Meiotic spindle assembly and chromosome segregation in oocytes

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Oocytes accumulate maternal stores (proteins, mRNAs, metabolites, etc.) during their growth in the ovary to support development after fertilization. To preserve this cytoplasmic maternal inheritance, they accomplish the difficult task of partitioning their cytoplasm unequally while dividing their chromosomes equally. Added to this complexity, most oocytes, for reasons still speculative, lack the major microtubule organizing centers that most cells use to assemble and position their spindles, namely canonical centrosomes. In this review, we will address recent work on the mechanisms of meiotic spindle assembly and chromosome alignment/segregation in female gametes to try to understand the origin of errors of oocyte meiotic divisions. The challenge of oocyte divisions appears indeed not trivial because in both mice and humans oocyte meiotic divisions are prone to chromosome segregation errors, a leading cause of frequent miscarriages and congenital defects.

Introduction

Sexual reproduction relies on the fusion of paternal and maternal haploid gametes—the sperm and the extremely large oocyte, respectively—forming a new diploid organism. Meiotic divisions contribute solely to the formation of haploid gametes. They consist of two successive divisions, without intervening DNA replication, meiosis I and II, which reduce the genetic content by half. It has been known for over a decade that female meiosis is highly prone to chromosome segregation errors, especially in humans (Hassold and Hunt, 2001; Hassold et al., 2007; Nagaoka et al., 2012). At least 10% of human pregnancies produce aneuploid embryos (presenting a gain or loss of entire chromosomes), inducing spontaneous abortions and congenital defects such as trisomies, for which incidence increases with maternal age (Nagaoka et al., 2012). In eukaryotes, the structure orchestrating chromosome alignment and segregation during cell division is the microtubule spindle. In mitotic cells, the microtubules that compose the spindle are mostly nucleated from centrosomes acting as major microtubule organizing centers (MTOCs). Canonical centrosomes are composed of a pair of centrioles surrounded by pericentriolar material (PCM) that possesses microtubule nucleation activity. The microtubule slow-growing end (minus end) is tethered to the PCM of the centrosome, whereas the fast growing (plus end) is directed toward chromosomes. At mitosis entry, centrosomes separate on opposite sides of the nuclear envelope, defining the future spindle poles and allowing bipolar spindle formation. Whereas the majority of male gametes retain centrosomes containing centrioles, in oocytes of most metazoan species, centrioles are eliminated before meiotic divisions (Szollosi et al., 1972; Manandhar et al., 2005). Thus, spindle morphogenesis and positioning are atypical in these cells. The lack of centrosomes could favor the asymmetric partitioning of the cytoplasm by reducing the distance between the pole of the spindle that is anchored to the cortex and the cell cortex. Indeed, astral microtubules, a subpopulation of microtubules connecting the spindle pole to the cortex in most mitotic cells, are absent in most oocytes because of the lack of centrioles. However, as a result of the large size of oocytes, even when centrosomes are retained, oocytes can still divide extremely asymmetrically, as in starfish. In these oocytes, centriole-containing centrosomes participate in chromosome capture once chromosomes are close enough to be reached by microtubules. Chromosome gathering is, however, achieved by a contractile actin mesh that delivers chromosomes to the spindle (Lénárt et al., 2005). Interestingly, the lack of centrioles imposes atypical modes of spindle assembly in oocytes that we are going to review in this study.

Centrosome-independent microtubule nucleation

In mitosis, the spindle is formed by microtubules that are nucleated from canonical centrosomes. Although centrosome-mediated spindle formation is dominant in most mitotic cells, mitosis can still take place in the absence of centrosomes, showing that other centrosome-independent pathways can participate in spindle formation (Khodjakov et al., 2000; Basto et al., 2006; Azimzadeh et al., 2012; Bazzi and Anderson, 2014). These centrosome-independent pathways become dominant in cells lacking centrosomes such as oocytes. Indeed, because most oocytes lack canonical centrosomes, they use alternative

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Abbreviations used: aMTOC, acentriolar microtubule organizing center; CDK1, cyclin-dependent kinase 1; CPC, chromosomal passenger complex; MTOC, microtubule organizing center; NEBD, nuclear envelope breakdown; NuMa, nuclear mitotic apparatus; PCM, pericentriolar material; SAF, spindle assembly factor; TACC, transforming acidic coiled coil.

