Role of *Filia*, a maternal effect gene, in maintaining euploidy during cleavage-stage mouse embryogenesis

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During oogenesis, mammalian eggs accumulate proteins required for early embryogenesis. Although limited data suggest a vital role of these maternal factors in chromatin reprogramming and embryonic genome activation, the full range of their functions in preimplantation development remains largely unknown. Here we report a role for maternal proteins in maintaining chromosome stability and euploidy in early-cleavage mouse embryogenesis. *Filia*, expressed in growing oocytes, encodes a protein that binds to MATER and participates in a subcortical maternal complex essential for cleavage-stage embryogenesis. The depletion of maternal stores of Filia impairs preimplantation embryo development with a high incidence of aneuploidy that results from abnormal spindle assembly, chromosome misalignment, and spindle assembly checkpoint (SAC) inactivation. In helping to ensure normal spindle morphogenesis, Filia regulates the proper allocation of the key spindle assembly regulators (i.e., AURKA, PLK1, and γ-tubulin) to the microtubule-organizing center via the RhoA signaling pathway. Concurrently, Filia is required for the placement of MAD2, an essential component of the SAC, to kinetochores to enable SAC function. Thus, Filia is central to integrating the spatiotemporal localization of regulators that helps ensure euploidy and high-quality cell cycle progression in preimplantation mouse development. Defects in the well-conserved human homologue could play a similar role and account for recurrent human fetal wastage.

aneuploidy | mitotic kinases | spindle assembly checkpoint

After fertilization, the mouse embryo divides 3 times before compacting at 8 cells to form a morula at embryonic day 2.5 (E2.5). The subsequent formation of a fluid-filled blastocele at 32 cells establishes the early blastocyst (E3.5), which implants on the wall of the uterus at ≈E4.5. This period of early embryogenesis encompasses 3 major events: activation of the embryonic genome at the 2-cell stage (1), formation of initial cell lineages (2), and establishment of embryonic axes (3). In addition to early embryonic gene products, proteins encoded by maternal genes that accumulate during oogenesis and persist during early embryogenesis are critical for successful preimplantation development. Such maternal effect genes are well described in other model organisms (4–7) but have become an investigative focus in mammals only recently (8).

Genetic ablation in mice has identified several maternal effect genes, including *Stella* (official symbol, *Dppa3*), *Zar1*, and *Brg1* (official symbol, *Smarca4*), that are required for the very earliest stages of development. Maternally produced *Stella* is indispensable for the maintenance of methylation involved in the epigenetic programming after fertilization (9–11). *Zar1* transcripts are uniquely expressed in oocytes, but the protein persists in 1-cell stage zygotes, where it is required for the successful union of paternal and maternal haploid genomes (12). *Brg1* is expressed in both oocytes and embryos, and conditional depletion of maternally expressed *Brg1* impairs the embryonic genome activation in mouse 2-cell embryos (13). The absence of other maternal proteins, including MATER (official symbol, *Nlrp5*), PADI6, and FLOPED (official symbol, *Ooep*), blocks embryos at the 2-cell stage (14–16), reminiscent of treatment of embryos with α-amanitin, which inhibits activation of the embryonic genome (1). Even after activation of the embryonic genome, maternal factors continue to play important roles during preimplantation development, as indicated by impaired early embryogenesis after genetic ablation of *Tcl1* and *Npm2* (17, 18); however, the functions and molecular mechanisms through which they affect development remain unknown.

In addition to individual maternal proteins, the characterization of Filia as a ≈50-kD binding partner for MATER (14) established the presence of a maternal effect complex in the subcortex of eggs and preimplantation embryos (19). Filia is encoded by a single-copy gene that is expressed uniquely in growing oocytes of adult animals. As with the majority of egg mRNAs (20, 21), *Filia* transcripts are degraded during meiotic maturation and ovulation, but the cognate protein persists during preimplantation development. Filia and MATER participate in a subcortical maternal complex (SCMC) that is essential for preimplantation development (16). The SCMC contains at least 4 components (FLOPED, MATER, TLE6, and Filia). *Floped*$^{tm/tm}$ and *Mater*$^{tm/tm}$ females have normal ovarian histology and ovulate eggs that can be fertilized; however, embryos progress poorly beyond the first cleavage, and null females are sterile (14, 16). The severity of the phenotype makes it uncertain whether the molecular defects seen in embryos lacking the SCMC are causative or reflective of imminent cell death. Here we report the less catastrophic *Filia* null phenotype, providing mechanistic insight into its role as an upstream regulator/integrator in ensuring the fidelity of chromosome segregation during preimplantation mouse development.

