Review

Mechanisms of kinetochore-microtubule attachment errors in mammalian oocytes

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Proper kinetochore-microtubule attachment is essential for correct chromosome segregation. Therefore, cells normally possess multiple mechanisms for the prevention of errors in kinetochore-microtubule attachments and for selective stabilization of correct attachments. However, the oocyte, a cell that produces an egg through meiosis, exhibits a high frequency of errors in kinetochore-microtubule attachments. These attachment errors predispose oocytes to chromosome segregation errors, resulting in aneuploidy in eggs. This review aims to provide possible explanations for the error-prone nature of oocytes by examining key differences among other cell types in the mechanisms for the establishment of kinetochore-microtubule attachments.

Key words: aneuploidy, chromosome segregation, kinetochore, microtubule, oocyte.

Introduction

Correct chromosome segregation during meiosis is essential for accurate inheritance of the parental genome by the next generation. In females, oocytes undergo meiosis during which chromosomes are segregated into the egg. Chromosome segregation errors during meiosis in oocytes produce aneuploid eggs, and fertilization of the aneuploid egg with a sperm results in severely compromised embryonic development. In humans, chromosome segregation errors during the first meiotic division (meiosis I) in oocytes are a leading cause of pregnancy loss and congenital disease such as Down syndrome (trisomy 21) (Hassold & Sherman 2000; Chiang et al. 2012; Nagaoka et al. 2012; Jones & Lane 2013). The frequency of chromosome segregation errors during meiosis I in oocytes is extremely high; studies using comparative genomic hybridization (CGH) array analysis with oocytes donated by patients under fertility treatment suggest that the error rate of meiosis I exceeds 40%, depending on maternal age (Fragouli et al. 2011; Gabriel et al. 2011; Geraedts et al. 2011; Handyside et al. 2012).

Why is chromosome segregation so error prone in human oocytes? To understand the error-prone nature of oocytes, comparisons between oocytes and other cell types using mouse oocytes and cultured somatic cells as models could be instructive. Oocytes are highly specialized cells and provide a cellular basis to accomplish meiosis, fertilization and early embryogenesis, which are key events for reproduction and development. These specialized functions are likely accomplished by a set of oocyte-specific factors, which could be cell-intrinsic or -extrinsic. Given that the error-prone nature of oocytes could be explained by factors that are differently regulated in other cell types, it is possible that oocyte-specific features for their specialized functions are linked to the error-prone nature of oocytes. This review describes differences in the processes for the establishment of correct kinetochore-microtubule attachments, a prerequisite for correct chromosome segregation, between mitosis in somatic cells and meiosis I in mammalian oocytes. I introduce current ideas regarding how these differences could explain frequent errors in kinetochore-microtubule attachments in mammalian oocytes and discuss the possibility that these differences are attributable to oocyte-intrinsic factors or features. This review does not examine age-related defects that predispose oocytes to chromosome segregation errors,
but interested readers are referred to several excellent reviews (Chiang et al. 2012; Nagaoka et al. 2012; Jones & Lane 2013; Webster & Schuh 2017).

**Overall chromosome dynamics during meiosis I in mouse oocytes**

In mammals, oocytes undergo premeiotic S-phase, followed by meiotic prophase, including the leptotene, zygotene and pachytene stages, before arresting at the dictyate stage in fetal ovaries. This cell cycle arrest at meiotic prophase is maintained for a long period including the oocyte growth phase in which the oocyte grows into an extraordinarily large cell (approximately 80 µm in diameter in mouse oocytes and approximately 130 µm in human oocytes), and the arrest is released shortly before ovulation in adult stages (Chiang et al. 2012; Nagaoka et al. 2012; Jones & Lane 2013; Webster & Schuh 2017). Fully grown oocytes have a characteristic nucleus called the germinal vesicle (GV) in which chromosomes are compacted around the nucleolus and nuclear membrane. These GV-stage oocytes are competent for M-phase entry; they enter M-phase around the time of ovulation or when they are experimentally isolated and cultured in vitro (Jaffe & Norris 2010).