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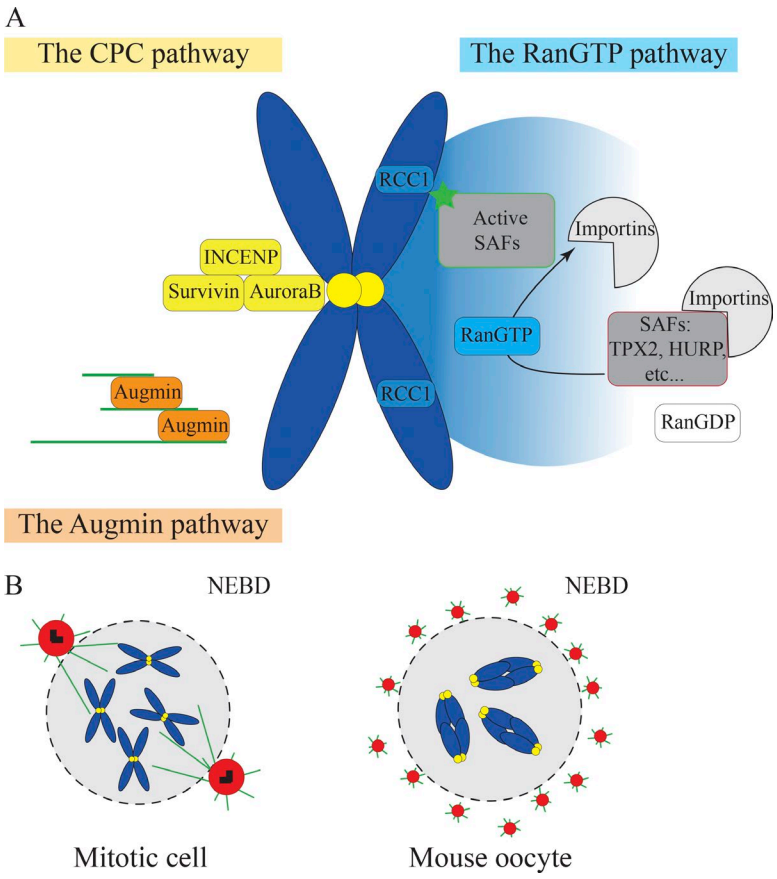

Figure 1. **Pathways replacing centrosomes for microtubule nucleation in oocytes.** (A) The three microtubule nucleation pathways: the RanGTP pathway, CPC pathway, and Augmin pathway. (B) Microtubule nucleation by centrosomes in mitotic cells (left) and by multiple aMTOCs in mouse oocytes (right). For A and B, DNA is in blue, microtubules in green, kinetochores in yellow, pericentriolar material in red, and centrioles in black.

pathways to nucleate microtubules (Fig. 1 A). Among them, the RanGTP pathway has been very well described (Fig. 1 A). The small Ran GTPase (Ras-like nuclear protein) is present in a gradient around chromosomes both in mitotic and meiotic cells. The RanGTP active form is produced by the Ran guanosine exchange factor regulator of chromosome condensation 1 (RCC1) that is localized on chromosomes (Kalab et al., 1999). This gradient locally activates spindle assembly factors (SAFs), such as, for example, targeting protein for Xklp2 (Tpx2), that participate in microtubule nucleation, interaction, and stabilization as well as motor activities (Meunier and Vernos, 2016). These SAFs interact with importins via their NLS and are kept inhibited. The RanGTP gradient is proposed to promote the dissociation of SAFs from their inhibitory binding to importins, causing their local activation and release (Gruss et al., 2001; Nachury et al., 2001). In human oocytes, RanGTP inhibition seems to delay microtubule nucleation and impair spindle formation (Holubcová et al., 2015). However, human oocytes used in this study were atretic (oocytes from patients receiving in vitro fertilization that did not spontaneously resume meiosis in response to hormonal treatment), and thus, they might not behave similarly to healthy human oocytes. Differently, inhibition of RanGTP delays but does not impair spindle assembly in mouse and *Drosophila melanogaster* oocytes (Dumont et al., 2007; Cesario and McKim, 2011). This suggests that although the RanGTP pathway is involved in microtubule nucleation for spindle assembly in the absence of centrosomes, other pathways seem important. Among these, the Augmin pathway (Fig. 1 A) generates new microtubules along preexisting microtubules (Sánchez-Huertas and Lüders, 2015). The Augmin complex is composed of eight proteins (named HAUS 1–8) able to recruit γ-tubulin to the sides of microtubules within the spindle (Goshima et al., 2008; Lawo et al., 2009; Uehara et al., 2009). In *Xenopus laevis* egg extracts, Augmin depletion results in reduced microtubule nucleation and multipolar spindle formation, suggesting a role of the Augmin complex in spindle bipolarization (Petry et al., 2011). In fruit flies, Augmin compensates for the lack of centrosomes by promoting microtubules nucleation at meiotic spindle poles (Colombié et al., 2013). Similarly, the chromosomal passenger complex (CPC) pathway (Fig. 1 A) is also involved in microtubule stabilization and spindle assembly in *Xenopus* egg extracts and *Drosophila* oocytes (Sampath et al., 2004; Kelly et al., 2007; Tseng et al., 2010; Radford et al., 2012; Das et al., 2016). The CPC is associated with kinetochores and is composed of the Aurora B/C kinase, the inner centromeric protein (INCENP), Survivin, and Borealin (Dumont and Desai, 2012). In *Caenorhabditis elegans* oocytes, Katanin increases the density of small microtubules by severing preexisting ones and could thus contribute to microtubule formation by amplifying microtubule nucleation via other pathways (Srayko et al., 2006).