Results

Reduced Fecundity of *Filia* Null Females. To investigate the function of Filia in early embryogenesis, mouse lines lacking the protein were established using DNA recombineering (22) and targeted ablation in embryonic stem cells [supporting information (SI) Fig. S1*A–E*]. *Filia*$^{+/tm}$ intercrosses produced 275 pups from 32 litters (mean, 8.6 ± 2.0 pups/litter) with expected Mendelian ratios (Table 1); however, *Filia*$^{tm/tm}$ females mated with normal, heterozygous, or homozygous null males produced litters of 4.1 ± 2.2 pups (44 litters), 3.3 ± 2.4 pups (13 litters), and 4.6 ± 2.7 pups (38 litters), respectively (Table 1). The inability of a normal paternal allele to rescue the ≈50% decrease in fecundity and the presence of *Filia* transcripts in oocytes, but not embryos (19), implicated *Filia* as a maternal effect gene. The mixed BL6/Sv129 genetic background of the *Filia* null females may contribute to variable litters size (0–5 litters) during ≈6 months of mating and to the variable time from mating to delivery, which ranged from ≈20 days to 120 days, with an average of ≈30 days (normal, ≈20 days).

Impaired Preimplantation Embryonic Development in *Filia* Null Females. The decrease in fecundity did not result from abnormal oogenesis, ovulation, or fertilization (Fig. S2 *A* and *B*), and, unlike

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Table 1. Fertility of *Filia*$^{tm/tm}$ mice

Mice	Male *Filia*$^{tm/tm}$	Male *Filia*$^{tm/+}$	Male *Filia*$^{+/+}$
Female *Filia*$^{tm/tm}$	4.6 ± 2.7 (38)*	3.3 ± 2.4 (13)	4.1 ± 2.2 (44)
Female *Filia*$^{tm/+}$	8.3 ± 2.1 (28)†	8.6 ± 2.0 (32)‡	ND§

*Litter size ± SEM (number of litters).
†Genotype, *Filia*$^{tm/tm}$:*Filia*$^{tm/+}$:*Filia*$^{+/+}$::121:111:0; gender, male:female::113:119.
‡Genotype, *Filia*$^{tm/tm}$:*Filia*$^{tm/+}$:*Filia*$^{+/+}$::69:135:71; gender, male:female::133:142.
§ND, not determined.

the absence of FLOPED or MATER, which caused the disappearance of SCMC components (16), the SCMC persisted in eggs and embryos derived from *Filia* null females (Fig. S2 *C* and *D*). Although similar numbers of 2-cell embryos were recovered from normal and null females at E1.5, dramatically fewer morulae or blastocysts were collected from null mice at E2.5 or E3.5, respectively (Fig. 1*A*). Consistent with these in vivo observations, when 2-cell embryos collected at E1.5 from null and normal mice were cultured for an additional 3 days, significant decreases in the percentages of embryos reaching the morula and blastocyst developmental stages were seen (Fig. S3).

To more precisely define the developmental impairment, E2.5 embryos were flushed from *Filia* null females after mating with normal males. Embryos were variably affected and ranged from 1-cell zygotes to morulae, with most delayed at the 3- to 4-cell stage (Fig. 1*B* and *C*). Embryos were then assigned to 1 of 3 groups (group I, 3–4 cells; group II, 5–8 cells; group III, precompaction and postcompaction morulae) and cultured for 48 h. Embryos from group III had no block to development. Embryos from group II had slightly reduced development, with 67% (n = 15) reaching the blastocyst stage. In group I, most of the embryos (79%; n = 142) continued cell division, 64% underwent compaction, and 42% formed blastocele cavities as developmentally appropriate after 1 day or 2 days of culture, respectively (Fig. 1*D*). However, these morula- and blastocyst-like structures contained fewer cells than normal (E3.5 morula: ≈8 cells compared with ≈16–32 cells in controls; E4.5 blastocyst: ≈15 cells compared with >36 cells in controls). Thus, the absence of maternal Filia appears to delay embryonic progression rather than impose a complete arrest in early development. We confirmed this by examining the progression of the first and second mitotic cell divisions, which were delayed for 6–8 h in embryos lacking Filia (Fig. 1*E* and *F*). The delay was prompted by a prolonged G2 phase, as indicated by the presence of the cell cycle marker phosphohistone H3$^{Ser\text{-}10}$ (data not shown), suggesting that the absence of maternal Filia compromised cell cycle progression at the G2/M transition.

Aneuploidy in Embryos Derived from *Filia* Null Females. Two populations emerged from the evaluation of E2.5 embryos derived from *Filia*$^{tm/tm}$ females, one that was largely unaffected and progressed to blastocysts in a timely manner and the other that had a variety of molecular defects that resulted in poor embryonic development. The frequent observation of micronuclei (Fig. 2*A*) in the latter population suggested a defect in chromosome stability. Therefore, we examined the karyotype of the delayed embryos from *Filia* null females, but restricted our analysis to hyperploidy to avoid chromosome spread preparation artifacts that could result in hypoploidy. As anticipated, a significantly higher rate of hyperploidy (41–42 chromosomes) was observed in embryos from *Filia* mutant females (25.0%; n = 72) compared with normal controls (1.4%; n = 72) (Fig. 2*B*). We note that pseudoeuploidy with coexisting monosomy and trisomy would not be detected by counting chromosomes, and that our results likely underestimate the actual rates and extent of aneuploidy.

