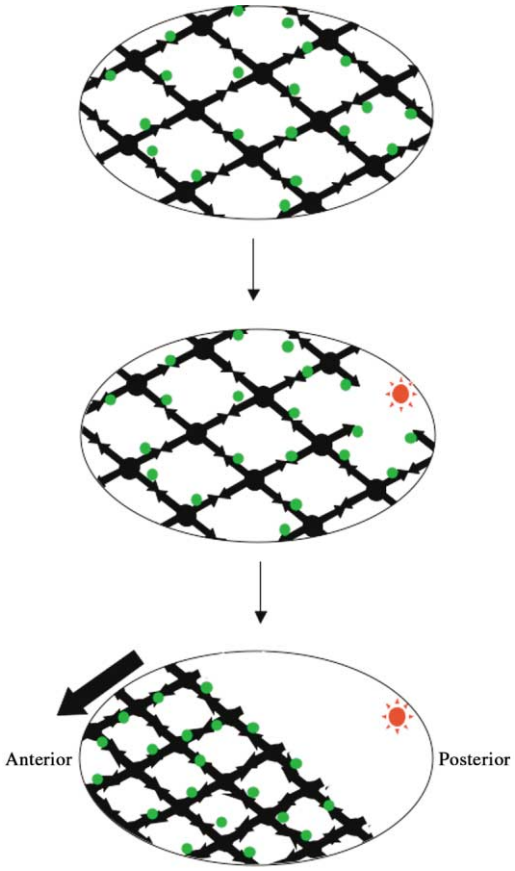

Figure 1. Polarization of the *C. elegans* Zygote

Polarization begins with local disruption of the actomyosin network (black) by the MTOC (red). The resulting asymmetric contraction carries PAR-6 complexes (green) away from the MTOC.

What initiates the contraction? Zygotes lacking a functional MTOC do not stop forming NMY-2 foci around the sperm pronucleus and do not undergo an asymmetric contraction (Munro et al., 2004). Munro and colleagues propose that local weakening of the actomyosin network by the MTOC would be sufficient to cause the network to collapse and contract toward the opposite pole. How the MTOC weakens the network, however, is not known. Microtubules have been implicated in the modulation of actin dynamics in many systems (Rodriguez et al., 2003). A recent study, however, showed that depletion of tubulin by RNAi does not prevent polarization of the *C. elegans* zygote, suggesting that MTOC components other than microtubules are involved (Sonneville and Gonczy, 2004).

What connects the anterior PARs to the network also is not known. Localization of PAR-3 to the cortex requires microfilaments (Severson and Bowerman, 2003), consistent with a link to the actin cytoskeleton. Munro et al. noted that cortical proteins not required for polarity (e.g., E-cadherin) also localize to the anterior, suggesting that cortical flows sweep resident proteins indiscriminately. The PAR proteins, however, are not simply passive passengers. Depletion of anterior PARs reduces the rate and extent of cortical flow (Munro et al., 2004) and also severely disrupts the reciprocal movement of the central cytoplasm (Cheeks et al., 2004). Mobilization of cytoplasmic flow is one of the mechanisms by which PAR proteins regulate the localization of protein complexes in the cytoplasm (Cheeks et al., 2004). Thus, by modulating actomyosin dynamics, PAR proteins not only promote their own transport but also the transport of complexes in the cytoplasm, in effect propagating the influence of the sperm MTOC throughout the embryo.

Cortical flow sweeps the anterior PARs away from the sperm MTOC, but what keeps them from coming back? The sperm pronucleus/MTOC eventually leaves the cortex to meet the maternal pronucleus in the middle of the embryo and begin mitosis. At that time, perhaps in response to entry into M phase, the anterior NMY-2:GFP foci disassemble abruptly (Munro et al., 2004). Remarkably, excess NMY-2:GFP and PAR-6:GFP are retained in the anterior cap, apparently trapped by a boundary that encircles the middle of the zygote. This boundary is characterized by high levels of NMY-2:GFP and low levels of both posterior and anterior PARs. What forms the boundary? Earlier studies showed that the posterior PAR protein, PAR-2, is required after, but not before, pronuclear meeting to maintain polarity (Cuenca et al., 2003). Munro et al. found that in *par-2(RNAi)* embryos, NMY-2:GFP foci still flow anteriorly before pronuclear meeting, but the boundary does not form or is displaced posteriorly, and NMY-2:GFP and PAR-6:GFP flow back toward the posterior after pronuclear meeting. How PAR-2, a RING finger protein, contributes to the boundary is not clear. Munro et al. suggest that PAR-2 inhibits recruitment of NMY-2 to the cortex. Paradoxically, PAR-2 promotes cortical localization of PAR-1, and PAR-1 binds directly to NMY-2 (Schneider and Bowerman, 2003). Another possibility is that balanced competition between anterior and posterior PARs at the boundary creates an area where neither class of PARs can stably associate with the cortex. Such an edge effect could impart unique properties to the cortex in this region.

Munro et al. also report NMY-2:GFP flows in cells that develop apicobasal PAR asymmetries, suggesting that cortical flows mobilize PAR complexes in cells other than the zygote. Actin-powered cortical flows have been observed in migrating cells and amphibian oocytes (Rodriguez et al., 2003), but possible effects on PAR proteins have not yet been investigated. In *Drosophila*, nonmuscle myosin II colocalizes with PAR-3 and PAR-6 on the apical side of neuroblasts but, unlike in *C. elegans*, is not required for PAR localization (Barros et al., 2003). Instead, nonmuscle myosin II is required to restrict factors on the *basal* side of the cell, suggesting a possible conserved role in boundary demarcation and the creation of separate cortical domains. The interplay between PAR proteins, the actin cytoskeleton, and actin motors promises to be an exciting area of research in years to come.

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Selected Reading

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