In addition to these microtubule nucleation pathways, mouse oocytes contain acentriolar MTOCs (aMTOCs) capable of nucleating microtubules (Fig. 1 B). At nuclear envelope breakdown (NEBD), the nucleation capacity of these aMTOCs is low, but it increases throughout meiosis I. Indeed, levels of the RanGTPase effector TPX2 (Wittmann et al., 2000) rise progressively during meiosis I (Brunet et al., 2008), which intensifies the extent of phosphorylation of the aMTOC protein transforming acidic coiled coil 3 (TACC) and increases microtubule nucleation activity at aMTOCs (Still et al., 1999; Bayliss et al., 2003; Eyers et al., 2003; Tsai et al., 2003; Kinoshita et al., 2005; Brunet et al., 2008). These aMTOCs are perinuclear

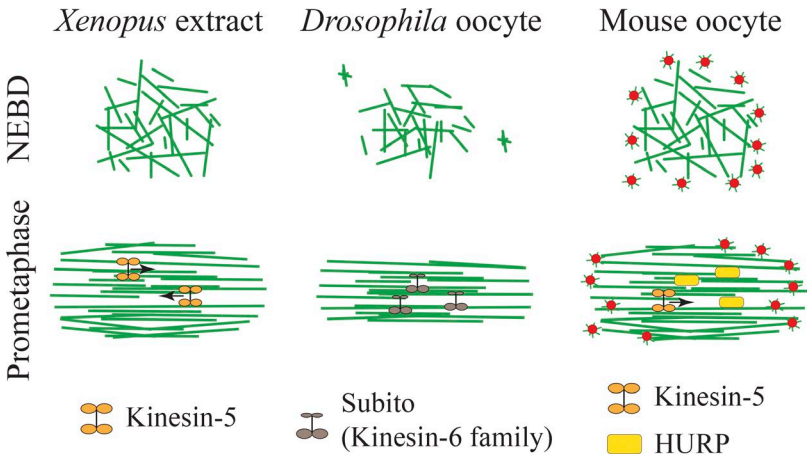

Figure 2. **Spindle bipolarization.** Organization of microtubules into a bipolar array via microtubule motors and microtubule-associated proteins in *Xenopus* egg extracts, *Drosophila*, and mouse oocytes between NEBD and prometaphase. Microtubules are in green and aMTOCs in red.

before meiotic divisions (Fig. 1 B) so that they can be readily distributed around the chromatin when NEBD occurs (Łuksza et al., 2013). Although the exact composition of these structures is not exhaustively known, they contain classical PCMs such as γ-tubulin and pericentrin and are likely bona fide PCMs (Gueth-Hallonet et al., 1993; Carabatsos et al., 2000). In mitotic cells, the PCM size is regulated by centrioles such that microtubule nucleation is carefully regulated (Kirkham et al., 2003; Conduit and Raff, 2010; Gopalakrishnan et al., 2012; Woodruff et al., 2015). In mouse oocytes, the size of the PCM seems to scale with the cell volume, but the regulatory mechanisms at play are unknown (Łuksza et al., 2013). Surprisingly, such acentriolar MTOCs are not detected on the nuclear envelope in prophase I or at later stages in spindle poles from *Xenopus*, *C. elegans*, *Drosophila*, and human atretic oocytes (Gard, 1991; Matthies et al., 1996; Srayko et al., 2006; Holubcová et al., 2015). Although all of these microtubule nucleation pathways are essential for spindle assembly in the absence of nucleation by centrosomes, little is known about their relative contribution in oocytes and how they interact.

