Mother centrioles are kicked out so that starfish zygote can grow

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Most oocytes eliminate their centrioles during meiotic divisions through unclear mechanisms. In this issue, Borrego-Pinto et al. (2016. *J Cell. Biol.* http://dx.doi.org/10.1083/jcb.201510083) show that mother centrioles need to be eliminated from starfish oocytes by extrusion into the polar bodies for successful embryo development.

Canonical centrosomes contain a pair of centrioles, often made of nine triplets of microtubules and surrounded by the pericentriolar material (PCM). They are the major microtubule organizing centers in most cells, which organize the microtubule spindle required to segregate chromosomes during cell division. Yet, most oocytes get rid of their centrioles. The biological significance of oocyte centriole riddance remains a mystery. Removing centrioles in oocytes could prevent some species, like *Xenopus*, from undergoing parthenogenetic development (Tournier et al., 1991). Also, eliminating the maternal centrioles is required to prevent the zygote from having an abnormal number of centrioles after fertilization, as sperm contribute two centrioles (motile sperm cells require centriole-based flagellar assembly and must retain their centrioles until fertilization [Manandhar et al., 2005]). In *Drosophila*, *Xenopus*, nematode, mouse, and human oocytes, egg centrioles are eliminated during meiotic prophase before oocyte asymmetric divisions (Szollosi et al., 1972; Manandhar et al., 2005; Januschke et al., 2006). Apart from the involvement of a helicase of undefined substrates, the pathway leading to centriole elimination has not been identified (Mikeladze-Dvali et al., 2012).

In contrast, starfish oocytes, like sea urchin or mollusk, eliminate their centrioles later in meiotic divisions (Nakashima and Kato, 2001; Shirato et al., 2006). Centrioles are replicated in a semiconservative manner during the S phase of the cell cycle. The old centriole, named the mother, is characterized by the presence of distal and subdistal appendages and serves as a template for the assembly of a new daughter centriole, lacking appendages (Bornens and Gönczy, 2014). However, to become haploid, oocytes undergo two consecutive divisions with no intervening DNA replication. Hence, centrioles are not duplicated between the two meiotic divisions and oocytes keep their number of centrioles limited to four. This also means that starfish oocytes assemble their first meiotic spindle in the presence of a pair of centrioles at each pole (Fig. 1 A). Out of the four centrioles contained in the oocyte, two (one mother and one daughter centriole) are extruded into the first polar body during the first asymmetric division. Subsequently, the second meiotic spindle is formed with only one centriole per pole (Fig. 1 A), and one centriole is extruded in the second polar body. Previous work suggested that the poles of the second meiotic spindle in starfish are not functionally equivalent (Uetake et al., 2002). In this issue, Borrego-Pinto et al. find that the mother centriole retains the ability to nucleate asters but is specifically guided into the second polar body for extrusion, whereas the daughter centriole is inactivated and then eliminated within the oocyte.

To investigate the mechanism of centriole elimination in the starfish *Patiria miniata*, Borrego-Pinto et al. (2016) first isolated homologues of centrosomal proteins and constructed fluorescent protein fusions to several centriolar proteins to track centriole fate in 3D time-lapse imaging during oocyte asymmetric divisions. Using specific markers of mother versus daughter centrioles, they established that, in meiosis I, the two spindle poles are equivalent, being constituted of a pair of mother and daughter centrioles. At anaphase I, one pair of mother/daughter centrioles is extruded into the first polar body. Importantly, the authors described an asymmetry in metaphase II, with the second meiotic spindle always having the mother centriole facing the cortex and the daughter centriole deep inside the cytoplasm (Fig. 1 B).

Borrego-Pinto et al. (2016) went on to identify the origin of this asymmetry. They show that the mother centriole, but not the daughter one, starts being rapidly transported toward the plasma membrane before completion of meiosis I spindle disassembly in a microtubule- and dynein-dependent manner, as its trafficking could be impaired by the dynein inhibitor ciliobrevin D (Firestone et al., 2012). In a second step, the mother centriole is anchored to the plasma membrane through the second meiotic division. Interestingly, electron microscopy of starfish oocytes revealed electron-dense material as well as vesicles between the mother centriole and the plasma membrane, suggesting that the mother centriole's plasma membrane anchorage occurs via its appendages (Reiter et al., 2012; Stinchcombe et al., 2015). Whether the mother centriole migrates to the cortex with its appendages facing or opposite the plasma membrane has not been addressed. However, it is reasonable to assume that, in a viscous environment such as the oocyte cytoplasm, a motion with the appendages up would be favored (Fig. 1 B). Moreover, whereas the migration of the mother centriole to the plasma membrane requires microtubules, its

