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Nuclear and Spindle Positioning during Oocyte Meiosis

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Abstract

Female meiosis is unique in that an asymmetrically positioned meiotic spindle expels chromosomes into tiny, non-developing polar bodies. The extrusion of chromosomes into polar bodies is always mediated by meiotic spindles that are attached to the oocyte cortex by one pole. The asymmetric, cortical positioning of the oocyte meiotic spindle preserves the volume and contents of the oocyte. Recent work in *C. elegans* and mouse has provided mechanistic details of spindle positioning in oocytes.

Introduction

Sexual reproduction requires the generation of gametes with unreplicated haploid genomes from germ cells with replicated diploid genomes. In oocytes, half of the genome is expelled into a tiny non-developing cell called a first polar body during anaphase of meiosis I, then half of the remaining genome is expelled into a second polar body during anaphase of meiosis II. These highly asymmetric divisions preserve the size and cytoplasmic contents of the oocyte for the fertilized zygote. Hormonal signaling drives immature oocytes arrested in G2/prophase into M-phase oocytes in a process called maturation. In many species, the oocyte nucleus (called a germinal vesicle) moves from a central location to a predetermined spot on the cortex called the animal pole either before or during the maturation process. This nuclear migration places the assembling spindle close to the cortex. After nuclear envelope breakdown, many species exhibit further translocation of the meiotic spindle to the cortex, often resulting in a spindle whose pole to pole axis is parallel to the cortex. Subsequent rotation of the spindle results in an orientation perpendicular to the cortex. The perpendicular orientation with one spindle pole attached to the cortex at anaphase is extremely conserved across animal phyla and presumably facilitates the expulsion of half the chromosomes into a polar body. A complete failure in chromosome expulsion results in a pentaploid embryo after fertilization and such embryos do not survive. Some insects naturally do not extrude polar bodies and instead avoid polyploidy by destroying the three “extra” chromosome sets segregated during a 2-spindled anaphase II [1]. In these organisms, meiotic spindles are still attached to the cortex by one pole at anaphase and it is the chromosomes pushed toward the cortex that are targeted for destruction [2,3].

Here we review experiments addressing the mechanisms of germinal vesicle migration, spindle migration and spindle rotation carried out in echinoderms, *Xenopus*, *C. elegans* and mouse. Studies in different species reveal both similarities and differences and each species

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offers some experimental advantage. Physical manipulation of germinal vesicles has been most fruitful in echinoderms and *Xenopus* whereas genetics and live imaging with fluorescent protein fusions have been most productive in *C. elegans* and mouse.

Nuclear migration

Germinal vesicle migration from a central position to a predetermined spot at the cortex of an obviously polarized oocyte often precedes spindle migration. For example, the sea cucumber oocyte has a predetermined animal pole where the maternal centriole-containing centrosomes are anchored. Microtubules extend from the animal pole centrosome to the nucleus during migration and these microtubules are required for nuclear migration [4]. In this case, nuclear migration may be mediated by the minus-end directed microtubule motor, cytoplasmic dynein, on the nuclear envelope moving toward microtubule minus ends at the cortically anchored centrosome as proposed for pronuclear migration [5,6]. Consistent with this pulling mechanism, the animal pole cortex of the sea cucumber oocyte invaginates toward the nucleus during migration [4]. The same mechanism may anchor the germinal vesicle at the cortex of starfish oocytes as microtubule depolymerization reduces the amount of centrifugal force required to displace the germinal vesicle away from the animal pole cortex [7]. Neither germinal vesicle migration nor anchoring at the cortex is affected by actin depolymerization in these echinoderms.

Centrioles and astral microtubule arrays are not present in diakinesis stage *C. elegans* oocytes [8] but microtubules are used to position the germinal vesicle in the center of the immature oocyte (Fig. 1) [9] and the microtubule motor, kinesin-1, is required to move the germinal vesicle to the cortex during maturation (Fig. 1) [10]. Although microtubules fill the cytoplasm of these oocytes [9,11], the mechanism of nuclear migration is not clear because the polarity of the microtubules extending between the nucleus and cortex has not been resolved. Unlike the sea cucumber oocyte, the *C. elegans* oocyte is not obviously polarized before this migration event. Instead, the germinal vesicle appears to migrate away from the source of maturation hormone which is secreted by sperm in the spermatheca (Fig. 1) [12,13]. In kinesin 1-depleted embryos, the distance between the germinal vesicle and the cortex distal to the spermatheca does not change, but the germinal vesicle still ends up asymmetrically positioned, presumably due to asymmetric growth of the oocyte toward the spermatheca [10].