Upon entry into M-phase of meiosis I, which is accompanied by nuclear envelope breakdown (NEBD; also called GV breakdown, GVBD), chromosomes are condensed and microtubule polymerization is activated (Dumont et al. 2007; Schuh & Ellenberg 2007) (Fig. 1). Polymerized microtubules initially self-assemble an apolar spindle and relocate chromosomes to the surface of the ball-like spindle. The spindle gradually establishes bipolarity, pushing the minus-ends of antiparallel microtubules into opposite directions (Schuh & Ellenberg 2007; Breuer et al. 2010). As the spindle bipolarizes, the chromosomes move toward the spindle equator through the spindle surface, resulting in a characteristic chromosome arrangement called the prometaphase belt (Kitajima et al. 2011). The microtubules then stretch the chromosomes, orienting homologous kinetochores into opposite spindle poles. Stably stretched chromosomes align at the spindle equator, resulting in a chromosome arrangement called the metaphase plate. Finally, homologous chromosomes are separated and segregated toward opposite spindle poles, allocating one set of chromosomes into an egg and the other into a polar body (Fig. 1). The egg is arrested at metaphase of meiosis II, a stage for sperm entry.

**Gradual spindle assembly without centrosomes**

One essential process for correct chromosome segregation in both mitosis and meiosis is the formation of a bipolar spindle. In mitosis of somatic cells, two centrosomes containing centrioles serve as locations where a majority of spindle microtubules are polymerized (Prosser & Pelletier 2017) and thus, immediately determine two spindle poles after entry to M-phase. This process provides a spatial organization in which microtubules can attach chromosomes to opposite spindle poles, which is essential for equal chromosome segregation into two daughter cells.

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Fig. 1. Acentrosomal spindle assembly in oocytes. Multiple microtubule-organizing center (MTOC) clusters are located around the nucleus and in the cytoplasm in germinal vesicle (GV)-stage oocytes. Upon nuclear envelope breakdown (NEBD), the MTOCs are fragmented and distributed around chromosomes, forming an apolar spindle. The spindle gradually sort anti-parallel microtubules and push their minus-ends into opposite poles, which is accompanied with MTOC regrouping into the two poles and chromosome congression into the spindle equator. The spindle microtubules then stretch chromosomes by pulling their homologous kinetochores toward opposite spindle poles. The chromosomes are eventually segregated into an egg and a polar body.
In oocytes, the process of bipolar spindle formation is unique. Mammalian oocytes have no centrioles (Szollosi et al. 1972) and thus, have no centrosomes that predetermine two spindle poles. In mouse oocytes, multiple clusters of microtubule-organizing centers (MTOCs) are distributed around the nucleus and in the cytoplasm before entry into M-phase (Calarco 2000; Schuh & Ellenberg 2007; Clift & Schuh 2015) (Fig. 1). Upon entry into M-phase, the MTOCs are fragmented into small pieces and randomly distributed around chromosomes (Clift & Schuh 2015). A majority of microtubules are polymerized at these MTOCs, depending on the activity of RanGTP, and they initially self-assemble an apolar spindle (Schuh & Ellenberg 2007). The fragmented MTOCs are gradually regrouped and segregated toward two spindle poles, depending on the activity of Kif11 (an orthologue of Xenopus Eg5) (Breuer et al. 2010; Clift & Schuh 2015), a kinesin that crosslinks anti-parallel microtubules and pushes their minus-ends into opposite directions (Kapitein et al. 2005). The segregated MTOCs are distributed around the spindle poles, which are broader than those defined by point centrosomes in mitosis (Breuer et al. 2010; Clift & Schuh 2015). This gradual spindle bipolarization needs a lengthy period of 3–5 h in mouse oocytes in an in vitro culture (Dumont et al. 2007; Schuh & Ellenberg 2007; Breuer et al. 2010; Clift & Schuh 2015).

