CHAPTER FIVE

Oocyte Meiotic Spindle Assembly and Function

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Abstract

Gametogenesis in animal oocytes reduces the diploid genome content of germline precursors to a haploid state in gametes by discarding ¾ of the duplicated chromosomes through a sequence of two meiotic cell divisions called meiosis I and II. The assembly of the microtubule-based spindle structure that mediates this reduction in genome content remains poorly understood compared to our knowledge of mitotic spindle assembly and function. In this review, we consider the diversity of oocyte meiotic spindle assembly and structure across animal phylogeny, review recent advances in our understanding of how animal oocytes assemble spindles in the absence of the centriole-based microtubule-organizing centers that dominate mitotic spindle assembly, and

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discuss different models for how chromosomes are captured and moved to achieve chromosome segregation during oocyte meiotic cell division.

1. INTRODUCTION

A nearly universal feature of animal life is the fusion of two haploid gametes to create a diploid zygote. Most animal cells except gametes have two closely related but genetically distinct copies of each chromosome, called homologous chromosome pairs, one inherited from each parent. The fundamental achievement of gametogenesis is to reduce the diploid genome of germline precursors to a haploid state through two specialized cell divisions, called meiosis I and II (Fig. 1) (Dumont & Desai, 2012; Howe & FitzHarris, 2013; Müller-Reichert et al., 2010; Ohkura, 2015). When two gamete genomes unite after the fertilization of an egg by a sperm, diploidy is restored and life begins anew.

This review focuses on recent advances in our understanding of how an egg reduces its genome content to a haploid state, and more specifically on oocyte meiosis I, the first of the two sequential divisions that produce haploid gametes. While we devote more attention to the nematode *Caenorhabditis elegans*, reference and comparison to other model organisms—including a mammal (*Mus musculus*), an amphibian (*Xenopus laevis*), and an insect (*Drosophila melanogaster*)—will highlight similarities and differences among these widely studied models. After introducing meiosis and how it differs from mitosis (Fig. 1), we will focus on three topics. First, we will consider the diversity of meiotic mechanisms across animal phyla, and our limited understanding of when, how, and why such differences evolved. We will then address structural features of the meiotic cell division machinery—the oocyte meiotic spindle—that distinguish it from the mitotic spindle, and discuss recent progress in understanding the molecular mechanisms that mediate spindle assembly early in oocyte meiosis I. Finally, we will review the spindle dynamics that align and segregate chromosomes later in meiosis I to produce a haploid genome.

2. MAINTAIN PARITY OR PARE DOWN: MITOTIC VERSUS MEIOTIC CELL DIVISION

During both meiotic and mitotic cell division, chromosomes are captured and moved by microtubules, hollow tubes that are 25 nm in diameter

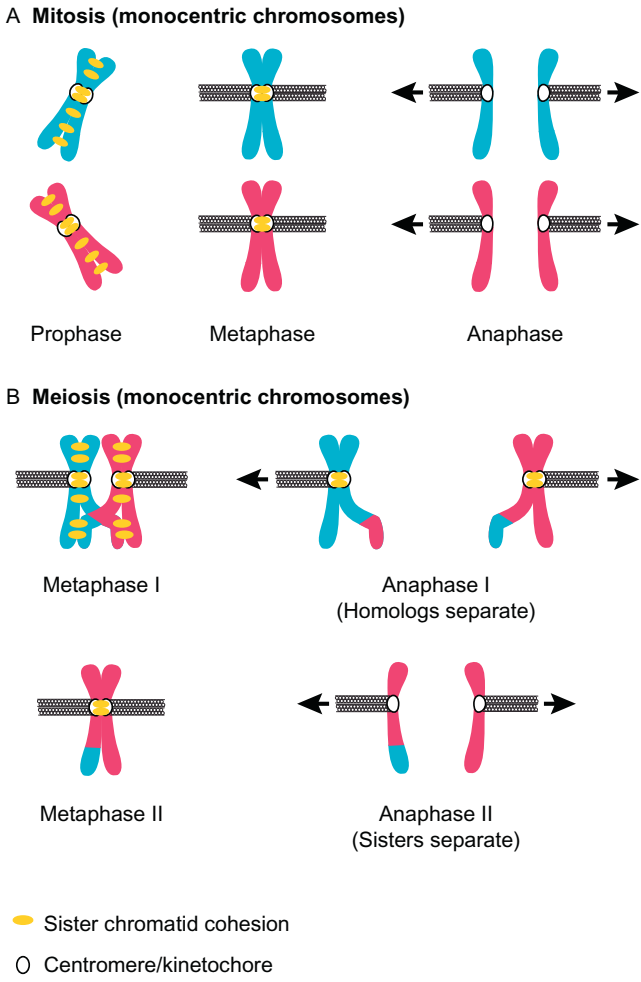

Figure 1 Distinct patterns of chromosome segregation occur during meiosis and mitosis. The progression of a single pair of homologous chromosomes (blue and red) through mitosis (A) and meiosis (B) is shown. Before either division, DNA replication duplicates both homologs. Subsequently, each homolog is composed of two sister chromatids tethered by sister chromatid cohesion (yellow ovals). (A) In many organisms, cohesion along chromosome arms is released during mitotic prophase. At metaphase, sisters remain linked by centromeric cohesion. Bipolar attachment of sister kinetochores to microtubules from opposite spindle poles aligns each homolog on the metaphase plate. In anaphase, centromeric cohesion is released and sister chromatids segregate toward opposite poles. (B) During prophase of meiosis I, homologous chromosomes become linked by reciprocal exchange of DNA during crossover recombination. Both sister kinetochores of one homolog capture microtubules from the same spindle pole; the sister kinetochores of the other homolog attach to microtubules from the opposite spindle pole (for simplicity, microtubule contact with both sister chromatid kinetochores is not depicted). In anaphase of meiosis I, release of cohesion between chromosome arms allows homologs to separate; sisters remain tethered by centromeric cohesion. In anaphase II, centromeric cohesion is released, allowing sisters to separate.

and are formed by the polymerization of α- and β-tubulin dimers (Fig. 2A). Microtubules are highly dynamic: they alternate between periods of rapid growth and shrinkage at their plus end, while being more stable at their minus end (Kirschner & Mitchison, 1986; Mitchison & Kirschner, 1984).

Much of our understanding of microtubule and chromosome dynamics during cell division has come from studies of mitosis (Hirano, 2015; Walczak et al., 2010). Before mitotic cell divisions, semiconservative DNA replication duplicates each chromosome in the genome. The resulting copies of each homolog, called sister chromatids, are held together by a ring-shaped protein complex called cohesin, proposed to encircle each pair of sister chromatids. During mitosis, microtubules assemble into a bipolar structure called the spindle and capture each sister chromatid through large protein complexes called kinetochores (Fig. 2B). A kinetochore assembles on each chromatid, and ultimately the sister kinetochores attach to microtubules from opposite spindle poles. Once these so-called "amphitelic attachments" have formed, each pair of sisters aligns on the metaphase plate, a plane midway between the two poles. Next, cell cycle regulated proteolysis breaks open the cohesin rings, freeing the sister chromatids, and allowing their movement toward opposite poles in response to microtubule-based pulling forces (Ohkura, 2015; Petronczki et al., 2003). Each daughter cell receives one copy of each homolog and ploidy is unchanged (Fig. 1A).