Maturing mammalian oocytes, like those of *C. elegans*, have no centrioles [14] and are not obviously pre-polarized [15]. As in *C. elegans*, maturation-hormone dependent movement of the germinal vesicle from a central position to a cortical position has been documented in blue fox oocytes matured in vivo [16]. In contrast, mouse oocytes matured in vitro undergo nuclear envelope breakdown with the germinal vesicle near the center of the oocyte [17]. Barrett and Albertini [18] have provided evidence that the mouse germinal vesicle is positioned near the cortex when gap junctions between oocyte and cumulus cells are maintained. However, this remains controversial [19] and meiotic spindles that assemble near the center of the mouse oocyte are able to migrate to the cortex and extrude polar bodies [17]. Likewise, in kinesin 1-depleted *C. elegans* oocytes, spindles assemble further from the cortex than in wild-type, but a partially-redundant dynein-dependent mechanism eventually positions spindles at the cortex [20–22]. In contrast, physical displacement of the germinal vesicle away from the animal pole cortex causes defects in polar body extrusion in the pre-polarized oocytes of sea cucumber [4] and *Xenopus* [23].

Chromosome capture

In most oocytes, the germinal vesicle has a much greater diameter than the pole-pole length of the meiosis I spindle, thus necessitating special mechanisms to collect the chromosomes

from an enormous volume of 3D space onto a tiny spindle. In *Xenopus*, the germinal vesicle has a diameter of 400 μm [24] while the initial pole-pole length of the meiosis I spindle is 17 μm [25]. In these oocytes, a unique transient microtubule array (TMA) forms on the vegetal surface of the germinal vesicle during nuclear envelope breakdown. This structure is thought to both collect the chromosomes from the large nuclear volume and transport the chromosomes closer to the cortex where the spindle assembles [25]. The starfish germinal vesicle is 80 μm in diameter while the microtubules nucleated from the prometaphase centrosomes extend only 20 μm from the animal pole cortex. In these oocytes, a contractile actin meshwork collects the chromosomes onto the spindle [26]. In *C. elegans*, the germinal vesicle is 12 – 15 μm in diameter whereas the meiosis I spindle is initially only 8 μm long. In this case, chromosomes appear to be collected from the large nuclear volume by a cloud of microtubules that fill the volume of the nucleus during germinal vesicle breakdown and which re-organize into a more compact bipolar spindle [9,27].

Spindle migration

Because of the size difference between the *C. elegans* germinal vesicle and the bipolar spindle, the spindle assembles on average 4 μm away from the cortex [9,22], even though the germinal vesicle was contacting the cortex before germinal vesicle breakdown [10]. Shortly after assembly, the 8 μm long meiotic spindle translocates at a velocity of 1.0 $\mu\text{m}/\text{min}$ in a sideways fashion ending at the cortex with the spindle axis parallel to the cortex (Fig. 2). Translocation of the meiotic spindle in *C. elegans*, in contrast to mice, does not require F-actin. Treatment with latrunculin A or depletion of profilin by RNAi blocked polar body extrusion but had no effect on the rate of movement of chromosomes to the cortex [9].