In human oocytes, the bipolar spindle assembly requires an even longer period (approximately 16 h) (Holubcová et al. 2015). A key difference between human and mouse oocytes is the absence of MTOC clusters in human oocytes. Live imaging experiments using human oocytes that could not be used for in vitro fertilization revealed that a majority of microtubules are polymerized around chromosomes, which depends on chromosome-mediated activation of RanGTP (Holubcová et al. 2015). The polymerized microtubules self-assemble initially an apolar spindle, which is slowly converted into a bipolar spindle. The transition from an apolar to a bipolar state exhibits unstable bipolarity in the spindle. A bipolar spindle frequently fails to maintain its bipolarity and becomes apolar or multipolar. This spindle instability in human oocytes, which is more pronounced than that observed in mouse oocytes, may be attributable to the absence of MTOC clusters (Holubcová et al. 2015).

The microtubule organization in an apolar spindle is not suited for the formation of proper kinetochore-microtubule attachments because kinetochores are at risk for microtubule attachments in random directions (Kitajima et al. 2011; Davydenko et al. 2013). Assuming that a fraction of kinetochore-microtubule attachments form in the apolar spindle and could fail to be removed through spindle bipolarization, such attachments would result in merotelic attachment in which a single kinetochore is erroneously attached by microtubules to opposite spindle poles (Holubcová et al. 2015; Webster & Schuh 2017). Thus, the gradual assembly of the acentrosomal spindle provides an explanation for the higher frequency of errors in kinetochore-microtubule attachments at the early stages of M-phase in oocytes than that in centrosomal somatic cells and spermatocytes.

**Less focused spindle poles**

The acentrosomal bipolar spindle in oocytes exhibits a barrel-like shape, which is different not only from centrosomal spindles in somatic cells but also from acentrosomal spindles in mouse early embryos. The barrel-shaped acentrosomal spindle in mouse oocytes has a broad distribution of MTOCs around the spindle poles (Fig. 1), whereas acentrosomal spindles in embryos at the 8-cell stage exhibit well-focused spindle poles with a confined distribution of MTOCs (Courtois et al. 2012). Thus, the absence of centrosomes alone does not account for the less focused spindle poles in oocytes. The difference in the spindle poles between oocytes and 8-cell-stage embryos is at least partly attributed to a difference in cytoplasmic size. Every cell division during early embryogenesis reduces cytoplasmic size by approximately half, which is accompanied by a reduction in spindle volume and focusing of spindle poles (Courtois et al. 2012). If half the cytoplasmic volume is artificially removed from oocytes, the oocyte assembles a smaller spindle with a restricted distribution of MTOCs at the poles (Kyogoku & Kitajima 2017). Conversely, if the cytoplasmic volume is increased through cell fusion with an enucleated oocyte, the fused oocyte exhibits a larger spindle with a broader distribution of MTOCs at the poles. The spindle with broad MTOC distributions at the poles is prone to chromosome misalignment, likely through improper attachments of kinetochores with microtubules emanating from the broadly distributed MTOCs within one pole (Kyogoku & Kitajima 2017). These data suggest that the large cytoplasm, a unique feature of fully grown oocytes, is limiting for the spatial focusing of spindle poles and for the efficiency of chromosome alignment, a prerequisite for correct chromosome segregation.