The mechanics of meiosis I are fundamentally distinct from those of mitosis. As in mitosis, DNA replication precisely copies each homolog before meiosis I (Fig. 1B). In prophase of meiosis I, the duplicated homologs pair and become joined by an elaborate protein structure called the synaptonemal complex (Ohkura, 2015; Petronczki et al., 2003; Zickler & Kleckner, 2015). The homolog pairs (called bivalents) then become covalently linked through reciprocal exchange of DNA during crossover recombination. Much as in mitosis, microtubules of the oocyte meiotic spindle establish attachments to the bivalents. However, during meiosis I the two sister kinetochores of each duplicated homolog are captured by the same pole, not by opposite poles as occurs during mitosis. Microtubules from the opposite pole capture the sister kinetochores of the other homolog. Once bipolar attachments form and each bivalent aligns on the metaphase plate, a subset of meiotic cohesin complexes are destroyed, allowing the homologs to segregate to opposite poles (Ohkura, 2015; Petronczki et al., 2003). The two sister chromatids of each homolog remain tethered by the surviving cohesin complexes. Meiosis II then proceeds much like mitosis: sister kinetochores are captured by opposite poles and move apart when the remaining cohesin complexes are

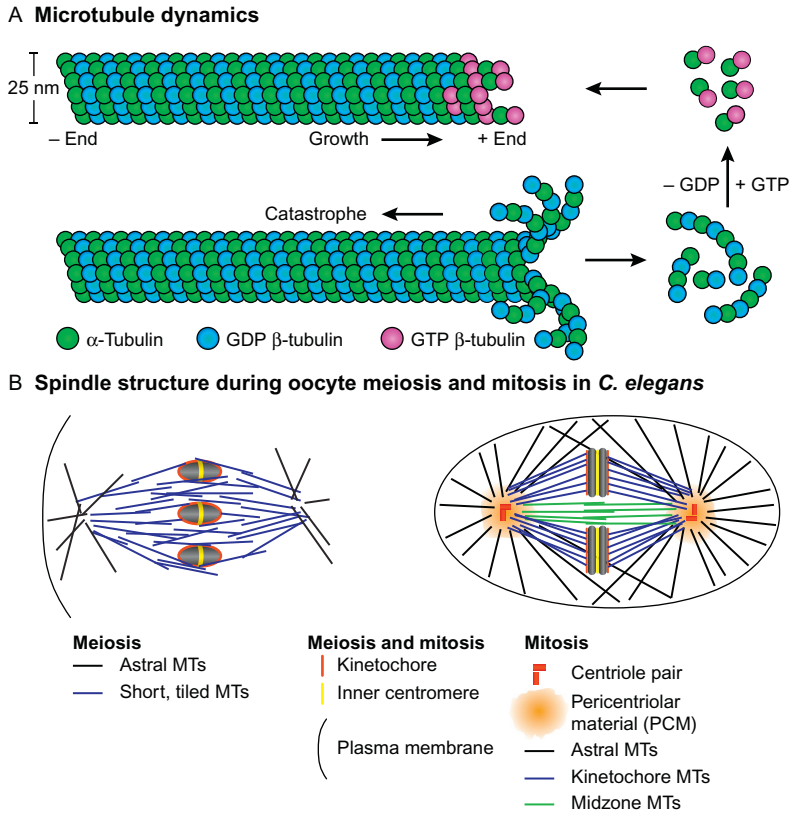

Figure 2 Microtubule dynamics and spindle structures during oocyte meiosis and mitosis. (A) Microtubules are hollow tubes 25 nm in diameter formed by lateral association of 13 protofilaments, linear polymers composed α- and β-tubulin heterodimers. Due to the ordered assembly of α/β-tubulin heterodimers, microtubules have intrinsic polarity. One end (the minus end) has α-tubulin as the terminal subunit. This end is relatively stable. The other end (the plus end) has β-tubulin as the terminal subunit. This highly dynamic end switches between rapid growth and shrinkage depending on whether the exposed β-tubulin subunits are bound to GTP (growth) or GDP (shrinkage). During growth, GTP-tubulin adds to the plus end, forming a GTP cap. GTP is hydrolyzed to GDP, and if GDP-β-tubulin is exposed on the plus end of a microtubule, catastrophic disassembly can occur. (B) Cartoons of spindle structure during meiosis and mitosis in *C. elegans*. During mitosis, long, radial microtubule arrays are nucleated in the pericentriolar material surrounding the centrioles. Some microtubules (blue) from each spindle pole are captured by kinetochores (red). Ultimately, these kinetochore/microtubule attachments align each chromosome on the metaphase plate. Other overlapping, antiparallel microtubules (green) become bundled together in a region called the midzone, a process that stabilizes mitotic spindle structure. In contrast, centrioles are degraded prior to assembly of the oocyte meiotic spindle. Short, often tiled, microtubules form near chromosomes and ultimately coalesce into a bipolar structure.

destroyed. While meiotic and mitotic cohesins share several subunits, certain subunits are unique to each type of division and account for the different patterns of chromosome segregation (Herran et al., 2011; Ishiguro et al., 2011, 2014; Klein et al., 1999; Lee & Hirano, 2011; Llano et al., 2012; McNicoll et al., 2013; Severson & Meyer, 2014; Severson et al., 2009).

In summary, meiosis I differs from mitosis in that homologs pair and then segregate to opposite spindle poles, but sister chromatids never separate. Thus, each daughter cell inherits a single homolog composed of two sister chromatids, rather than a single chromatid from both homologs as occurs during mitosis. Meiosis II then simply reduces genome content to a single chromatid from each inherited homolog. While recent advances have improved our understanding of the mechanisms that tether and release sister chromatids to reduce ploidy, this review will focus on the assembly and function of the microtubule-based oocyte meiosis I spindle.

3. CENTROSOMES: THE STARS OF MITOTIC SPINDLE POLES

While the spindles that form in mitotic cells and in oocytes are both bipolar, they can differ substantially in how they form and in microtubule organization (Fig. 2B). In animal cells, centrosome-based cytoplasmic microtubule-organizing centers (MTOCs) dominate mitotic spindle assembly and structure (Nigg & Raff, 2009). At each spindle pole, a centrosome nucleates, anchors, and organizes microtubules, accounting for spindle bipolarity. At the inner core of each centrosome is a pair of short, orthogonally oriented, and very stable microtubule-based structures called centrioles (Gönczy, 2012). One centriole of the pair is an older, mature structure—the "mother"—while the other is newly constructed—the "daughter"—and not yet fully empowered to replicate or organize a new centrosome. To form centrosomes, the centrioles recruit a pericentriolar matrix (PCM) composed of an extensive network of coiled–coil proteins and other associated proteins that expands dramatically early in mitosis, a process called centrosome maturation (Prosser & Pelletier, 2015; Woodruff et al., 2014).

The PCM includes microtubule-related structures called γ-tubulin ring complexes (γ-TURCs), which include a ring of γ-tubulin subunits that nucleate the polarized assembly of α/β-tubulin dimers into microtubules (Oakley et al., 2015). Consequently, microtubules grow with their more stable minus ends anchored in the centrosome and their more dynamic plus ends projecting outward. Because γ-TURCs are distributed throughout

the PCM of each centrosome, the microtubules they nucleate project outward in all directions, forming large and radially symmetric asters. Ultimately, some microtubules from each centrosome capture and align chromosomes at the metaphase plate, and then mediate chromosome segregation to the poles during anaphase.

Importantly, centrosome duplication is tightly regulated during the cell cycle (Firat-Karalar & Stearns, 2014). After mitosis, each daughter cell receives one pair of centrioles that separate. Each centriole then produces a new, orthogonally oriented daughter centriole, restoring the seeds of bipolarity for the next mitotic cell division (Gönczy, 2012). Intriguingly, centrioles also serve as the basal bodies from which cilia and flagella grow at the surface of interphase cells. These roles—as centrosome organizers or basal bodies—appear to be mutually exclusive in all animal cells.

4. THE CURIOUS STRUCTURE OF (SOME) OOCYTE MEIOTIC SPINDLES: ACENTRIOLAR POLES

In contrast to the spindles formed during mitosis, oocyte meiotic spindles in many animals—including humans and the laboratory models of mice, frogs, fruit flies, and nematodes—entirely lack centrioles (Dumont & Desai, 2012; Howe & FitzHarris, 2013; Müller-Reichert et al., 2010; Ohkura, 2015). Nevertheless, bipolar spindles still assemble in the absence of these key organizers of the mitotic spindle. Moreover, even oocytes that retain centrioles at meiotic spindle poles typically lose them during or shortly after meiosis, and thus most animal eggs ultimately lack functional centrioles. Over a century ago, Boveri (a codiscoverer of centrioles) noted the absence of centrioles in eggs and that, other than centrioles, sperm provide little that is unique during fertilization. He therefore proposed that eliminating oocyte centrioles might prevent parthenogenetic development, with the sperm-derived centrioles restoring complete cell status to the egg. Consistent with this hypothesis, spindle assembly during the first embryonic mitosis in most animals is mediated by centrioles derived from the sperm basal body. However, both oocytes and sperm are acentriolar in mice and other rodents, and even the mitotic divisions in early mouse embryos lack centrioles (Manandhar et al., 2005; Szollosi et al., 1972). Thus, Boveri's notion of the centriole as an essential mitotic spindle organizer is clearly not absolute. Exceptional cases aside, however, centriole elimination during oogenesis or maturation ensures that cell fusion during fertilization does not increase centrosome count.