In *C. elegans* embryos, meiotic spindle translocation requires microtubules. Depletion of tubulin by RNAi results in chromosomes that remain stationary in the center of the embryo. These embryos exit M phase and form pronuclei with normal timing indicating that failed migration is not due to checkpoint activation or cell death. Spindle translocation is also blocked in oocytes depleted of the microtubule-severing enzyme katanin [9] and in embryos depleted of kinesin-1 [22]. Since kinesin-1 is a plus-end directed microtubule motor, it might seem intuitive that spindle translocation would be a result of kinesin-1 directly transporting the meiotic spindle to the cortex along microtubules with plus-ends at the cortex. However, kinesin-1 heavy chain, light chain and an interacting protein do not localize on the meiotic spindle or surrounding area near the spindle. Cytoplasmic microtubules densely populate the entire *C. elegans* meiotic embryo with a fraction of these microtubules extending diagonally inward from the cortex. The polarity of these microtubules has not been directly determined, however, analysis of kinesin-1 dependent movement of yolk granules supports the presence of a population of cytoplasmic microtubules with their minus-ends anchored at the cortex and plus-ends extending inward [10]. This arrangement of microtubules has been demonstrated directly by hook decoration in *Xenopus* oocytes [28]. Thus kinesin-1 may play an indirect role in spindle translocation. Since kinesin-1 is required for positioning the germinal vesicle at the cortex, it is possible that depletion of kinesin-1 results in meiotic spindles assembled at a distance from the cortex that is too far for some unknown translocation mechanism to function. Another possibility is that after positioning the germinal vesicle at the cortex, kinesin-1 also functions to anchor the spindle to the cortex in meiosis I and II.

In mouse oocytes, the meiosis I spindle assembles near the center of the oocyte, then translocates in a pole-first orientation at 0.12 $\mu\text{m}/\text{min}$ over an average distance of 17 μm so that perpendicular orientation at the cortex is achieved without a discrete rotation event (Fig. 3A) [17]. The spindle migrates to the nearest point on the cortex, even if displaced after migration initiates, with no apparent pre-determined animal pole [29]. When microtubules

are depolymerized with nocodazole, mouse meiotic chromosomes still migrate to the cortex [17] and with increased instantaneous velocity [30]. Spindle migration is blocked by actin depolymerizing drugs [17], an actin-stabilizing drug [30], loss of the actin nucleator Formin 2 [30,31], inhibition of the actin regulator, cdc42 [32], or inhibition of myosin II [29] suggesting an actin-dependent motility mechanism. Elucidation of the arrangement and polarity of actin filaments during spindle migration is thus essential to define a mechanism.

Recently, a meshwork of inter-connected actin filaments filling the entire cytoplasm of the mouse oocyte has been demonstrated both in fixed preparations by phalloidin staining and with a GFP-fusion to an actin-binding protein in living oocytes. The density of this meshwork increased in front of the migrating spindle as it approached the cortex [29,33]. Using a different fluorescent actin-binding peptide, Li *et al.* [30] observed a completely different actin cytoskeleton consisting of a cloud of actin initially surrounding the spindle and left behind as the spindle migrates. This large-scale arrangement of actin is reminiscent of the actin comets that form behind *Listeria* and propel *Listeria* by polymerization-driven pushing forces [34]. Li *et al.* were unable to detect this actin cloud behind the translocating spindle by a second method and this cloud was not detected by Schuh and Ellenberg [29] or by Azoury *et al.* [33].

Schuh and Ellenberg proposed that myosin II anchored on the spindle pole moves on the interconnected actin bundles extending toward the nearest point on the cortex to generate a pulling force (Fig. 3BII). In contrast, Li *et al.* proposed that actin polymerization pushes the spindle from behind (Fig. 3BIII). Invagination of the cortical actin cytoskeleton toward the approaching spindle pole and movement of actin structures toward the leading spindle pole [29] are consistent with a pulling force between the spindle and cortex, and inconsistent with a polymerization-driven mechanism. The biochemistry of myosin II, however, suggests a different way of generating a pulling force between the leading spindle pole and the cortex. The active form of myosin II is a bipolar thick filament consisting of numerous myosin hexamers arranged in an anti-parallel fashion to allow inward sliding or contraction of anti-parallel actin filaments [35,36]. Anchoring of myosin on a cargo and movement on cytoplasmic actin filaments, as proposed by Schuh and Ellenberg, is more commonly ascribed to other myosin classes like myosin V [37]. Myosin II could mediate spindle migration by cross-bridging anti-parallel actin filaments and thereby contracting the actin network that extends between one spindle pole and the cortex (Fig. 3BI). These models differ in the polarity of actin filaments which has not been elucidated in mouse oocytes. The localization of myosin II also might distinguish between these mechanisms. Schuh and Ellenberg showed staining with a phospho-myosin regulatory light chain antibody indicating localization at spindle poles as well as throughout the cytoplasm where the actin network is. Similar immunofluorescence by Dumont *et al.* [31] revealed staining of cytoplasmic foci but did not reveal spindle pole staining. Thus the mechanism of actin-driven spindle migration in mouse oocytes like the mechanism of microtubule-driven spindle migration in *C. elegans* both await improved resolution of filament polarity and motor localization.