**Chromosome cohesion**

Chromosome cohesion is essential for accomplishing correct chromosome segregation in both mitosis and meiosis (Nasmyth & Haering 2009). Cohesion between
sister chromatids, which contain DNAs duplicated during S-phase, is mediated by four-subunit protein complexes called cohesins (Fig. 2). In somatic cells, a cohesin complex is composed of structural maintenance of chromosomes (SMC) proteins Smc1\(^{a}\) and Smc3, the kleisin subunit Rad21, and either STAG1/SA1 or STAG2/SA2 (Losada et al. 1998, 2000; Waizenegger et al. 2000). In mitosis of somatic cells, sister chromatids are segregated into daughter cells. This process requires the establishment of sister kinetochore biorientation in which sister kinetochores are stably attached by microtubules to opposite spindle poles before entry into anaphase onset (Lampson & Cheeseman 2011; Sarangapani & Asbury 2014). The stabilization of kinetochore-microtubule attachment requires tension across paired kinetochores (Nicklas & Koch 1969; King & Nicklas 2000). Cohesin-mediated cohesion between sister chromatids enables generation of tension across sister kinetochores by providing a resistant force against the microtubule-mediated forces that pull sister kinetochores toward opposite spindle poles. Improper attachments that fail to pull sister kinetochores into opposite spindle poles lack tension, and such tension-less attachments are unstable and subsequently removed for error correction (Lampson & Cheeseman 2011; Sarangapani & Asbury 2014). Thus, cohesin is required for error correction and stabilization of proper kinetochore-microtubule attachments.

**Meiotic chromosome cohesion**

In meiotic cells, meiosis-specific cohesin subunits, such as the kleisin subunits Rec8 and Rad21L, Smc1\(^{b}\), and STAG3/SA3, are expressed and form meiotic cohesin complexes (Prieto et al. 2001; Revenkova et al. 2001; Lee et al. 2003; Ishiguro et al. 2011; Lee & Hirano 2011). Rec8-containing meiotic cohesins are responsible for the maintenance of cohesion between sister chromatids that are generated during premeiotic S-phase (Tachibana-Konwalski et al. 2010). In addition, Rec8-cohesins also support chiasmata, physical links generated by meiotic recombination between homologous chromosomes, through mediating sister chromatid cohesion along chromosome arms (Fig. 2). Thus, Rec8-cohesin supports the meiosis-specific structure of chromosomes in which both homologous chromosomes and sister chromatids are held together.

In contrast to mitosis in which sister chromatids are segregated, during meiosis I, homologous chromosomes are segregated. To accomplish this meiosis I-specific pattern of chromosome segregation, homologous kinetochores but not sister kinetochores must be pulled by spindle microtubules into opposite spindle poles prior to the onset of anaphase (Watanabe 2012). As in mitosis, the stabilization of kinetochore-microtubule attachments requires tension. Supported by Rec8-cohesins along chromosome arms, chiasmata enable the generation of tension across homologous kinetochores when they are correctly pulled by microtubules into opposite spindle poles. Correct attachments are therefore stabilized, whereas improper attachments, which fail to generate tension, are unstable and are thus corrected. Thus, Rec8-containing cohesin is essential for the establishment of correct kinetochore-microtubule attachments that enable meiotic chromosome segregation. Moreover, Rec8-containing cohesin at kinetochores may mediate the establishment of the meiotic kinetochore geometry that facilitates correct microtubule attachments, as discussed later.

**Tension-sensing mechanisms in mitosis**

How do kinetochores sense tension? It is likely that multiple tension-sensing mechanisms function in
mitosis. Thus far, several lines of experimental evidence have been provided for at least three proposed models – the spatial position model, spatial separation model and kinetochore-intrinsic model (Lampson & Cheeseman 2011; Sarangapani & Asbury 2014; Monda & Cheeseman 2015).

First, the spatial position model suggests that tensionless kinetochore-microtubule attachments are destabilized when they are located in the vicinity of spindle poles (Monda & Cheeseman 2015). Because tensionless attachments would apply imbalanced spindle forces on kinetochores, the kinetochores would move toward one spindle pole. The Aurora A kinase, which is enriched at the spindle pole, generates a spatial gradient of its kinase activity around the pole and preferentially phosphorylates the substrates on kinetochores located in the vicinity of spindle poles (Fig. 3A) (Anna et al. 2015; Chmáčal et al. 2015). Aurora A phosphorylates kinetochore proteins that anchor microtubules, such as the subunits of the Ndc80 kinetochore complex (Anna et al. 2015), which exhibits reduced affinity for microtubules when phosphorylated (Cheeseman et al. 2006; DeLuca et al. 2006). Thus, this mechanism can preferentially destabilize kinetochore-microtubule attachments that fail to generate balanced tension using a spatial cue within the spindle.