But why eliminate centrioles before meiosis? Based on the limited comparative data available, it seems likely that primitive animal oocytes retained centrioles and centrosome-based meiotic spindle pole function, complete with astral microtubules. For example, during meiosis I in sea star and sea urchin (echinoderm) oocytes, each spindle pole harbors a pair of centrioles, as in mitosis, but centriole duplication does not occur after meiosis I. Each pole in meiosis II therefore has only a single centriole (Nakashima & Kato, 2001; Sluder et al., 1989). Experimental analysis of sea star oocytes indicates that the centrioles inherited by polar bodies are fully functional and replicable, whereas the single centriole that remains in the egg after meiosis II is apparently nonreplicable, and presumably eventually degenerates (Sluder et al., 1989; Uetake et al., 2002). The two pairs of centrioles derived from the sperm then mediate the first mitotic division of the zygote. Thus, in sea stars the oocyte's complement of replication-competent centrioles is discarded into polar bodies. Indeed, the failure of polar body emission enables parthenogenetic development due to the retention of functional, reproductive centrosomes (Washitani-Nemoto et al., 1994).

Echinoderms are deuterostomes, like vertebrates, and another echinoderm group, the sea cucumbers, also have centriolar spindles (Holland, 1981). However, chordates, including the tunicates, all appear to assemble acentriolar spindles during oocyte meiosis (Sawada & Schatten, 1988). Which mode is primitive for animals?

Many invertebrate protostomes also retain centrioles through oocyte meiosis. Centrioles have either been detected using transmission electron microscopy (TEM) or have been inferred to exist because of the presence of extensive astral microtubule arrays at meiotic spindle poles. Therefore, centriolar oocyte spindles may be the ancestral condition. In the clam *Spisula solidissima*, the fertilized oocyte contains three centrosomes during prometaphase of meiosis I—one from the sperm and two from the oocyte (Wu & Palazzo, 1999). As in echinoderms, one oocyte centrosome with paired centrioles is extruded into the first polar body at the end of meiosis I. A second oocyte centrosome, with an unpaired centriole, is extruded into the second polar body at the end of meiosis II. Thus, the zygote inherits one maternal centrosome with a single, unduplicated centriole and one paternal centrosome with a duplicated centriole. While the fate of the remaining oocyte centrosome is unknown, subsequent mitotic divisions use the sperm-derived centrioles. Remarkably, the sperm centrosome appears to be repressed during oocyte meiosis: both its γ-tubulin and α-tubulin become undetectable by metaphase of meiosis I. Similar suppression of the sperm

centrosome is observed in annelids and echinoderms (Stephano & Gould, 2000). *Spisula* likely typifies the entire Lophotrochozoan supergroup; although TEM has been applied to identify centrioles in only a few species in this group, the presence of large asters around meiotic spindle poles in oocytes of numerous Lophotrochozoan species implies their presence (Crowder et al., 2015b). Meanwhile, although insect and *C. elegans* oocytes feature well-studied acentriolar (and largely anastral) meiotic spindles, this is unlikely to be primitive for the Ecdysozoan supergroup as the oocyte meiotic spindles of some crustaceans have large astral, centriolar poles (Goudeau & Goudeau, 1986; Goudeau & Lachaise, 1980; Lindsay et al., 1992).

Although comparative data are limited, acentriolar spindle assembly pathways likely evolved multiple times in animals, and nearly exclusively for use in oocyte meiosis. This in turn suggests that similar selective pressures may have favored the transition to acentriolar oocyte spindles in these lineages. One adaptive advantage might have been to obviate the book-keeping involved in sorting centrioles from different sources into polar bodies versus eggs. Although this seems like a minor mechanistic burden, any distinctions that enable predictable centriole sorting could in principle be interpreted by other agents, making meiosis more susceptible to cheating (i.e., meiotic drive).

Another often cited rationale for acentriolar meiosis is that small and largely anastral meiotic spindles might minimize the size of polar bodies, the discard products of meiosis. The extreme asymmetry of the two divisions that occur during oocyte meiosis ensures that the zygote inherits almost all of the maternally synthesized gene products, biosynthetic machinery, and fuel that sustain early embryonic development. For example, in *C. elegans* oocytes, very small meiotic spindles assemble in close proximity to the cortex (Fig. 2). While the zygote itself is approximately 50×20 μm, the oocyte meiotic spindles initially are roughly 8 μm in length and by metaphase shorten to only 5 μm (Albertson & Thomson, 1993; McNally & McNally, 2005; McNally et al., 2006; Yang et al., 2003). By contrast, the first mitotic spindle is much larger (Fig. 2), with astral microtubules spanning the entire length and width of the one-cell zygote (Müller-Reichert et al., 2010). Only a small number of short astral microtubules have been detected during oocyte meiosis in *C. elegans*; these microtubules may be important for the microtubule motor-dependent translocation and rotation of the spindle prior to anaphase (Crowder et al., 2015a; Ellefson & McNally, 2009, 2011; Yang et al., 2005). Indeed, spindle positioning in *C. elegans* oocytes is microtubule-dependent but actin-independent (Yang et al., 2003). In contrast,

rotation of the acentriolar and likely asterless oocyte spindles in mouse oocytes requires actin and myosin but appears to be independent of any microtubule motors (Maro et al., 1984; Wang et al., 2011). It is not clear whether acentriolar oocyte meiotic spindles in *Drosophila* have any astral microtubules (Skold et al., 2005); however, asters are detectable in *Xenopus* oocytes (Gard, 1992), although it is not known if they are involved in spindle positioning.

While small anastral oocyte meiotic spindles may facilitate the extremely asymmetric division that discards ¾ of the replicated genome into tiny polar bodies, it is clear that this is not the only means to this end. The astral, centriolar meiotic spindles of mollusks and echinoderms accomplish divisions just as asymmetric as the anastral, acentriolar spindles of rodents and nematodes. Moreover, many insects dispense with polar body extrusion altogether, and the discard products of female meiosis are abandoned to their fate in the egg cytoplasm (Foe et al., 1993).

Other rationales for the acentriolar structure of oocyte meiotic spindles warrant consideration. A recent analysis in *C. elegans* indicates that the small spindle size reduces the frequency of aneuploidy due to missegregation of chromosomes (Cortes et al., 2015). Also, centriole loss might relate to the use of centrosomes to establish cell polarity, such as the anterior–posterior body axis in *C. elegans* (Munro & Bowerman, 2009). Perhaps, the premeiotic loss of oocyte centrioles eliminates conflicting signals for polarity establishment. Although nematode sperms are amoeboid and do not use centrioles and flagella for motility, they nevertheless provide the centrioles that polarize the egg and are used to initiate mitotic cell division in the zygote (Nelson et al., 1982). Finally, other key differences between spermatogenesis and oogenesis may explain the loss of centrioles in eggs. For example, the end product of spermatogenesis in most animals is four haploid, flagellated sperm cells. The spermatocyte meiotic spindle is as much a basal body distributor as it is a chromosome sorter: just as each spindle pole captures a haploid genome, each haploid genome captures a spindle pole, the essential seed for the motor that takes it to its fate. In contrast, eggs and polar bodies do not need to swim. Perhaps, Boveri was mistaken to nominate parthenogenesis as the big risk evaded by acentriolar meiosis: instead, what if polar bodies were *too* complete? Animal life cycles depend on the zygote as the one-cell bottleneck both to limit potential conflict among genetically heterogeneous cell lineages within the individual, and to ensure that alleles comprising the genome of each newly formed individual are maximally exposed to selection (Grosberg & Strathmann, 1998). Indeed, if sea star polar bodies inherit

enough cytoplasm, they can continue mitosis (Saiki & Hamaguchi, 1997). If such cells were incorporated into the embryo it would potentially violate these imperatives. The chance of this happening is remote in free-spawning invertebrates, but is perhaps higher in encapsulated, brooded, or placental embryos. Depriving polar bodies of centrioles might forestall this possibility. That this is a plausible risk is shown by the rare instances, in humans, of polar body fertilization, giving rise to a chimera or even a twin (Bieber et al., 1981).

Finally, consider the unusual way the freshwater clam *Corbicula leana*, a hermaphroditic triploid, begins a new life. Centrosomes with typical centriole pairs mediate assembly of the oocyte meiosis I spindle, but the spindle remains parallel to the plasma membrane and both poles and all maternal chromosomes are extruded simultaneously into a pair of polar bodies (Komaru et al., 2000). Thus, development in this species is androgenetic: the zygote chromosomes come entirely from the sperm. This is doubtless among the most extreme variants of meiosis, but underscores the extent to which this biological process, so central to all eukaryotic sexuality, evolves in concert with animal life histories.