Spindle bipolarization

Once microtubules are formed, the spindle must assemble in a bipolar fashion to accurately segregate chromosomes in two distinct groups. In mitotic cells, centrosomes are duplicated during interphase of the cell cycle, and cells enter mitosis with two centrosomes. At the onset of mitosis, the two centrosomes separate and nucleate microtubules (Fig. 1 B). Duplicated centrosomes thus form the spindle axis and promote rapid spindle bipolarization (Toso et al., 2009; Tanenbaum and Medema, 2010). In oocytes, spindle bipolarization does not rely on a bipolar axis predefined by the two separated centrosomes. Instead, spindle bipolarization is a sequential and slow process. It can take up to 12 min in *C. elegans*, 4 h in mouse, and 6.5 h in human atretic oocytes, which corresponds to around half of the transition time from NEBD to anaphase in these species (Dumont et al., 2007; Schuh and Ellenberg, 2007; Holubcová et al., 2015; Sumiyoshi et al., 2015) and 40 min in *Drosophila* oocytes (Sköld et al., 2005). In the absence of centrosomes, the establishment of a bipolar spindle depends on the sorting and stabilization of microtubules into a central array via microtubule motors and microtubule-associated proteins (Heald et al., 1996; Walczak et al., 1998). A crucial step in this process is the transformation of an unorganized ball of microtubules into a bipolar array presenting antiparallel microtubules in opposite orientations. This is achieved via the sorting and bundling of microtubules by plus end–directed microtubule motors (Fig. 2). Among them, Kinesin-5 (Eg5) was shown to be essential for the establishment and maintenance of spindle bipolarity in *Xenopus* extracts and mouse oocytes (Fig. 2) because its inhibition results in monopolar spindles (Walczak et al., 1998; Kapoor et al., 2000; Mailhes et al., 2004; Schuh and Ellenberg, 2007; Fitzharris, 2009). In *Drosophila,* the Kinesin-6 family member Subito facilitates spindle bipolarization (Fig. 2) by promoting the formation of a central microtubule array (Jang et al., 2005, 2007). In particular, CPC central spindle proteins such as INC ENP and Aurora B fail to localize to this central region in subito mutants. In mice, where oocytes assemble a meiotic spindle in the presence of multiple aMTOCs, these aMTOCs have to be properly organized to ensure correct spindle bipolarization. Before NEBD, aMTOCs are decondensed by Polo-like kinase 1 (PLK1); upon NEBD, they are spread along the nuclear envelope by a microtubule- and dynein-dependent mechanism; and after NEBD, aMTOCs are fragmented in smaller structures by Kinesin-5 (Łuksza et al., 2013; Clift and Schuh, 2015). This fragmentation process is essential for bipolar spindle formation, as a failure to fragment aMTOCs induces defects in bipolar spindle assembly and chromosome alignment. Next, concomitant to the formation of a central microtubule array, aMTOCs are progressively sorted along the central spindle into distinct poles between NEBD and 4 h after (Schuh and Ellenberg, 2007; Breuer et al., 2010). A key player in this process is the microtubule-associated protein and RanGTPase factor hepatoma up-regulated protein (HURP), which has a role very comparable to the one of Subito in *Drosophila* (Tsou et al., 2003). HURP is recruited by Kinesin-5 to the central spindle (Fig. 2) and permits aMTOCs sorting by facilitating microtubule stability in this region (Breuer et al., 2010). The stabilization of microtubules in the region of overlap of antiparallel microtubules provides tracks on which motors can bind aMTOCs as their cargo and transport them to spindle poles.