Spindle rotation

In many species, both meiosis I and meiosis II spindles initially adopt a parallel orientation at the cortex before undergoing a discrete rotation to a perpendicular orientation [9,25,38]. Rotation occurs only during meiosis II in mouse [39] and rat oocytes [40]. Spindle rotation followed by anaphase chromosome segregation effectively places the chromosomes destined for expulsion close to the cortex and places chromosomes destined for inheritance in the oocyte interior. The perpendicular spindle orientation resulting from rotation may facilitate extrusion of small polar bodies. In *C. elegans*, inhibition of meiotic spindle rotation leads to large polar bodies and aneuploid embryos [20,21].

In *C. elegans*, the meiosis I spindle remains parallel at the cortex and maintains a steady-state pole to pole length of 8 μm for 6 min before initiating a highly ordered series of movements that all depend on the anaphase promoting complex (APC/C). The spindle first shortens to 4.8 μm , homologous chromosomes then separate as the spindle shortens further to 2.3 μm , then the spindle narrows and lengthens to 5 μm during anaphase B [9]. The APC/C-dependence and relative timing of spindle rotation varies among other species. Vertebrate oocytes arrest in metaphase II due to natural APC/C inhibition. In mouse, the arrested spindle is parallel to the cortex and rotation only occurs after fertilization, indicating that rotation is APC/C-dependent as in *C. elegans* [41,42]. In contrast, *Xenopus* meiosis I spindles rotate during prometaphase so that the arrested oocytes have perpendicular metaphase spindles [25].

In *C. elegans*, spindle rotation is apparently independent of F-actin [9] or kinesin-1 [20,22], but requires the minus-end directed microtubule motor, cytoplasmic dynein. Partial depletion of dynein by RNAi results in meiotic spindles that remain parallel at the cortex through anaphase [20]. Meiotic spindle rotation also requires the function of the dynein regulator, LIN-5 (ortholog of mammalian NuMA), which is targeted to meiotic spindle poles through direct binding to ASPM-1 (abnormal spindle-like, microcephaly-associated) [21]. Cytoplasmic dynein is diffusely localized to the metaphase meiotic spindle, then just prior to rotation, dynein accumulates on meiotic spindle poles. Accumulation of dynein on meiotic spindle poles, like spindle rotation, requires the APC/C [9,20]. The mechanism by which dynein mediates rotation, however, is unclear.

In the absence of kinesin-1, spindles migrate late to the cortex, in a pole-first orientation, in a dynein-dependent and APC/C-dependent manner [20]. This result indicates that the dynein-dependent rotation mechanism can move one spindle pole toward the cortex, on a linear track, over a distance of up to 8 μm [20,22]. It is therefore reasonable to speculate that spindle rotation is mediated by transport of one spindle pole toward the cortex along a straight microtubule. If dynein is docked on spindle poles by its cargo-binding domain, dynein might walk on cytoplasmic microtubules with minus-ends anchored at the cortex. If *C. elegans* meiotic spindles have astral microtubules with minus ends anchored in the spindle pole and plus-ends extending toward the cortex, then dynein may transiently dock on the cortex and generate a brief pulling force before releasing from the cortex and motoring to the spindle pole. Thus defining the rotation mechanism depends on elucidating the orientation of microtubules extending between the spindle and cortex during rotation.