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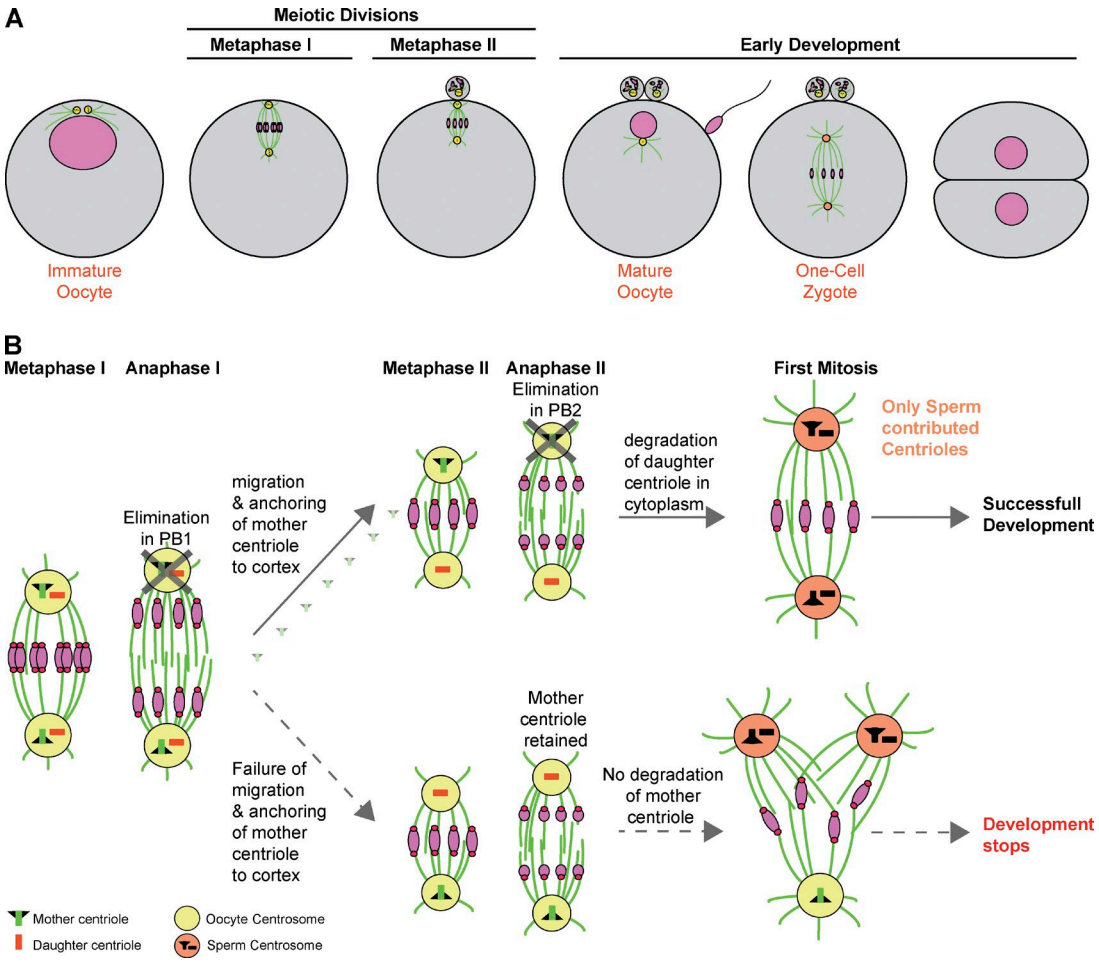

Figure 1. **Centriole elimination during meiotic maturation of starfish oocytes.** (A) Scheme of starfish oocyte meiotic divisions and early egg development. Oocyte divisions are asymmetric in size; meiotic spindles are off-centered in these large cells; and daughter cells are tiny, tailored to the chromatin mass, and named polar bodies. Microtubules are green, DNA is pink, maternal centrosomes are yellow, and sperm centrosomes are orange. (B) Fate of mother and daughter centrioles during meiotic divisions. Centrosomes are artificially enlarged to emphasize the centrioles. PB1 and PB2, first and second polar body, respectively. During anaphase I, the DNA and centrioles are segregated; one set of chromosomes and one pair of centrioles are extruded into PB1 during anaphase I. The remaining mother centriole separates from its paired daughter and rapidly moves toward the plasma membrane, where it is extruded in the second polar body (PB2) during anaphase II, leaving one set of oocyte chromatids to combine with the sperm chromatids. The remaining oocyte daughter centriole is inactivated and degraded after anaphase II. Therefore, only the sperm centrioles form the first mitotic spindle in the fertilized oocyte. Oocytes forced to retain a mother centriole form a tripolar aster upon fertilization, which stops development.