Interestingly, in human atretic oocytes in which spindle bipolarization is extremely slow, most spindles fail to maintain a bipolar shape but instead go through phases of multipolarity (Holubcová et al., 2015). Such unstable spindles are rarely observed in mitotic spindles or meiotic spindles from other species, except in oocytes from the *hurp*$^{-/-}$ strain (Breuer et al., 2010), thus raising the question of the nature of the regulatory mechanisms at play in this type of human oocyte favoring this instability.

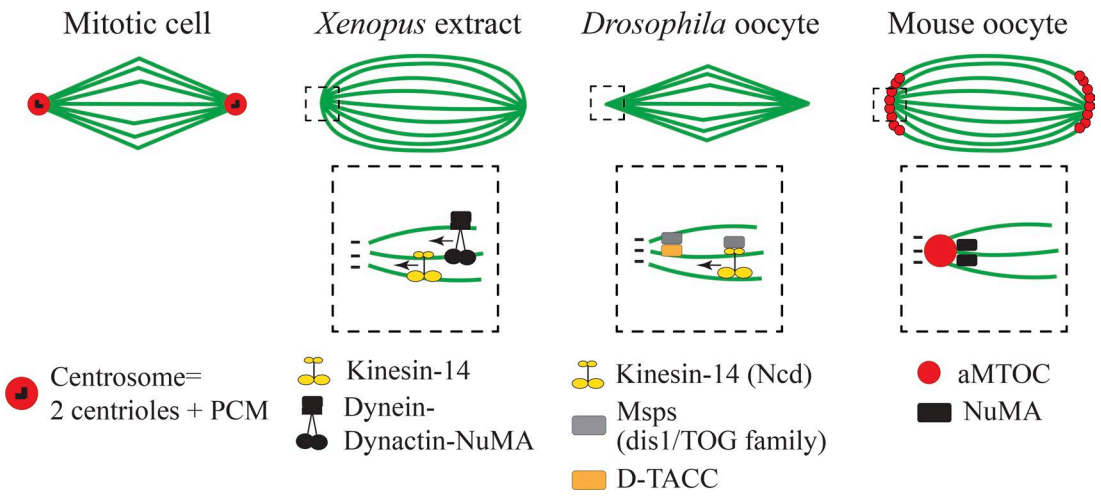

Figure 3. **Spindle pole formation and final spindle shape.** Top rows show spindle shape in metaphase in mitotic cells, *Xenopus* egg extracts, *Drosophila*, and mouse oocytes. The dashed square shows magnification of the spindle pole where microtubule motors and microtubule-associated proteins organize microtubule minus ends. Microtubules are in green.

Spindle pole formation

Spindle poles in mitosis are organized by a single centrosome (Fig. 3). Pole formation in oocytes is differentbecause it is not organized by a single entity. The formation of spindle poles, which is the region where microtubule minus ends are converging, relies on the activity of microtubule motors and microtubule-associated proteins (Fig. 3). *Drosophila* excepted, most oocytes present spindle poles that are less focused than in mitosis, having this typical barrel-shape aspect. Studies in *Xenopus* egg extracts have shown that Dynein and Kinesin-14 minus-end motors (Fig. 3) shape the poles by focusing microtubule minus ends in these regions (Heald et al., 1996; Walczak et al., 1998). In *Drosophila* oocytes, nonclaret disjunctional (Ncd; Kinesin-14) prevents pole splitting and multipolar spindle formation (Fig. 3; Endow and Komma, 1997; Sköld et al., 2005). Furthermore, Dynein in a complex with Dynactin and nuclear mitotic apparatus (NuMa) are essential to cross-link parallel microtubules (in the same orientation) and thus tether together microtubule minus ends at meiotic spindle poles in *Xenopus* egg extracts (Fig. 3; Merdes et al., 1996). Acentrosomal poles in *Drosophila* oocytes contain the microtubule-associated protein mini-spindles (Msps), which is a member of the defect in sister chromatid disjoining 1/tumor overexpression gene (dis1/TOG) family conserved in *C. elegans*, *Xenopus*, and humans. Msps is recruited to spindle poles by Kinesin-14 (Ncd) and D-TACC (Fig. 3), where it prevents loss of bipolarity possibly by stabilization of microtubules ends (Cullen and Ohkura, 2001). The *C. elegans* homologue ZYG-9 is enriched at spindle poles and required for spindle assembly (Matthews et al., 1998). Remarkably, the function of NuMa in tethering microtubule minus ends is conserved in acentriolar spindles. Indeed, the microtubule-associated protein NuMa accumulates at the poles in rabbit, human, and mouse oocytes (Yan et al., 2006; Alvarez Sedó et al., 2011; Kolano et al., 2012). In mouse oocytes, NuMa is required for the formation of barrel-shaped spindle poles as well as microtubule minus-end cohesion (Fig. 3) because its impairment causes hyperfocused poles that often lose microtubule connection (Kolano et al., 2012).