Second, the spatial separation model is based on the fact that Aurora B kinase, which partly shares kinetochore substrates with Aurora A and destabilizes kinetochore-microtubule attachment, is located at a...
centromeric domain between sister kinetochores (Tanaka et al. 2002; Andrews et al. 2004; Cimini et al. 2006). This domain, called the inner centromere, is spatially separated from kinetochore-microtubule attachment sites when sister kinetochores are correctly pulled toward opposite spindle poles. This spatial separation allows kinetochore substrates to avoid Aurora B-dependent phosphorylation (Liu et al. 2009; Welburn et al. 2009), leading to the stabilization of kinetochore-microtubule attachment (Fig. 3A). Thus, the spatial separation mechanism can selectively stabilize correct kinetochore-microtubule attachments by sensing tension-dependent changes in the spatial organization of sister kinetochores.

Last, the kinetochore-intrinsic model assumes that kinetochores have an intrinsic capacity to stabilize microtubule attachment when mechanical tension is applied (Sarangapani & Asbury 2014). In support of this model, purified kinetochores, which do not contain Aurora kinases, exhibit the capacity to stabilize microtubule attachment in response to mechanical tension (Akiyoshi et al. 2010). This capacity depends on the XMAP215 family member Stu2, which binds to the Ndc80 kinetochore complex, in budding yeast (Miller et al. 2016). How Stu2 strengthens tension-bearing kinetochore-microtubule attachments remains to be elucidated. Although kinetochore-intrinsic mechanisms for sensing tension are far from understood, it is likely that they contribute to the stabilization of correct kinetochore-microtubule attachments.

In summary, in mitosis, the establishment of correct kinetochore-microtubule attachment can be ensured by at least three different mechanisms. Importantly, however, the relative physiological importance of these different mechanisms remains unclear and may differ among different cell types and different stages within M-phase.

**Unique geometry of meiotic kinetochores**

Meiotic kinetochores have unique geometry to accomplish homologous chromosome segregation, a pattern specific for meiosis I (Watanabe 2012). In this meiotic geometry, sister kinetochores are spatially associated with each other, which presumably facilitates the orientation of microtubule-attachment surfaces of the sister kinetochores toward the same direction (Fig. 2). This sister kinetochore co-orientation would help microtubules attach the sister kinetochores to a same spindle pole (monopolar attachment). The monopolar attachment of sister kinetochores is needed to pull homologous kinetochores into opposite spindle poles, which is a prerequisite for correct homologous chromosome segregation. A protein required for the maintenance of sister kinetochore association in mammals is MEIKIN (Kim et al. 2015), which is specifically expressed in meiotic cells and localizes to kinetochores through binding to the constitutive kinetochore component CENP-C. MEIKIN recruits Polo-like kinase Plk1 to kinetochores, another factor needed for the maintenance of the sister kinetochore co-orientation (Kim et al. 2015). Although how MEIKIN and Plk1 maintain the sister kinetochore association is unclear, it is possible that they achieve this goal through regulating cohesion between sister kinetochores mediated by Rec8-cohesin. In mouse oocytes, artificial cleavage of Rec8 at kinetochores results in a loss of sister kinetochore association (Tachibana-Konwalski et al. 2013). In fission yeast, Rec8-cohesin mediates the cohesion between sister kinetochores, which is established specifically in meiotic cells (Sakuno et al. 2009). The maintenance of sister kinetochore cohesion depends on Moa1 and Plo1, the functional homologues of MEIKIN and Polo-like kinase, respectively (Yokobayashi & Watanabe 2005; Sakuno et al. 2009). In budding yeast, the monopolar complex is needed for monopolar attachment of sister kinetochores (Tóth et al. 2000). Structural studies suggest that monopolar crosslinks sister kinetochores to form meiosis I-specific kinetochore geometry (Corbett et al. 2010). Thus, the unique geometry of meiotic kinetochores is established by factors that physically mediate sister kinetochore association.