anchoring does not depend on microtubules or microfilaments, as shown by the continued tight association between the centriole and the membrane in the presence of microtubule- and/or actin-depolymerizing agents. This close anchoring via the centriole's appendages is reminiscent of the anchoring of centrioles forming cilia or at the immunological synapse in T cells (Stinchcombe et al., 2015). The precise mechanisms involved in mother centriole anchoring to the plasma membrane in starfish might be conserved in other systems that also require proximity between these two structures. It would be interesting to assess whether astral microtubules emanating from the mother centriole progressively depolymerize as the mother centriole approaches the plasma membrane to allow the intimate anchoring of the appendages with the plasma membrane. If so, Katanin, a microtubule-severing enzyme whose activity is regulated during meiotic divisions in the nematode oocyte, would be a good candidate to promote such a progressive destabilization (Srayko et al., 2000).

Future work will tell us why the daughter centriole does not experience such a migration event. This strongly argues for a functional asymmetry between the two types of centrioles. From the work of Borrego-Pinto et al. (2016), it appears that the daughter centriole is passively pushed inside the oocyte cytoplasm as a result of meiosis II spindle assembly and elongation. Dynein, which controls the migration of the mother centriole, could specifically associate with this centriole, like it does in *Saccharomyces cerevisiae*, by localizing preferentially to the spindle pole body (the yeast equivalent of the centrosome) facing the bud (Grava et al., 2006). Centrosome asymmetry has been described in several stem cell types (Roubinet and Cabernard, 2014) and this asymmetry is often rooted in its activity. However, Borrego-Pinto et al. (2016) show that the microtubule nucleation capacity of the daughter and mother centrioles is equivalent up to the metaphase II stage. It is only after fertilization and anaphase II that a difference in activity is detected between the mother and daughter centrioles. Thus, what underlies the asymmetry in behavior between the mother and daughter centrioles at anaphase I remains to be discovered. One possibility is that the presence of appendages in the mother centriole

allows the recruitment of specific factors, such as dynein, which in turn regulate mother centriole migration and anchoring.

Borrego-Pinto et al. (2016) also discovered that specific anchoring of the mother centriole to the plasma membrane, at which the second polar body will form, is the mechanism by which oocytes get rid of the remaining mother centriole. Importantly, actively removing the mother centriole after anaphase II is essential for zygotic development. Indeed, the researchers used the actin polymerization inhibitor cytochalasin D to prevent extrusion of the second polar body and artificially retain the mother centriole in the oocyte after anaphase II. When a mother centriole is retained, it keeps its microtubule nucleation capacity and participates in the first mitotic spindle pole organization of the fertilized egg, whereas the daughter centriole is inactivated and dismantled after anaphase II. As a consequence, because of the two centrioles contributed by the sperm cell, the mitotic spindle ends up being tripolar in the presence of an additional mother centriole, precluding correct chromosome segregation and further development (Fig. 1 B).

The origin of the difference in behavior between mother and daughter centrioles after anaphase II will require further investigation. To explain the loss in nucleation capacity of the daughter centriole, it will be important to check for the presence of various PCM components. Indeed, it is reasonable to assume that the daughter centriole loses its PCM association. PCM size scales with centriole size; thus, appendages of the mother centriole might possess an innate ability to maintain association with the PCM (Bobinnec et al., 1998; Delattre et al., 2004). A possible cell cycle–dependent enzymatic activity appearing after anaphase II might explain the rapid loss in microtubule nucleation capacity of the daughter centriole. It is surprising that the starfish zygote cannot cluster the mother centriole material with the centrioles from the sperm, unlike mouse oocytes, which, like cancer cells, are able to cluster PCM to regulate the total number of microtubule organizing centers (Kwon et al., 2008; Breuer et al., 2010). It will be interesting to determine whether starfish zygotes express proteins such as HURP or HSET, which are major players in extra-centrosome clustering (Kwon et al., 2008; Breuer et al., 2010).

Altogether, the results from Borrego-Pinto et al. (2016) address a major unresolved question: why do oocytes lose or inactivate their canonical centrioles during female meiosis? They show for the first time that maternal centrioles must be extruded from or inactivated in the starfish egg before fertilization so that they do not perturb mitotic spindle assembly. This is a very important step in our understanding of female gamete formation. Moreover, this work establishes starfish oocyte meiosis as a novel model system to study both functional and structural centrosome asymmetry, an essential component of asymmetric divisions.

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