In mouse oocytes, the discrete aMTOCs organize spindle poles (Fig. 3). After their bipolar sorting, aMTOCs progressively cluster together between 4 and 7 h after NEBD and will contribute to the cohesion and integrity of spindle poles (Kolano et al., 2012). Even though not addressed so far, if the sorting of aMTOCs fails to be optimal, the number of aMTOCs at each pole might not be identical and could thus favor force imbalance within the spindle compared with mitotic spindles where the poles are formed by equivalent centrosomes. This would resemble the process of clustering of extra-centrosomes in cancer cells in which unbalanced poles favor chromosome missegregation (Kwon et al., 2008; Breuer et al., 2010). In *C. elegans*, *Drosophila*, *Xenopus*, and humans, microtubule minus ends do not seem to be anchored to discrete aMTOC entities (Fig. 3). Although they are not anchored to detectable structures, their poles are shaped by a combination of factors as described above (in this section). In addition, most meiotic spindle poles, with the exception of *Drosophila*, have a broad shape compared with the more focused mitotic spindle poles, which could be related to the lack of tight organizers, the centrosomes (Fig. 3). Thus, meiotic spindle poles could possibly be less robust than the mitotic ones that are anchored to distinct centrosomes.

Chromosome alignment

After a bipolar spindle is formed, chromosomes align in the spindle equator. In mitosis, the "search and capture" model states that microtubules growing toward the chromosomes are rapidly captured and stabilized by the kinetochores, establishing stable kinetochore–microtubule attachments (Kirschner and Mitchison, 1986; Wollman et al., 2005). In oocytes, chromosome alignment is a much slower and progressive process that depends on the interaction of microtubules with chromosome arms and kinetochores. The interaction of chromosome arms with microtubules and microtubule motors, which also exist in the short prometaphase of mitotic cells, are thought to generate forces pushing chromosomes toward the spindle equator (Mazumdar and Misteli, 2005; Cheeseman and Desai, 2008; Cai et al., 2009; Wandke et al., 2012). In *C. elegans*, the kinesin-like protein KPL-19 localizes to a nonkinetochore chromatin region where microtubules contact chromosomes and could promote the motion of chromosomes toward the equator (Wignall and Villeneuve, 2009).

An EM study has suggested that mouse oocytes establish extremely delayed kinetochore–microtubule attachments

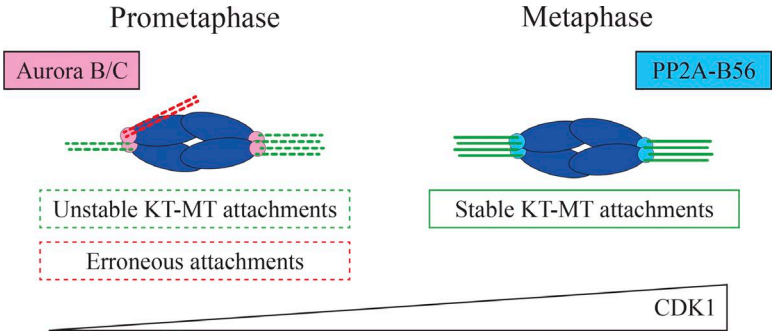

Figure 4. **Establishment of stable kinetochore–microtubule attachments in mouse oocytes.** Mouse oocytes form stable kinetochore–microtubule (KT-MT) attachments only at late metaphase I. Aurora B/C phosphorylation destabilizes kinetochore–microtubule attachments, whereas PP2A-B56 dephosphorylation activity stabilizes the attachments. This process is regulated by a progressive increase in CDK1 activity. DNA is in blue.

