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SPOTLIGHT

MRCK ensures cortex-chromatin "social distancing" to enable egg spindle rotation

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During the second meiotic cell division, egg cells discard one set of chromatids to the polar body to produce a large haploid gamete. Meiotic spindle rotation is a critical step to ensure proper polar body extrusion. In this issue, Bourdais et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202211029) have identified MRCKβ as an essential kinase for efficient spindle rotation. MRCK activates cortical myosin II rings overlying the spindle to prevent the notoriously sticky interaction between the cell cortex and chromatin to facilitate spindle rotation. Furthermore, Bourdais et al. found that the same MRCK-myosin II pathway also operates in zygotes to promote parental genome unification.

To maintain correct ploidy over generations, it is critical to produce haploid gametes from diploid precursor cells during meiosis. Cells committed to meiosis undergo a single round of DNA replication and then two rounds of cell division. In many female animals, meiotic cell division is highly asymmetric, ultimately producing a large egg and small polar bodies. This asymmetric division allows the egg to retain a large quantity of cytoplasm, which is critical to successfully complete embryogenesis after fertilization by sperm (1; Fig. 1 A).

Cellular cytoskeletons play multiple essential roles during cell division, and meiosis is no exception. Microtubules form the spindle machinery to segregate chromosomes, while actin filaments (F-actin), together with nonmuscle myosin (myosin II) motors, exert contractile force to cleave cells to complete cell division. In addition to this well-established role in cytokinesis, the actin cytoskeleton also contributes to spindle positioning in female meiosis. An Arp2/3-induced F-actin network drives cytoplasmic flow to exert pushing forces that help to localize spindles close to the cell cortex, which is an essential prerequisite to achieve asymmetric division (2).

Mouse eggs in the second round of meiotic cell division (meiosis II) are a particularly interesting system to study spindle positioning. Meiosis II spindles are initially parallel to the cell cortex, which requires the spindle to subsequently rotate to a perpendicular position to enable extrusion of one set of chromatids to the polar body and retention of the other set in the egg (Fig. 1 A). Upon anaphase II onset, sister chromatids are segregated to the spindle poles, forming two cortical actin caps overlying each set of segregating chromatids. RhoA/ROCK-dependent myosin II activation generates contractile tension within the actin cytoskeleton to exert pushing forces on the central spindle, which was proposed to trigger spontaneous symmetry breaking and spindle rotation from parallel to perpendicular (3, 4). This actomyosin contractility-mediated pushing force drives furrow ingression, eventually leading to cytokinesis and polar body extrusion. In early anaphase, each of the cortical actin caps is marked by characteristic ring-shaped activated myosin II, but the specific contributions of these rings to spindle rotation in female meiosis was completely unknown.

Myosin II activation is mediated via phosphorylation of the myosin regulatory light chains (MRLC) on specific residues including Serine 19, which is principally catalyzed by three kinase families: the calcium/calmodulin-dependent myosin light chain (MLCK), the Rho-associated coiled coil kinases ROCK1 and ROCK2, and the myotonic dystrophy-related CDC42-binding kinases MRCKα, MRCKβ, and MRCKγ (5). In addition to being differentially activated by their varying upstream regulators, each of the kinase families likely partitions to distinct subcellular locations due to differences in protein domains. The linear arrangement of protein kinase C conserved region 1, pleckstrin homology, citron homology, and Cdc42/Rac interactive binding domains likely act to concentrate MRCK proteins at the inner face of the plasma membrane adjacent to the cortical actin cytoskeleton (6).

In this issue, Bourdais et al. (7) challenged the generally accepted view that ring myosin II activation is driven by MLCK in mouse oocytes (8) and discovered that MRCKβ is the major kinase responsible for this activation (7). MRCK inhibition selectively eliminated ring myosin II activation while preserving RhoA/ROCK-dependent MRLC phosphorylation at cleavage furrows. This observation allowed the authors to characterize the hitherto mysterious role of