The intriguing variation of meiotic mechanisms across animal phylogeny seems largely neglected, despite its obvious possible adaptive significance. Nematodes provide one avenue for exploring this remarkable example of evolutionary cell biology. Their oocytes are amenable to live imaging, and many different species have been isolated, often with fully sequenced genomes (Brauchle et al., 2009; Farhadifar et al., 2015; Phillips & Bowerman, 2015). Moreover, there is substantial cryptic genetic variation that impacts embryonic viability among wild *C. elegans* populations (Paaby et al., 2015). Perhaps, some of this genotypic variation influences oocyte spindle assembly and might shed light on the phenotypic variation observed at the family and phyla levels.

5. THE CURIOUS STRUCTURE OF OOCYTE MEIOTIC SPINDLES, PART 2: TILED MICROTUBULES

Another remarkable difference between mitotic and at least some oocyte meiotic spindles is the continuous versus discontinuous nature of the microtubules that span the distance between the poles and the spindle midzone and chromosomes (Fig. 2B). Although mitotic spindles may include some short tiled microtubules (Goshima & Kimura, 2010), long microtubules typically extend from each mitotic centrosome to the midzone

and beyond (White & Glotzer, 2012). In some cases, the long microtubules form crosslinked, antiparallel bundles that stabilize the mitotic spindle and ultimately constitute the central spindle and midbody remnant, structures that promote the initiation and completion of cytokinesis, respectively (White & Glotzer, 2012). By contrast, electron tomography studies in *C. elegans* oocytes have shown that individual microtubules do not reach from the poles to the midzone or to the chromosomes (Srayko et al., 2006). Rather a series of short tiled microtubules—presumably bundled together by other proteins—span these distances (Fig. 2B). Similarly, in both *Drosophila* oocytes and *Xenopus* oocyte extracts, meiotic spindles appear to be composed of short tiled microtubules (Burbank et al., 2006; Skold et al., 2005; Yang et al., 2007). Remarkably, the plus ends of microtubules in these arrays grow both from the poles toward the midzone and from the midzone toward the poles (Liang et al., 2009; Yang et al., 2007). In sum, mitotic and oocyte meiotic spindles have remarkably distinct structures and dynamics.

Why oocyte meiotic spindles should be composed of short, discontinuous microtubules is not known, although such an arrangement could provide a traction mechanism for the poleward movement of chromosomes during anaphase as has been proposed for mitotic spindles (Goshima & Kimura, 2010). Nevertheless, it seems ironic that the relatively small oocyte meiotic spindles have such tiling, while the often much larger mitotic spindles include longer, untiled microtubules. One explanation for this curious structure has come from *C. elegans*, where the AAATPase called katanin is critical for assembling oocyte meiotic spindles (Clark-Maguire & Mains, 1994; Connolly et al., 2014; Mains et al., 1990; McNally & McNally, 2011; McNally et al., 2014; Srayko et al., 2000, 2006; Yang et al., 2003). Unlike regulators of microtubule growth that influence polymerization, katanin severs microtubules along their length (Hartman et al., 1998; McNally & Vale, 1993; Srayko et al., 2006). Because acentriolar oocyte meiotic spindles lack the γ-TURCs that nucleate microtubule assembly, microtubule severing might generate substrates for polymerization during oocyte meiosis. Indeed, electron tomography has shown that oocyte spindles in *C. elegans* mutants lacking katanin have fewer but longer microtubules than do wild-type spindles (Srayko et al., 2006). Thus severing apparently promotes microtubule assembly, and perhaps also promotes the use of tiled, discontinuous microtubules in oocyte spindles. Although katanin has been implicated in the scaling of mitotic spindle size relative to cell size in *Xenopus tropicalis* embryos (Loughlin et al., 2011), roles for katanin during oocyte meiotic division have not been found outside of *C. elegans*.

6. ACENTRIOLAR OOCYTE MEIOTIC SPINDLE ASSEMBLY: MICROTUBULE ORIGINS

The role of katanin in generating more microtubules during *C. elegans* meiosis highlights a fundamental issue. Because oocytes in some animals, including humans, lack centrioles and their associated PCM as MTOCs, understanding the origin of oocyte meiotic spindle microtubules is of fundamental importance. A PCM-independent pathway for microtubule nucleation was first discovered when DNA-coated plastic beads, added to *Xenopus* oocyte extracts, were shown to nucleate microtubules that assembled into bipolar spindles in the absence of centrosomes (Heald et al., 1996). This DNA-based microtubule nucleation activity requires the Ran GTPase (Clarke & Zhang, 2008). In the absence of Ran activity, nuclear importins bind to and negatively regulate factors that nucleate microtubule assembly. Chromatin generates a gradient of active Ran that negatively regulates the importins, releasing the bound microtubule nucleators, and thereby promoting microtubule assembly near chromosomes.

While the discovery that chromatin and Ran GTPase can promote microtubule assembly independently of centrosomes has provided insight into spindle assembly during both meiosis and mitosis, oocyte meiotic spindle assembly is at most only partially dependent on this pathway. In *Xenopus* extracts, depletion of Ran only delays oocyte meiotic spindle assembly, and meiosis I spindles assemble in the absence of Ran in mice, *C. elegans* and *Drosophila* (Askjaer et al., 2002; Bamba et al., 2002; Cesario & McKim, 2011; Dumont et al., 2007).

More recently, two additional pathways that contribute to oocyte meiotic spindle assembly have been identified. One requires the augmin complex, which acts through γ-tubulin to nucleate microtubules that branch off the lateral surfaces of existing microtubules. The second pathway requires the chromosomal passenger complex (CPC), which regulates multiple steps in mitotic cell division. The CPC pathway has been proposed to stabilize chromatin-nucleated microtubules (Tulu et al., 2006), and to be required when Ran activity is reduced or absent (Maresca et al., 2009). Although γ-tubulin associates with CPC-dependent MTOCs in mammalian cell culture (Tulu et al., 2006), it remains unclear whether the CPC pathway requires γ-tubulin (Petry & Vale, 2015).

The importance of augmin and the CPC for oocyte spindle assembly appear to differ from organism to organism. Both influence oocyte meiotic

spindle assembly in *Xenopus* extracts and *Drosophila* oocytes (Colombie et al., 2008, 2013; Goshima & Kimura, 2010; Meireles et al., 2009; Petry et al., 2011; Radford et al., 2012; Sampath et al., 2004). However, while CPC components are required for oocyte meiotic spindle assembly in mouse and *C. elegans* (Dumont et al., 2010; Sharif et al., 2010), augmin does not appear to be conserved in *C. elegans* (Edzuka et al., 2014), and a role for augmin in mice has not been described.

As with augmin and the CPC, the importance of γ-tubulin for oocyte spindle assembly may vary from organism to organism. In mouse oocytes, γ-tubulin is present at early MTOC foci (Calarco, 2000; Clift & Schuh, 2015; Gueth-Hallonet et al., 1993; Ma et al., 2010; Palacios et al., 1993). While a requirement for γ-tubulin has not been described, mouse oocyte meiotic spindle assembly is severely disrupted in the absence of NEDD1, which is required for γ-tubulin recruitment to early MTOC foci (Ma et al., 2010). Similarly, the fly ortholog of Nedd1, called Dgp71WD, also is required for oocyte meiosis I spindle assembly (Reschen et al., 2012), although the defects in Dgp71WD mutants are more severe than those observed in mutants lacking γ-tubulin (Hughes et al., 2011). While defects in oocyte spindles have not been observed after RNAi-knockdown of *C. elegans* γ-tubulin alone, it is present diffusely throughout the oocyte spindle, and reducing γ-tubulin function in a *mei-1*(−)*/katanin* mutant results in more severe spindle defects with further loss of microtubule density, compared to *mei-1*(−) single mutants (McNally et al., 2006).

The role of γ-tubulin during oocyte spindle assembly clearly warrants further investigation. Although examples of γ-tubulin-independent microtubule assembly have not yet been conclusively identified (Petry & Vale, 2015), ever more examples of γ-tubulin-dependent nucleation of non-centrosomal microtubules are being identified, and minus-end stabilization of microtubules initiated at centrosomes but subsequently released to function elsewhere has also been demonstrated (Feldman & Priess, 2012; Hendershott & Vale, 2014; Keating et al., 1997; Musch, 2004; Oakley et al., 2015; Ori-McKenney et al., 2012; Petry & Vale, 2015; Tanaka et al., 2012; Wang et al., 2015; Yalgin et al., 2015). Whether these other processes contribute to oocyte meiotic spindle assembly remains poorly understood. Nevertheless, it seems likely that multiple pathways contribute to microtubule nucleation and organization during oocyte meiosis, although the relative importance of each pathway may vary from organism to organism.