**Effect of meiotic kinetochore geometry on spatial separation-based tension-sensing mechanisms**

The unique geometry of meiotic kinetochores is essential for accomplishing the meiosis I-specific pattern of chromosome segregation but may influence the tension-sensing mechanisms for error correction and selective stabilization of kinetochore-microtubule attachments. In particular, among the three tension-sensing mechanisms in mitosis described above, the spatial separation model relies on the mitotic geometry of sister kinetochores (Fig. 3A). Nevertheless, a spatial separation mechanism similar to that in mitotic kinetochores may work on meiotic kinetochores (Watanabe 2012). In support of this idea, in fission yeast meiosis I, the inner centromere at which Aurora kinase is located appears to be spatially separated from kinetochore-microtubule attachment sites when homologous kinetochores are pulled into opposite spindle poles (Sakuno et al. 2011). However, in mouse oocytes, Aurora B and Aurora C (a parologue expressed in meiotic cells) remain in close proximity to kinetochore-microtubule attachment sites even after homologous kinetochores are
pulled into opposite spindle poles (Fig. 3B) (Yoshida et al. 2015). Acute inhibition of Aurora B and C on the stretched chromosomes increases stable correct kinetochore-microtubule attachments and decreases incorrect attachments (Yoshida et al. 2015). These results suggest that the activity of Aurora B and C lowers the stability of correct kinetochore-microtubule attachments and allows the formation of incorrect attachments on stretched chromosomes. Thus, it is likely that the contribution of the spatial separation mechanism for the stabilization of microtubule attachments on meiotic kinetochores in mammalian oocytes is low compared with that on mitotic kinetochores in somatic cells. This hypothesis explains why meiotic kinetochores in oocytes exhibit more frequent attachment errors than do mitotic kinetochores in somatic cells.

Spindle pole-based mechanisms correct kinetochore-microtubule attachments during prometaphase

Given that the contribution of the spatial separation mechanism on meiotic kinetochores to the stabilization of microtubule attachments is small in oocytes, what are the principal mechanisms? As in mitosis, several lines of evidence suggest that spindle pole-based mechanisms destabilize kinetochore-microtubule attachments during meiosis I in acentrosomal oocytes. High-resolution live imaging studies indicate that during prometaphase I, chromosomes frequently exhibit vigorous movements between spindle poles, likely due to imbalanced spindle forces applied to kinetochores through improper kinetochore-microtubule attachments (Kitajima et al. 2011). These attachments are destabilized as kinetochores approach the spindle poles during meiosis I in acentrosomal oocytes (Chmáatal et al. 2015). Accordingly, the vast majority of chromosomes can stay no longer than 30 min within 4 μm of the spindle poles (Kitajima et al. 2011; Chmáatal et al. 2015; Kyogoku & Kitajima 2017). This removal of attachments near the spindle pole would allow the chromosome to reorient and move toward the spindle equator, which would promote subsequent attempts for the formation of correct kinetochore-microtubule attachments (Chmáatal et al. 2015).

A mechanism for coordinated stabilization of kinetochore-microtubule attachments in oocytes