(K-fibers), 1 to 2 h before anaphase (Brunet et al., 1999). However, even though stable K-fibers appear to be formed late in mouse oocytes, this does not exclude the possibility that microtubules could establish earlier contacts with kinetochores. Indeed, kinetochore–microtubule attachments are observed after calcium or cold treatment 3 to 4 h before anaphase (Lane et al., 2012). Yet K-fiber stability varies until late metaphase I (Fig. 4). A study with high-resolution live microscopy revealed that almost all kinetochores undergo multiple steps of error correction before engaging into stable bipolar attachments (Kitajima et al., 2011). Thus, K-fibers may not have been well preserved during EM fixation procedures and failed to be detected at earlier stages (Brunet et al., 1999). It may be interesting to reanalyze in more detail the timing of apparition of K-fibers by EM. The delay in K-fiber formation depends on cyclin-dependent kinase 1 (CDK1) activity (Fig. 4), which increases very gradually throughout meiosis I (Davydenko et al., 2013). A precocious increase in CDK1 activity leads to premature stable kinetochore–microtubule attachments and lagging chromosomes at anaphase. Aurora B/C phosphorylation activity destabilizes the attachments, whereas protein phosphatase 2A-B56 (PP2A-B56), recruited at kinetochores by an increase in CDK1 activity, stabilizes kinetochore–microtubule attachments (Fig. 4; Yoshida et al., 2015). Using a genetic approach, it has been shown in mouse oocytes that Aurora C corrects erroneous kinetochore attachments (Balboula and Schindler, 2014). In addition, kinetochore microtubule stability is regulated by their position within the spindle, as they can undergo Aurora A–dependent destabilization near spindle poles (Chmátal et al., 2015). It is thought that a delay in K-fiber formation would prevent the stabilization of erroneous attachments before bipolar spindle formation, a very slow and unsteady process in meiosis I.

A recent study has shown that stable K-fibers formation is also slow in *Drosophila* oocytes (Głuszek et al., 2015) but depends on an alternative mechanism. The catastrophe-promoting complex Sentin–EB1 (end-binding protein 1) is responsible for delaying stable K-fiber attachments by regulating microtubule end dynamics. Mutant oocytes for *sentin* present more stable K-fibers early on in meiosis I, which is deleterious for bivalent segregation. Thus, one could speculate that slow K-fiber formation might be beneficial in the context of spindles organized from multiple aMTOCs or from chromosomes that might produce more merotelic attachments than spindles organized from centrosomes (when one kinetochore is attached to the two spindle poles).

Chromosome segregation

Once chromosomes are aligned on the spindle equator, pulling by K-fibers drives chromosome separation. In mitotic cells, chromosome separation is driven first by shortening of the kinetochore–microtubule attachments (anaphase A) and then by spindle elongation (anaphase B). In mouse oocytes, the opposite happens: first, the spindle elongates by a Kinesin-5–dependent mechanism, and then kinetochore–microtubule attachments shorten (FitzHarris, 2012). Interestingly, in nematodes, K-fibers align chromosomes but are not required for chromosome separation at anaphase (Dumont et al., 2010). Instead, it is proposed that microtubule assembly between chromosomes promotes their separation. This is consistent with the fact that spindle poles almost completely disappear at anaphase in this species. In addition, *C. elegans* chromosomes are holocentric presenting kinetochores ensheathing the entire chromosome length (Oegema et al., 2001). Although the presence of holocentric chromosomes could favor microtubule nucleation between chromosomes at anaphase, it could also promote the formation of merotelic attachments. Whether this kinetochore-independent separation mechanism is conserved in mammalian oocytes is still unknown, even though spindles lacking K-fibers are still able to undergo anaphase in mouse oocytes (Deng et al., 2009).

In mitosis, sister kinetochores are attached to opposite poles before segregation (bi-oriented), and cohesins (protein complexes holding the sister chromatid together) are cleaved at anaphase, leading to separation. In meiosis, sister kinetochores are attached to the same pole (mono-orientation), whereas homologous chromosomes are attached to opposite poles (Watanabe, 2012). At anaphase I, the meiotic-specific cohesin Rec8 is protected from cleavage at centromeres, permitting the separation of homologous chromosomes but not the separation of sister chromatids. Loss of cohesion is a leading cause of age-associated chromosome segregation errors (Chiang et al., 2010; Lister et al., 2010). The recently discovered kinetochore factor meiotic kinetochore factor (MEIKIN) is conserved from yeast to humans and required for both mono-orientation and cohesion protection (Kim et al., 2015). This suggests that MEIKIN could be a novel candidate implicated in age-associated chromosome segregation errors.

Why lose centrioles?