Given the growing number of proteins known to promote centrosome-independent microtubule assembly during oocyte meiosis, we also need to

understand where these factors act. While a gradient of active Ran GTPase can stimulate microtubule assembly around chromosomes, where and how nucleation occurs is not clear. Intriguingly, it appears that some spindle microtubules assemble at sites removed from chromosomes. Cytoplasmic microtubule assembly occurs in mouse, fly, and worm oocytes, and may also occur at the oocyte nuclear envelope in mice and at the oocyte cortex in humans, *Drosophila*, and *C. elegans* (Battaglia et al., 1996; Calarco, 2000; Ellefson & McNally, 2009; Han et al., 2015; Luksza et al., 2013; McNally & McNally, 2005; Schuh & Ellenberg, 2007; Sumiyoshi et al., 2015; Yang et al., 2003; Zou et al., 2008). While γ-tubulin and pericentrin may be important for cytoplasmic microtubule assembly in mouse oocytes, and dynein has been implicated in promoting their movement toward the assembling spindle (Luksza et al., 2013), how microtubules are nucleated at more distant sites and are then transported toward the spindle remain poorly understood and are ripe topics for further investigation.

7. ACENTRIOLAR OOCYTE MEIOTIC SPINDLE ASSEMBLY, PART 2: POLE COMPOSITION

The absence of centrosomes and their impressive microtubule-organizing activity leaves a mechanistic void in our understanding of how oocyte spindles achieve the bipolar structure required to segregate chromosomes in opposite directions. Why are there two poles instead of one, and why only two poles and not more? This intriguing problem is by no means unique to animal oocytes, as higher plants lack centrioles throughout all of their cell divisions (Mineyuki, 2007), but here we limit our discussion to animal oocytes.

While the poles that ultimately form in many animal oocytes lack centrioles, they nevertheless contain PCM components associated with mitotic centrioles. In mouse oocytes, the poles contain pericentrin, γ-tubulin, and Cep192 (Fig. 3), all PCM components in mitosis (Calarco, 2000; Clift & Schuh, 2015; Gueth-Hallonet et al., 1993; Ma et al., 2010; Palacios et al., 1993). In *C. elegans*, the bipolar oocyte spindle poles that ultimately form also contain mitotic PCM proteins, although their dynamics over time in many cases remain poorly understood and some of them also are present elsewhere in the spindle. These include the calponin homology protein ASPM-1, the microtubule severing complex katanin, the kinesin-12 and -13 family members KLP-18 and KLP-7, the minus-end directed microtubule motor dynein, and the NUMA homolog LIN-5 (Connolly et al., 2014,

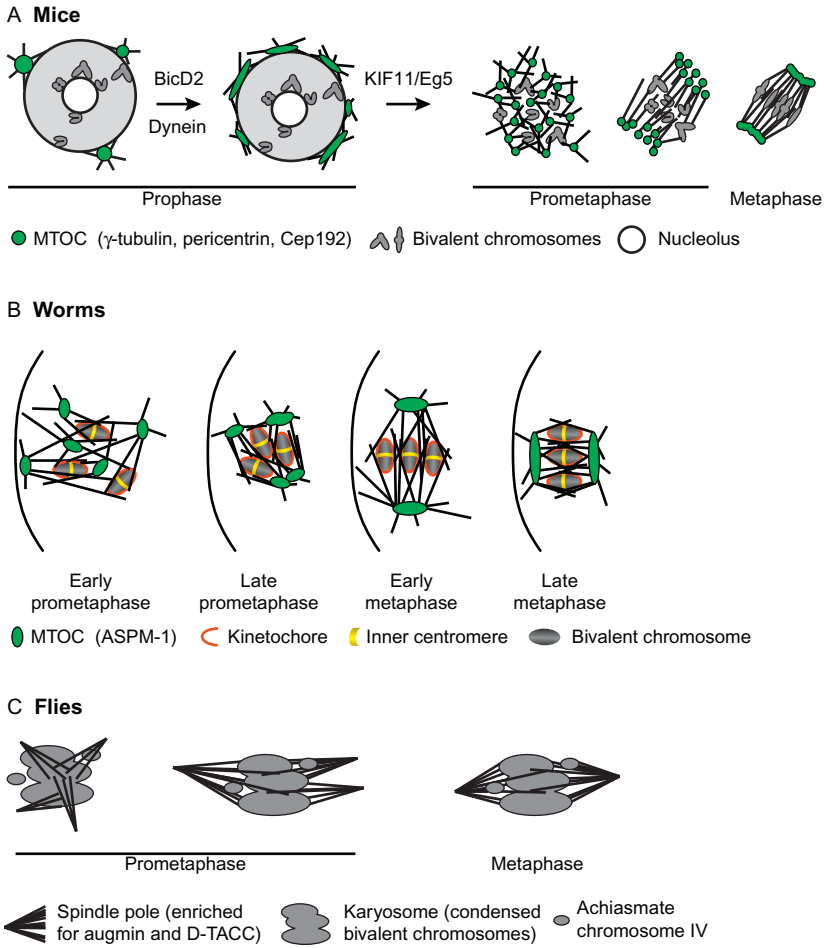

Figure 3 Assembly of meiotic spindles in mouse, worm, and fly oocytes. Live imaging of microtubules and chromosomes during oocyte meiosis in these three model organisms has revealed similarities and differences in spindle assembly. When spindle assembly begins, homolog pairs have undergone crossover recombination to form bivalents, with the exception of *Drosophila* chromosome 4, which does not recombine. (A) In mouse oocytes, small microtubule-organizing centers (MTOCs) form near the nuclear envelope prior to NEB. Subsequently, MTOCs are stretched into thin ribbons, often becoming fragmented. This stretching process requires dynein and BicD2, which anchors dynein at the nuclear envelope. After NEB, the Eg5 homolog KIF11 promotes further MTOC fragmentation, yielding an average of approximately 26 MTOCs per oocyte. These MTOCs then coalesce as bivalents congress, forming a bipolar spindle by metaphase. (B) At NEB in *C. elegans* oocytes, most bivalents are located near the nuclear envelope. Following NEB, a cloud of short microtubules assembles around the bivalents, which subsequently cluster together. Concurrently, several foci of the microtubule-scaffolding protein ASPM-1 appear, presumably present at small MTOCs. The ASPM-1 foci coalesce into two poles as

2015; Ellefson & McNally, 2011; Gomes et al., 2013; McNally et al., 2006, 2014; O'Rourke et al., 2007; van der Voet et al., 2009; Wignall & Villeneuve, 2009). Thus far, γ-tubulin has not been clearly detected at poles but appears to be present diffusely throughout the oocyte spindle (McNally et al., 2006). While less is known about pole composition in *Drosophila*, augmin subunits and the conserved microtubule-associated proteins (MAPs) minispindles and D-TACC have been detected at mature poles (Colombie et al., 2013; Cullen & Ohkura, 2001). *Drosophila* γ-tubulin appears to be somewhat enriched at oocyte spindle poles but also colocalizes with microtubules throughout the prometaphase meiosis I spindle (Endow & Hallen, 2011; Hughes et al., 2011).

8. ACENTRIOLAR OOCYTE MEIOTIC SPINDLE ASSEMBLY, PART 3: POLE ASSEMBLY

A landmark advance in our understanding of oocyte meiotic spindle dynamics came from live cell imaging of pole assembly in mouse oocytes, which showed that multiple small pole foci coalesce to form a bipolar spindle structure (Schuh & Ellenberg, 2007). While earlier studies of fixed oocytes first documented this phenomenon (Carabatsos et al., 2000; Gueth-Hallonet et al., 1993; Palacios et al., 1993), Schuh and Ellenberg used live cell imaging to show that many small MTOCs are initially dispersed throughout the area surrounding the mouse oocyte chromosomes early in meiosis I. Over time these small foci coalesce to form a bipolar structure.