A marked difference between mitosis in somatic cells and meiosis I in oocytes is temporal changes in the stability of kinetochore-microtubule attachments through prometaphase and metaphase. In mitosis, the stability of kinetochore-microtubule attachments is thought to sharply increase when sister kinetochores establish biorientation (Lampson & Cheeseman 2011; Godek et al. 2015). In contrast, during meiosis I in oocytes, the stability of kinetochore-microtubule attachments gradually increases during prometaphase and metaphase (Brunt et al. 1999; Kitajima et al. 2011; Gui & Homer 2012; Lane et al. 2012; Davydenko et al. 2013), which takes approximately 6–12 h in mice and 15–20 h in humans. This gradual increase in the stability of kinetochore-microtubule attachments is controlled by temporal changes in Cdk1 activity (Davydenko et al. 2013). In contrast to mitosis in which Cdk1 activity reaches maximum immediately after entry to M-phase, in meiosis I, the Cdk1 activity of oocytes gradually increases during prometaphase and metaphase (Choi et al. 1991; Gavin et al. 1994; Polanski et al. 1998; Davydenko et al. 2013). Cdk1 phosphorylates the kinetochore component BubR1 (Elowe et al. 2010; Kruse et al. 2013), which binds and recruits the phosphatase PP2A-B56 to kinetochores upon phosphorylation (Huang et al. 2008; Elowe et al. 2010; Suijkerbuijk et al. 2012; Kruse et al. 2013; Yoshida et al. 2015). PP2A-B56 antagonizes the attachment destabilizers Aurora B and Aurora C on kinetochores (Foley et al. 2011; Suijkerbuijk et al. 2012; Yoshida et al. 2015). Thus, the gradual increase in Cdk1 activity results in a gradual stabilization of kinetochore-microtubule attachments during prometaphase and metaphase in oocytes (Fig. 3B). This pathway likely affects the stability of the attachments in a coordinated fashion among all chromosomes. Such coordinated stabilization of kinetochore-microtubule attachments is also observed in mitosis in somatic cells (Godek et al. 2015). In mitosis, however, coordinated stabilization sharply occurs at the transition of prometaphase to metaphase, depending on a progressive reduction in Cdk1 activity through Cyclin A degradation during prometaphase (Kabeche & Compston 2013). This process is in striking contrast to the gradual coordinated stabilization by the progressive increase in Cdk1 activity in meiosis I of oocytes.

Gradual increase in the stability of kinetochore-microtubule attachments allows temporal coordination with the progression of acentrosomal spindle assembly

Kinetochore-microtubule attachments are sharply stabilized in somatic cells where bipolar spindle formation is rapid. On the other hand, attachments are gradually stabilized in oocytes where bipolar spindle formation is...
slow. Thus, mitosis of somatic cells and meiosis I of oocytes are similar in that the timing of attachment stabilization matches the timing of bipolar spindle formation. It is possible that this similarity reflects the importance of temporal coordination of attachment stabilization with bipolar spindle formation (Fig. 3). In oocytes, at early prometaphase I, when the acentrosomal spindle has not yet established a bipolarized state, the activity of the Cdk1-BubR1-PP2A-B56 pathway is relatively low, and thus, Aurora B/C is allowed to phosphorylate kinetochore substrates (Fig. 3B). This high phosphorylation state prevents kinetochores from precocious stabilization of microtubule attachments in random directions in an apolar spindle. From late prometaphase I to early metaphase I, when the spindle bipolarizes, the activity of Cdk1-BubR1-PP2A-B56 is balanced with the activity of Aurora B/C at kinetochores (Fig. 3B). This balance would moderate the phosphorylation levels of Aurora B/C substrates at kinetochores, which would allow the formation of kinetochore-microtubule attachments with a relatively low stability. If kinetochores are moved toward spindle poles by imbalanced forces through these attachments, the attachments would be efficiently removed by the activity of Aurora A enriched around chromosome poles, allowing chromosomes to reorient and move toward the spindle equator. At late metaphase I, when the oocyte has a fully bipolarized spindle and fully stretched chromosomes, maximal activity of Cdk1-BubR1-PP2A-B56 allows the dephosphorylation of the kinetochore substrates (Fig. 3B), and thus, supports the formation of stable end-on kinetochore-microtubule attachments that are needed for homologous chromosome segregation. Thus, the gradual increase in the activity of the Cdk1-BubR1-PP2A-B56 pathway provides an intracellular environment suited for temporal coordination of the stabilization of kinetochore-microtubule attachments with the progression of spindle bipolarization. However, molecular mechanisms of how attachment stabilization and spindle bipolarization are coordinated in oocytes is poorly understood.