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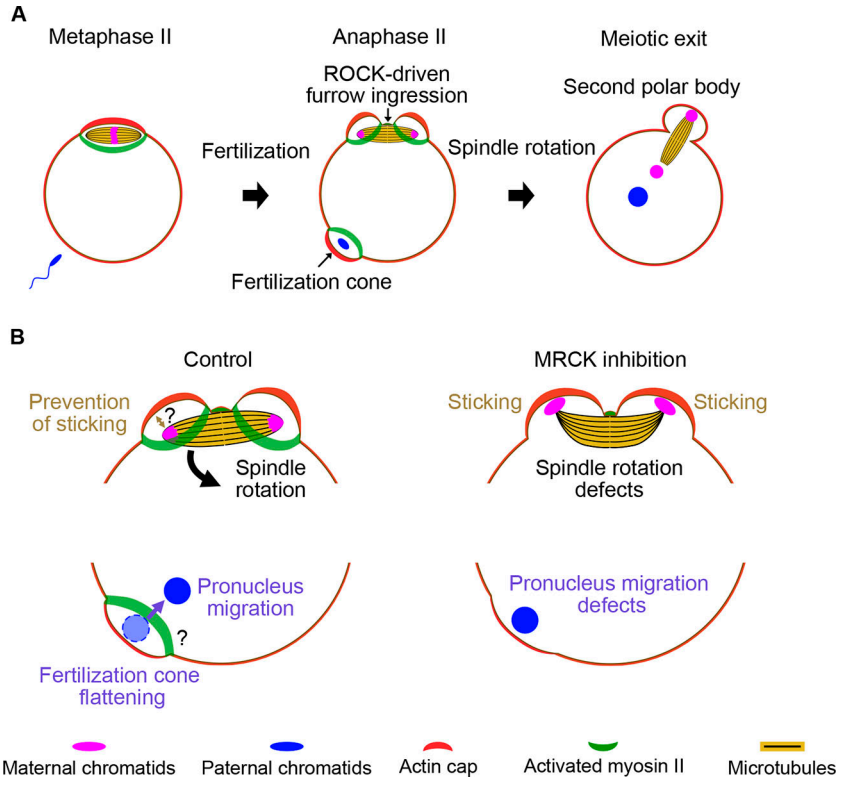

Figure 1. **Roles of MRCK and activated ring myosin II in mouse eggs and zygotes. (A)** Upon fertilization, spindle rotation leads to the extrusion of one set of maternal chromatids into the second polar body, forming diploid zygotes. **(B)** MRCK-mediated ring myosin II activation ensures spindle rotation and paternal pronucleus migration driven by fertilization cone flattening. In MRCK-inhibited eggs, spindle rotation is compromised due to segregated chromatids sticking to the cortex, resulting in failure of extrusion of the second polar body. MRCK inhibition also prevents fertilization cone flattening, leading to the cortical localization of the paternal pronucleus.

ring myosin II activation in mouse eggs. Inhibition of ring myosin II activation caused spindle rotation defects, eventually leading to failure to extrude the polar body (Fig. 1 B). Defects in polar body extrusion created eggs with two pronuclei (i.e., polyploid gametes), demonstrating MRCK's significant role in spindle rotation to maintain genome integrity. Interestingly, the authors noticed that segregating chromatids that remained attached to the cortex prevented spindle rotation. Occasional chromatid disengagement from the anaphase spindle allowed completion of spindle rotation. These observations demonstrated the deleterious consequences of contact between segregating chromatids and the cortex and revealed that MRCK maintains the physical separation between chromatin and cortex to facilitate spindle rotation.

In addition to meiosis II eggs, ring myosin II activation has also been observed in zygotes produced by the fusion of sperm with egg cells. Zygotes have a structure called the fertilization cone, which is an actin cap surrounded by phosphorylated ring myosin II that is formed on the cortex overlying sperm chromatids at the entry site (9, 10; Fig. 1 A). Upon fertilization and meiotic exit, paternal pronuclei are initially formed at the peripheral regions and later migrate toward the zygote center to closely appose the maternal pronucleus in preparation for the first mitotic cell division. A recent study proposed that pronuclear migration is triggered by fertilization cone flattening (11), but this hypothesis had not been directly tested. Bourdais et al. (7) found that MRCK inhibition in zygotes prevented the formation and flattening of the fertilization cone and the subsequent paternal pronucleus migration (Fig. 1 B; 7). These results indicate that the MRCK-driven myosin II activation has critical roles in completing gametogenesis and initiating embryogenesis.

This study raises several fundamental questions. First, why are chromosomes so sticky, and why do they resist detachment from the cortex? The molecular nature of the chromatin–cortex association is not known, nor how the MRCK-myosin II pathway prevents this interaction. Chromatin could be directly binding to the cell cortex or to the cortical actin cytoskeleton. Second, it is still unclear if spindle rotation is initiated by spontaneous symmetry breaking. Microtubule organizing centers have recently emerged as new players in spindle positioning in mouse meiosis I oocytes, supporting the actin-based positioning mechanism (12). It would be interesting to explore whether cytoskeletal fibers at spindle poles are directly involved in symmetry breaking. MRCK-inhibited eggs, which typically suppressed symmetry breaking, would be an ideal system to shed light on this question. Third, how does ring myosin II activation drive paternal nucleus migration? Based on their results, Bourdais et al. (7) speculate that fertilization cone flattening results from transient myosin II ring reorganization into a contractile cortical cap. To test this idea, high-resolution time-lapse imaging of ring myosin II dynamics at the flattening fertilization cone could be informative. Detailed molecular studies of the fertilization process are technically challenging but are critically important for the next giant leaps forward in the field of cell biology of sexual reproduction.

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