More recently, live imaging of Cep192 fusion to GFP has shown that early pole coalescence in mouse oocytes involves an early dispersal of fewer and larger MTOC foci into more and smaller foci, followed by a still mysterious coalescence (Clift & Schuh, 2015). An early phase of dispersal, prior to nuclear envelope breakdown, requires the polo kinase PLK1 and the minus-end directed microtubule motor dynein, in a process that stretches the early MTOCs out into ribbon-like structures that often fragment. After nuclear envelope breakdown, the late phase further fragments and disperses

bivalents congress onto the metaphase plate. Initially, the bipolar metaphase spindle is oriented parallel to the oocyte cortex, but then rotates to be perpendicular to the cortex. During rotation, the MTOCs widen as the spindle shortens dramatically. (C) In fly oocytes, the recombined bivalents form a compact structure called a karyosome. After NEB, microtubules accumulate around the karyosome and several dynamic, pole-like structures form. These microtubule foci coalesce to form the poles of the bipolar meiotic spindle. Augmin, γ-tubulin, and D-TACC are enriched at the poles.

the MTOCs. This latter phase requires the tetrameric kinesin-5 family member KIF11, which presumably promotes fragmentation through its antiparallel microtubule-sliding activity, much as it promotes oocyte meiotic spindle bipolarity in *X. laevis* and mitotic spindle bipolarity in mammalian cell culture (Sawin et al., 1992). When KIF11-dependent dispersal is disrupted, transient monopolar oocyte spindles assemble, although they eventually recover and appear to segregate chromosomes normally (Clift & Schuh, 2015).

Similar processes of pole coalescence have been described during invertebrate oocyte spindle assembly. Time-lapse imaging of a kinesin-GFP fusion that labeled microtubules in live *Drosophila* oocytes suggested that small MTOC foci, initially distributed throughout the oocyte nucleoplasm, coalesce during meiotic spindle assembly (Skold et al., 2005). Microtubule attachment to chromosomes and the crosslinking of such attached microtubules may further promote *Drosophila* oocyte spindle assembly. More recently in *C. elegans*, a GFP fusion to the pole marker ASPM-1 was used for live cell imaging of oocyte meiosis I and II spindle assembly. Multiple small ASPM-1 foci coalesced over time to form a bipolar structure, resembling MTOC dynamics in mouse oocytes (Connolly et al., 2015). While the mechanisms that promote the coalescence of early small pole foci in *C. elegans* also remain unknown, the *C. elegans* kinesin-12 family member KLP-18 promotes spindle bipolarity (Connolly et al., 2014; Segbert et al., 2003; Wignall & Villeneuve, 2009), presumably by promoting the sliding of antiparallel microtubules, much as has been documented for its mammalian orthologs (Tanenbaum et al., 2009; Vanneste et al., 2009). Additionally, the microtubule severing activity of MEI-1/katanin and ASPM-1 itself appear to have roles in pole assembly (Connolly et al., 2014; McNally et al., 2014). Mouse ASPM-1 also is required for oocyte meiotic spindle assembly and is localized to the spindle poles (Xu et al., 2012). Thus, at least some molecular mechanisms of acentriolar pole assembly appear conserved between invertebrates and vertebrates.

Although mice, frogs, nematodes, and fruit flies all appear to assemble oocyte meiotic spindles through the coalescence of multiple early foci, the details of this process vary in each system. In mice, the small MTOC foci initially were shown to coalesce into a ball-like structure, with the MTOCs clustered internally and the chromosomes distributed on the surface (Schuh & Ellenberg, 2007). Subsequently, some MTOCs were ejected peripherally and then coalesced into a bipolar organization, with chromosomes ultimately congressing to form the metaphase plate. However, the

more recent study using Cep192 as a marker for MTOC pole foci failed to detect a ball-like structure with MTOCs surrounded by chromosomes; rather the MTOCs and chromosomes were comingled (Clift & Schuh, 2015). In both studies, the subsequent dispersal of MTOCs was shown to require the kinesin-5 family member KIF11 (Fig. 3A) (Clift & Schuh, 2015; Schuh & Ellenberg, 2007). How the multiple, dispersed MTOCs coalesce to form two poles remains unknown.

In *Xenopus* extracts, discrete, small microtubule foci have not been reported as pole intermediates during the assembly of oocyte meiotic spindles. However, acentrosomal bipolar spindle organization does require a kinesin-5 family member (Eg5), as in mouse. Pole assembly also is thought to involve crosslinking of microtubule minus ends mediated by dynein and the coiled–coil protein NUMA (Heald et al., 1996; Merdes et al., 2000; Mitchison et al., 2005).

In *C. elegans*, live-imaging studies indicate that chromosomes are initially dispersed throughout a mass of microtubules, and the small ASPM-1 pole foci formed early in meiosis coalesce to form a bipolar structure (Fig. 3B) (Connolly et al., 2015). Unlike in mice and frogs, kinesin-5 (*C. elegans* BMK-1) is not required for bipolar spindle assembly during meiosis or mitosis (Saunders et al., 2007). Moreover, while the NUMA ortholog LIN-5, together with dynein, is required to position and orient the *C. elegans* oocyte meiotic spindle (Crowder et al., 2015a; Ellefson & McNally, 2009, 2011; van der Voet et al., 2009), roles for either protein in pole assembly have not been documented.

In *Drosophila*, the dynamics of pole coalescence remain less well characterized, but MAPs are clearly involved. Mutational inactivation of the conserved MAP minispindles results in assembly of tripolar meiosis I spindles (Cullen & Ohkura, 2001). Minispindles localization to spindle poles depends on another MAP, called D-TACC. A complex of minispindles and D-TACC, transported to microtubule minus ends by the kinesin Ncd, has been proposed to stabilize microtubule minus ends to promote pole assembly and spindle bipolarity (Cullen & Ohkura, 2001; Matthies et al., 1996). The *C. elegans* D-TACC ortholog TAC-1 acts in a complex with ZYG-9, an XMAP215 ortholog, to promote microtubule stability during mitosis (Bellanger & Gonczy, 2003), and although ZYG-9 is required for meiotic spindle assembly (Yang et al., 2003), how ZYG-9 influences oocyte spindle assembly is not well understood and the role of TAC-1 has not been addressed. Finally, *Drosophila* Subito, a member of the kinesin-6 family that crosslinks antiparallel microtubules, is not required for oocyte spindle

assembly. However, *subito* mutants display transient instability of spindle poles, suggesting that Subito-mediated central spindle integrity may be important for promoting spindle bipolarity (Colombie et al., 2013; Jang et al., 2007).

In sum, the molecular pathways that promote pole coalescence and spindle bipolarity appear to partially overlap, but significant differences are also apparent across animal phylogeny. While some of the variation likely reflects multiple, independently evolved appearances of acentriolar spindle assembly, systematic comparisons of the different gene requirements in each model system are needed to better assess the conservation, and divergence of oocyte spindle assembly mechanisms.

9. KINETOCHORE FUNCTION AND POLE COALESCENCE

While an early coalescence of MTOC foci has been implicated in mouse, nematode, and fly oocyte meiotic spindle assembly, the mechanism of MTOC coalescence remains unknown. However, a recent analysis of ASPM-1 dynamics during oocyte meiotic spindle assembly in *C. elegans* suggests that proper microtubule–kinetochore attachments contribute to the coalescence of early pole foci (Fig. 4A) (Connolly et al., 2015). In *C. elegans* mutants that lack the microtubule depolymerizing kinesin KLP-7, called MCAK in vertebrates, ASPM-1 foci fail to coalesce into a bipolar structure and instead often form tripolar or even tetrapolar structures. Both ASPM-1 and MEI-1/katanin mark the poles of *klp*-7(−) mutants, indicating that the supernumerary poles are molecularly similar to those of wild-type bipolar spindles, although these foci have not yet been shown to localize with MTOCs. The extra poles also are functional, as chromosomes often segregate into three discrete masses during anaphase. Thus, KLP-7 is required for the coalescence of early pole foci in *C. elegans* oocytes.