**Error correction of kinetochore-microtubule attachments during metaphase**

In mitosis, it is generally assumed that a majority of errors in kinetochore-microtubule attachment are corrected before the establishment of chromosome alignment. However, in meiosis I of oocytes even after the establishment of stable chromosome alignment at early metaphase, a substantial fraction of kinetochores on stably stretched chromosomes at the spindle equator have merotelic attachments (Kitajima et al. 2011; Yoshida et al. 2015) in which a single kinetochore is attached to microtubules from both spindle poles (Fig. 3). These errors must be corrected by the end of metaphase to prevent chromosome segregation errors (Lane & Jones 2014). Spindle pole-based mechanisms are likely ineffective in correcting these errors because the stretched chromosomes stay around the spindle equator during metaphase. How are these errors on the stretched chromosomes corrected during metaphase? Interestingly, when Aurora B/C are inhibited after chromosome stretching at early metaphase I, the merotelic attachments are corrected into stable monopolar end-on attachments, depending on the tension between homologous kinetochores (Yoshida et al. 2015). These data suggest that the dephosphorylation of kinetochore substrates, which would presumably stabilize attachments of microtubules emanating from any direction, is sufficient to subsequently induce selective stabilization of microtubule attachments to one spindle pole by sensing the tension between homologous kinetochores. Assuming that the attachments of microtubules to the nearest spindle pole are predominant over the attachments to the other spindle pole on one single kinetochore of the stretched chromosome, unselective stabilization of attachments by kinetochore dephosphorylation would be sufficient to increase tension across homologous kinetochores. One hypothesis is that the increase in the tension may potentiate kinetochore-intrinsic capacity to selectively stabilize microtubules that contribute to the tension but not microtubules that counteract the tension. If this hypothesis is correct, then a gradual increase in the phosphatase activity at kinetochores through the Cdk1-BubR1-PP2A-B56 pathway could serve as an upstream signal that promotes error correction of kinetochore-microtubule attachments during metaphase.

**Conclusion**

Although many mechanisms used for the regulation of kinetochore-microtubule attachments during mitosis in somatic cells are shared with meiosis in oocytes, recent progress in the studies of spindle and chromosome dynamics and their regulatory pathways revealed considerable variations, which provide possible explanations for the high frequency of kinetochore-microtubule attachment errors during meiosis in oocytes. The differences between mitosis in somatic cells and meiosis in oocytes include the acentrosomal nature of the meiotic spindle, meiosis-specific geometry of sister kinetochores, and oocyte-specific large cytoplasmic size. These oocyte-intrinsic features are likely linked to the specialized functions of oocytes. Oocytes function to segregate homologous chromosomes but not sister
chromatids during meiosis I for which meiosis-specific geometry of sister kinetochores is required. Oocytes also function to provide a large amount of maternal materials that support post-fertilization embryonic development for which large cytoplasmic size would be required. Although physiological importance of the absence of centrosomes in oocytes is unclear, inhibition of centrosome elimination during oogenesis results in failure of early embryonic development in Drosophila (Pimenta-Marques et al. 2016). In summary, in oocytes, the error-prone nature of kinetochore-microtubule attachments, which predispose these cells to chromosome segregation errors, may be considered a trade-off of the oocyte-specific capacity to accomplish specialized functions in reproduction and development. It is noteworthy that human oocytes appear to be more error prone than mouse oocytes, which may be attributed to factors that are not common to mammalian species, such as the absence of detectable MTOC clusters in human oocytes (Holubcová et al. 2015). Considering that excellent progress toward the understanding of kinetochore-microtubule attachment errors as well as age-related defects in oocytes has been recently made, it would not be surprising if future works provide novel evolutionary implications for the error-prone nature of oocytes.

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