Rotation of the mouse meiosis II spindle is blocked by actin-depolymerizing drugs [42] and myosin II-inhibiting drugs [41]. Rat meiotic spindle rotation is similarly blocked by an actin-depolymerizing drug and, in addition, by low concentrations of a microtubule-depolymerizing drug [40]. One possibility is that rodent meiotic spindle rotation is mediated by a dynein-dependent cortical pulling mechanism as in *C. elegans* and that an intact actomyosin cortical cytoskeleton is required to keep the cortex rigid so that one spindle pole moves to the cortex instead of cortical fragments moving toward the spindle pole. In this scenario, *C. elegans* meiotic embryos may have an actin-independent mechanism to maintain cortical rigidity as is the case in *S. cerevisiae* [43] or the latrunculin treatments reported by Yang *et al.* [9] may not have depolymerized all of the F-actin.

Summary and Future Directions

Oocyte meiotic spindles are oriented perpendicular to the cortex at anaphase in all animals but variations exist in the mechanisms leading up to this final orientation. Echinoderms use centriole-based astral microtubule arrays. *C. elegans* uses the microtubule motor kinesin-1 to initially place the meiotic spindle in a parallel orientation at the cortex, then uses

cytoplasmic dynein to rotate the spindle to a perpendicular orientation. In mouse, an actin and myosin-based system is used to move the meiosis I spindle to the cortex in a pole-first orientation. In all cases, the detailed molecular mechanisms have not yet been elucidated, in part because filament polarity has not been determined.

An ongoing question is whether the spindle pole that is transported toward the cortex during rotation or during pole-first migration is predetermined. There is currently no molecular marker that displays an asymmetric localization pattern before spindle rotation or pole-first migration. Thus it is not clear whether inheritance is always stochastic or if there are mechanisms determining which set of chromosomes are inherited [44].

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Figure 1.

Migration of the germinal vesicle during oocyte maturation in *C. elegans*. Images of oocytes within living worms expressing mCherry::histone (chromosomes and nucleoplasm) and GFP::PH (plasma membrane). (A) Second most mature oocyte (-2) with germinal vesicle positioned in the center of the oocyte. (B) Most mature oocyte (-1) showing movement of the germinal vesicle towards the oocyte cortex distal to the spermatheca. (C) Most mature oocyte just prior to GVBD, showing germinal vesicle positioned close to the oocyte cortex distal to the spermatheca. Sp denotes location of the spermatheca. Red arrows indicate direction of germinal vesicle movement.

Figure 2.

Meiotic spindle positioning in the *C. elegans* embryo.. (A) Images of meiotic embryos within living worms expressing GFP::tubulin (microtubules, green) and mCherry::histone (chromosomes, red). Time=0 is at germinal vesicle breakdown. The embryo cortex is outlined in yellow dashed lines for clarity. Upper row, low magnification showing spindle position within the embryo. Lower row, high magnification images showing spindle orientation. Sp denotes the spermatheca. (B) Animation of images in A. Microtubules (green), chromosomes (red), and sperm (orange). The meiotic spindle is assembled a short distance from the cortex after germinal vesicle breakdown (GVBD), then translocates to a parallel orientation at the cortex after fertilization. The metaphase meiotic spindle remains at a constant 8 μ m length and parallel position until activation of the anaphase-promoting complex (APC). After APC activation the meiotic spindle shortens and then rotates 90° to a perpendicular position. Following rotation the meiotic spindle continues to shorten in its pole-to-pole axis during early anaphase chromosome segregation, then elongates in an anaphase B-like process during extrusion of the polar body. Time of activation of the anaphase-promoting complex is denoted with a red line. Text and black lines below images indicate molecular components required for different positioning events

Figure 3. Spindle migration in the mouse oocyte. (A) Animation of spindle movement toward the cortex in a maturing mouse oocyte. The meiosis I spindle assembles near the center of the oocyte and migrates toward the nearest site on the cortex in a pole-first orientation, thereby eliminating a need for spindle rotation. Metaphase II spindles are parallel to the cortex and rotate after fertilization. B. Diagram of possible mechanisms of actin-dependent spindle translocation. I) contraction of anti-parallel actin filaments connecting the cortex to the spindle by bipolar myosin II mini thick filaments, II) cargo-like transport of the spindle along actin filaments with barbed ends oriented toward the cortex, with myosin II localized

at the spindle pole and III) actin polymerization-driven motility from the rear. Actin - red, microtubules-green, chromosomes – blue, myosin II - black. Outer circle = zona pellucida.