In vertebrates, MCAK regulates the microtubule–kinetochore attachments that mediate mitotic chromosome segregation during anaphase (Ems-McClung & Walczak, 2010; Kline-Smith et al., 2004). In the absence of MCAK in human cell culture lines, improper syntelic (kinetochores of both sister chromatids attached to the same pole) and merotelic (kinetochore of one sister chromatid attached to both poles) microtubule–kinetochore attachments are observed. In wild-type cells, MCAK associates with kinetochores prior to anaphase, and its depolymerase activity may destabilize inappropriate microtubule–kinetochore attachments. In *klp*-7(−)/*MCAK* mutant *C. elegans* oocytes, the persistence of such improper attachments

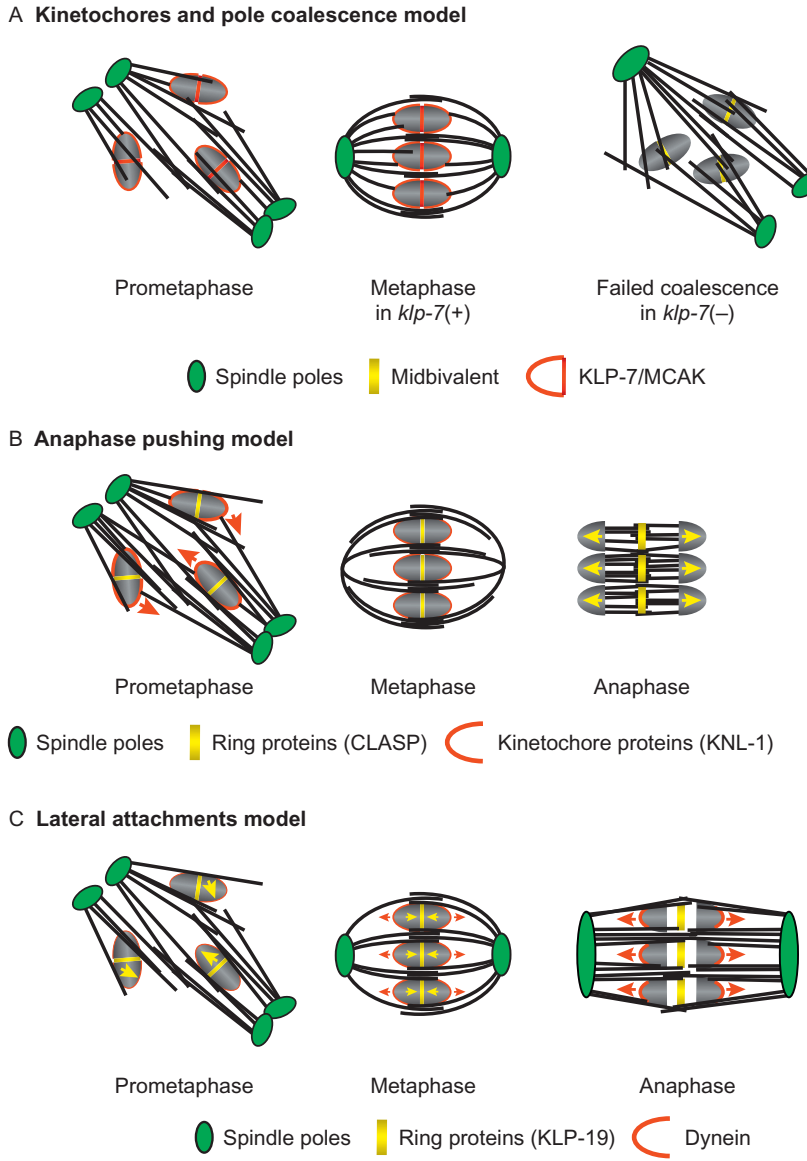

Figure 4 Models for bipolar spindle assembly and chromosome congression and segregation in *C. elegans*. Three models are shown that address pole assembly (A) and chromosome congression and segregation (B and C) in *C. elegans* oocytes. (A) Proper microtubule–kinetochore attachments permit the coalescence of early pole foci to form a bipolar oocyte spindle. In this model, bivalent alignment and spindle bipolarity require that the two homologs of each bivalent attach specifically to microtubules from opposite spindle poles. Incorrect attachments (see text) are eliminated by the microtubule

(Continued)

during oocyte meiosis might lead to abnormal tension within the assembling spindle, and this imbalance in forces has been proposed to interfere with the coalescence of ASPM-1 foci into a bipolar structure (Connolly et al., 2015). Consistent with such a model, partial knockdown of components of the Ndc80 complex that mediates microtubule–kinetochore attachment rescues spindle bipolarity in *klp*-7(−) mutants.

While the importance of microtubule–kinetochore attachment for oocyte spindle assembly in other species remains largely unknown, roughly 90% of the chromosomes in mouse oocytes transiently form improper syntelic or merotelic attachments (Kitajima et al., 2011). Moreover, knockdown of the Ndc80 complex in both mouse and nematode oocytes results in spindle assembly defects (Dumont et al., 2010; Sun et al., 2010, 2011), suggesting that a role for kinetochores is conserved. Finally, expression of a dominant negative allele of *Drosophila* KLP10A, a kinesin-13/MCAK family member, results in disorganized or extra oocyte spindles poles (Zou et al., 2008), suggesting that a role for kinesin-13/MCAK in limiting pole number also may be conserved. It will be interesting to test whether MCAK-mediated removal of improper microtubule–kinetochore attachments is important for oocyte spindle pole coalescence in other organisms.

Figure 4—Cont'd depolymerase KLP-7/MCAK. Failure to disrupt these incorrect attachments in mutants with reduced KLP-7 function results in an imbalance in spindle tension that interferes with the coalescence of early pole foci and thus results in the assembly of multipolar spindles. (B) Microtubule–kinetochore interactions orient and align bivalents on the metaphase spindle, but at the metaphase-to-anaphase transition, the polar microtubule arrays disassemble, and a new population of microtubules forms between homologs and pushes them apart independently of kinetochores. The assembly of these microtubules is mediated by CLASP and other factors present in ring-shaped structures between each homolog pair at metaphase. When homologs separate, the rings are left behind at the metaphase plate. (C) Lateral interactions between microtubules and microtubule motor proteins mediate both chromosome congression to the metaphase plate and anaphase segregation to the poles. The chromokinesin KLP-19, localized to midbivalent rings, interacts with spindle microtubules to produce a polar ejection force that aligns bivalents at the metaphase plate. Subsequently, during anaphase, polar microtubules interact with dynein, a minus-end directed microtubule motor that accumulates at increasing levels on the poleward/lateral regions of bivalents as meiosis progresses. Dynein-directed motility mediates the poleward movement of chromosomes during anaphase.

10. A PUSHY VIEW OF KINETOCHORE FUNCTION AND CHROMOSOME DYNAMICS DURING OOCYTE MEIOSIS

The influence of microtubule–kinetochore attachment on oocyte spindle pole coalescence in *C. elegans* brings us to an intriguing controversy regarding the importance of kinetochores during oocyte meiosis (Fig. 4B and C). An important early observation was that depleting either components of the Ndc80 complex, responsible for the microtubule attachment activity of the kinetochore, or core kinetochore components such as KNL-1, had remarkably minor effects on meiotic spindle assembly and chromosome organization (Dumont et al., 2010). In live-imaging studies of microtubule and chromosome dynamics, using fusions of GFP and mCherry to β-tubulin and a histone, respectively, kinetochore disruption was shown to cause only minor perturbations in spindle morphology, chromosome congression to the metaphase plate, and anaphase chromosome segregation to the poles. During meiosis I in wild-type *C. elegans* oocytes, spindles shorten substantially prior to anaphase, and during anaphase the poles rapidly disassemble and most spindle microtubules are detected between the separating chromosomes (Fig. 4B). Moreover, knockdown of factors required for the assembly or stability of these anaphase microtubules, such as the microtubule-stabilizing protein CLS-2/CLASP, resulted in both a substantial loss of the interchromosomal microtubules and severe defects in anaphase chromosome movements (Dumont et al., 2010). These findings led Dumont and Desai to propose a model in which microtubule polymerization between the segregating chromosomes pushes the chromosomes apart during anaphase of meiosis I and II, with little if any requirement for microtubule–kinetochore interactions and poleward pulling forces during anaphase (Fig. 4B).

Whether the apparent lack of a substantial role for kinetochores during anaphase in *C. elegans* oocytes is relevant to other species is not known. However, TEM analysis of fixed mouse oocytes during meiosis I and II clearly indicate that microtubules attach to kinetochores, and the kinetochore regions lead the anaphase movements of meiotic chromosomes toward the poles (Brunet et al., 1999). Microtubule–kinetochore attachments have also been observed in human oocytes, although the correction of improper syntelic and merotelic attachments appears to be remarkably inefficient (Holubcova et al., 2015). Furthermore, knockdowns of Ndc80

complex components in mouse oocytes cause substantial defects in spindle organization and chromosome segregation (Sun et al., 2010, 2011), and perturbations of mouse meiotic spindle assembly lead to activation of the kinetochore-based spindle assembly checkpoint (Ma et al., 2010; McGuinness et al., 2009). Nevertheless, very few microtubule–kinetochore attachments form prior to metaphase of meiosis I in mouse oocytes and microtubule–kinetochore attachments may not be required for chromosome congression to the metaphase plate (Brunet et al., 1999). Although mutational inactivation of γ-tubulin disrupts microtubule–kinetochore attachments in *Drosophila* oocytes, the role of these attachments in chromosome segregation is unknown (Hughes et al., 2011). Systematic investigations of how microtubule–kinetochore attachments, and kinetochore function more generally, influence spindle assembly and chromosome movement are needed to fully assess and compare the role of these structures during oocyte meiotic cell division in different animal species.