Lack of centrioles in oocytes imposes atypical modes of spindle formation that might contribute to the inherent high rate of chromosome segregation errors observed in meiosis. A puzzling observation is that whereas centrioles and PCMs are lost in oocytes of most metazoan species, mouse oocytes still retain multiple discrete PCMs or aMTOCs that can participate in bipolar spindle formation. In contrast to most species, sperm centrioles degenerate in rodents during spermatogenesis and thus are not contributed by the sperm at fertilization (Woolley and Fawcett, 1973; Manandhar et al., 1998). Instead, centrioles progressively

assemble de novo in early embryos (Gueth-Hallonet et al., 1993). How centrioles are generated in rodent early embryos is not known. Nevertheless, these discrete PCMs could serve as templates for a later generation of centriole-containing centrosomes in the embryo.

Whether they possess discrete PCM foci at their poles or not, oocyte meiotic spindles appear to be fragile with steps of assembly that are slow and even unstable, as in humans. In addition, their shapes are often peculiar. Female meiotic spindles of many species are small and do not closely scale to cell size unlike mitotic spindles (Crowder et al., 2015). In mouse after fertilization and until centrioles assemble de novo at the 64-cell stage (Gueth-Hallonet et al., 1993), the spindle transitions from a meiotic shape to a mitotic one: the aMTOCs number sequentially decreases, poles become more focused, and the length of the spindle scales with the size of the cell (Courtois et al., 2012). This raises the question of the contribution of the centrosome in spindle size scaling. Furthermore, the large size of oocytes could dilute some components required for spindle morphogenesis and thus contribute to the fact that spindle size does not strictly correlate with cell size.

Still, very little is known about why and how centrioles are eliminated in oocytes of most species. One hypothesis is that centriole elimination prevents multipolar spindle formation in the first embryonic division after introduction of the sperm centrioles upon fertilization. However, in rodents, the sperm does not contribute with a centriole. Another hypothesis would be that it prevents parthenogenesis (egg activation in the absence of fertilization) because injection of centrosomes in *Xenopus* eggs induces activation without fertilization (Tournier et al., 1989). Recent studies have started to unravel how centrioles are removed in oocytes. In starfish, meiotic divisions take place in the presence of centriole-containing centrosomes. Mother centrioles are eliminated by extrusion into polar bodies, and the remaining daughter centriole is degraded in the cytoplasm (Borrego-Pinto et al., 2016). In the fruit fly, centriole elimination is a progressive process that ends up just before meiotic spindle assembly. It is dependent on PLK1 because its loss triggers PCM down-regulation, which leads to centriole removal. Centriole maintenance by perturbing this process results in spindle assembly defects in oocytes and early embryos and thus to female sterility (Pimenta-Marques et al., 2016). The absence of canonical centrosomes constitutes one of the many factors that could contribute to the innate susceptibility of oocyte to produce errors in chromosome segregation. However, despite its contribution to oocyte aneuploidy, centriole elimination must likely be crucial for gamete fitness of most metazoan species.

Conclusion

Recent advances have shed light on the mechanisms of spindle assembly in both mitosis and meiosis. It appears that oocytes use the same nucleation pathways as mitotic cells, namely the RanGTP, Augmin, and CPC pathways, with the exception that they are dominant in this study, in the absence of a centrosome pathway. Although they share common pathways, meiotic spindles are not just mitotic spindles without centrosomes, and these pathways are likely regulated in a meiosis-specific manner. Yet one can speculate that in the absence of centrosomes, the initial conditions might be key parameters influencing the entire process of spindle assembly with consequences on chromosome segregation. Oocytes have to face circumstances in which the critical mass of microtubules to capture and gather chromosomes could be limiting early on when they are polymerized only locally around chromosomes. This effect could be amplified by the fact that oocytes present huge nuclei (30 µm wide in the mouse and up to 450 µm in *Xenopus*), such that the volume at which the spindle starts assembling is gigantic compared with one of mitotic cells. It might be so that the critical concentration for tubulin to polymerize might be much more difficult to reach than in somatic cells when the nucleus breaks down, reinforcing the importance of pathways acting as catalyzers/amplifiers of tubulin polymerization locally around chromosomes. How these pathways, and yet-to-be-discovered ones, interact to promote early stages of spindle assembly has not been thoroughly addressed and remains an important question for future studies.

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