11. AN ALTERNATIVE MODEL FOR CHROMOSOME MOVEMENTS IN *C. ELEGANS* OOCYTES: SIDES MATTER

While kinetochore-independent microtubule polymerization has been proposed to push chromosomes apart during meiotic anaphase in *C. elegans* oocytes (Dumont et al., 2010), two other studies have suggested a very different model for chromosome congression and segregation (Muscat et al., 2015; Wignall & Villeneuve, 2009). In this model (Fig. 4C), kinetochores are proposed to have little if any role during either congression or segregation. Instead, lateral attachments of polar microtubules to chromosomes, and microtubule motor-mediated pushing and pulling forces, were proposed to move chromosomes during both congression to the metaphase plate and anaphase poleward segregation, respectively.

The first of these two studies demonstrated that the chromokinesin KLP-19 localizes to rings that encircle the midregions of each bivalent, that this midbivalent accumulation of KLP-19 requires the CPC, and that KLP-19 is important for chromosome congression to the metaphase plate (Wignall & Villeneuve, 2009). Knockdown of KLP-19 had relatively modest effects on chromosome orientation relative to the spindle poles, but congression of chromosomes to the metaphase plate was often defective. Moreover, when KLP-19 was knocked down in mutants that make monopolar spindles, chromosomes remained clustered around the monopole. In contrast, chromosomes were usually found close to microtubule ends, far from the

monopole, in oocytes with wild-type levels of KLP-19. Wignall and Villeneuve therefore proposed that lateral associations of spindle microtubules with meiotic chromosomes, together with KLP-19 motor activity, creates a polar ejection force that moves chromosomes toward the spindle midzone. Upon reaching the midzone, chromosomes encounter overlapping, antiparallel microtubules, and a balance of forces result in no net movement toward either pole, aligning chromosomes at the metaphase plate. Polar ejection forces have also been proposed to move chromosomes toward the metaphase plate in *Drosophila* oocytes, in the context of augmin-mediated microtubule polymerization, although whether these forces involve lateral microtubule attachments or end-on attachment at kinetochores was not addressed (Colombie et al., 2013).

In a more recent study, the KLP-19 rings in *C. elegans* oocytes were shown to detach from bivalents at the metaphase-to-anaphase transition, and this detachment was proposed to promote a dynein-dependent reversal in the direction of chromosome movement during anaphase (Muscat et al., 2015). Because dynein has multiple roles in meiotic spindle assembly, chemical inhibitors and conditional alleles were used to disrupt dynein function during chromosome segregation while minimizing effects on spindle assembly. Although the conditions used likely only partially impair dynein function, lagging chromosomes were observed in most oocytes. Dynein also was shown to be required for the poleward chromosome movement in mutants with monopolar spindles. Because the majority of contacts between chromosomes and microtubules appear to occur at the sides of chromosomes rather than at the poles, Muscat et al. proposed that lateral attachments account not only for congression of chromosomes to the metaphase plate, but also for dynein-mediated poleward movement during anaphase (Fig. 4C). In this model, cell cycle-dependent progression in KLP-19 location and possibly in a transition from plus-end directed motor activity to minus-end directed motor activity, accounts for the reversal in the direction of movement at the metaphase-to-anaphase transition.

The two competing models for how chromosomes move to the poles during anaphase in *C. elegans* oocytes (Fig. 4B and C) both have their strengths and weaknesses. In support of the Wignall and Villeneuve model that lateral attachments mediate chromosome congression and anaphase segregation, most of the oocyte spindle microtubules in *C. elegans* oocytes indeed pass by the chromosomes laterally, with very few microtubules appearing to terminate at the poleward surfaces. KLP-19 depletion disrupted chromosome congression in bipolar spindles and outward movement of chromosomes on monopolar spindles, and reducing dynein function had

the opposite effect—lagging chromosomes were observed during anaphase in bipolar spindles and chromosomes failed to move centripetally in monopolar spindles. However, it is important to note that KLP-19 accumulates on both the lateral and poleward faces of meiotic bivalents and on meiotic spindle microtubules (Powers et al., 2004). Similarly, dynein is broadly distributed throughout the meiotic spindle apparatus (Crowder et al., 2015a; Ellefson & McNally, 2009, 2011; van der Voet et al., 2009). In spite of the care taken to disrupt dynein function at the time of chromosome segregation, the methods used likely targeted all dynein, leaving open the possibility that the lagging chromosomes observed resulted from spindle defects rather than from a failure to move chromosomes along lateral microtubules. Finally, *C. elegans* chromosomes are holocentric, with centromeres and kinetochores dispersed throughout the chromosomes (Cheeseman et al., 2004; Dumont et al., 2010; Hagstrom et al., 2002; Howe et al., 2001). Thus even microtubules that pass laterally by microtubules might make contact with kinetochores.

In support of the Dumont and Desai end-on pushing model, all studies of microtubule dynamics during oocyte meiosis in *C. elegans* agree that the polar microtubules observed during prometaphase and early metaphase almost completely disappear during chromosome segregation in anaphase I, while the density of interchromosomal microtubules greatly increases. Moreover, knockdown of CLS-2 and several other factors (HTP-1/2 and BUB-1) clearly disrupted both the assembly of the interchromosomal microtubule network and the poleward movement of homologs (Dumont et al., 2010). However, CLS-2, HTP-1/2, and BUB-1 all associate with lateral and poleward faces of meiotic bivalents in prometaphase and metaphase, as described above for KLP-19, and their depletion results in severe defects in spindle assembly and chromosome congression and/or orientation on the metaphase plate (Dumont et al., 2010). Thus, it remains possible that the defects in anaphase movement are a consequence of the failure to align and orient bivalents within an organized network of microtubules such that they can form productive connections that allow their segregation toward opposite poles, rather than a failure to form a robust interchromosomal microtubule network that pushes chromosomes toward the poles.

It seems likely that a mix of multiple microtubule–microtubule and microtubule–chromosome interactions collaborate and compete with one another to establish the balance of forces that ultimately creates a bipolar spindle, aligns chromosomes at the middle of this spindle, and moves homologs toward opposite spindle poles during anaphase of meiosis I. Elimination

of any one mechanism may compromise some but not all movements. Indeed, a recent publication suggests that both lateral and end-on microtubule attachments contribute to chromosome positioning and movement during Drosophila oocyte meiosis I (Radford et al., 2015). More extensive genetic studies that simultaneously reduce the function of different combinations of these alternative pathways may clarify how they are integrated to execute meiotic cell division. But ultimately, a thorough understanding of the pleiotropic functions of these proteins will likely require techniques that selectively inactivate individual proteins in specific subcellular regions at specific times. Given recent advances in optogenetics, such approaches may soon become possible.

12. ADVANCING OUR UNDERSTANDING OF OOCYTE MEIOTIC SPINDLE ASSEMBLY: GENETICS AND LIVE CELL IMAGING

Compared to mitosis, our understanding of oocyte meiotic cell division lags considerably. Classical genetic screens in both *C. elegans* and *Drosophila* have revealed important players in this fundamental process, and a recent RNA interference screen in mouse oocytes promises to provide further insight (Pfender et al., 2015). While genetic screens and live cell imaging thus far indicate that there are common features among organisms, the molecular pathways that mediate meiotic spindle assembly appear to be numerous and diverse. To advance our understanding, more systematic genetic analyses are needed.

But genetic analysis has its limits. For example, many of the genes required for meiotic spindle assembly have earlier essential requirements. Null alleles of genes required for meiosis are often lethal because of mitotic requirements, precluding definitive genetic investigation of gene requirements during oocyte meiosis. While RNA interference, temperature sensitive mutations, and chemical inhibitors can bypass earlier requirements, all are inherently compromised by uncertainty as to how completely or specifically they eliminate gene functions.

The challenges to achieving a conclusive mechanistic understanding of meiotic spindle assembly and function in any one system extend well beyond a need for further genetic studies. Meiotic spindles are often small and in some cases relatively inaccessible to live cell imaging, although recent advances in light microscopy and the application of electron tomography will provide new insight. In addition, the further application of live cell-

imaging techniques, with genome editing technology to generate endogenous fluorescent protein fusions, holds much promise. Indeed, a systematic labeling of all meiotic spindle components with fluorescent protein markers, coupled with simultaneous live cell imaging of two or more proteins, is very much needed to provide a foundation of wild-type spindle assembly for comparison to mutant phenotypes. The pace of progress in this field has picked up considerably in the past five years, and recent advances in genetics and microscopy make rapid and significant further progress imminent.

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