To determine whether a defect in spindle assembly contributed to the aneuploidy, we imaged the mitotic spindles of embryos from *Filia* null females by immunofluorescence microscopy. Strikingly,

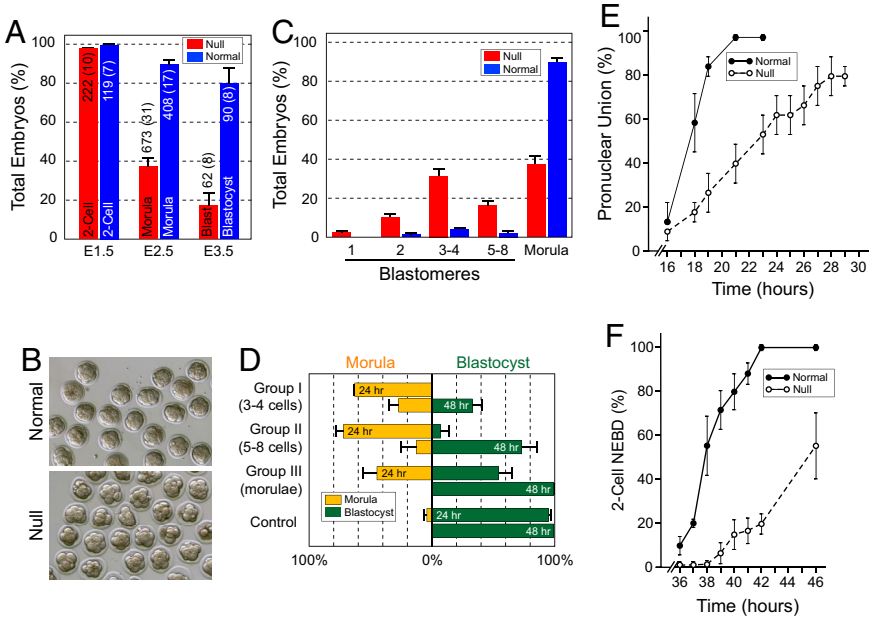

Fig. 1. Impaired preimplantation development of embryos from *Filia*$^{tm/tm}$ females. (*A*) In vivo development of embryos from adult normal (blue bar) and *Filia* null (red bar) females. The numbers on or above the bars reflect the number of embryos (number of females) analyzed. (*B*) Representative pictures of the embryos recovered at E2.5 from normal and *Filia* null mice. (*C*) Embryos (n = 673) recovered at E2.5 from 31 *Filia* null females had delayed embryonic development, ranging from 1-cell zygotes to 8 cells, with the major delay at the 3- to 4-cell stage (red bar). In contrast, most embryos from normal females were at the morula stage (blue bar). (*D*) In vitro culture of embryos from normal and *Filia* null females recovered at E2.5. (*E*) E0.5 zygotes from normal and *Filia* null females were cultured (175 from null females; 119 from normal controls). Beginning 16 h after fertilization at midnight, the percentage of 1-cell zygotes with a pronuclear union was determined morphologically. (*F*) Same as (*E*), except that nuclear envelop breakdown (NEBD) was determined morphologically in 2-cell embryos (195 from null females; 124 from normal controls) beginning 36 h after fertilization.

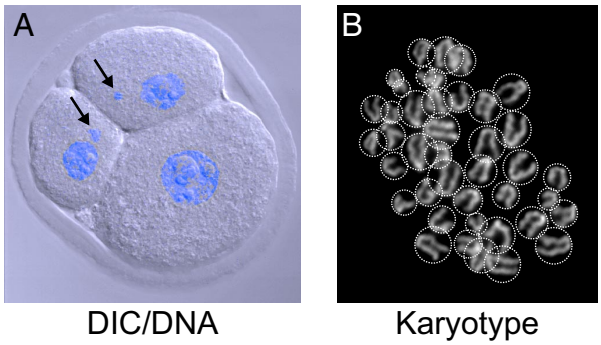

Fig. 2. Aneuploidy in embryos from *Filia* null females. (*A*) Micronuclei (arrows) in embryos from *Filia* mutant females. (*B*) Metaphase spread of a blastomere derived from a delayed *Filia* null embryo with 41 chromosomes (dotted circles).

69.2% of mitoses (n = 104) in delayed embryos from *Filia* null females had improper spindles, compared with 10.8% (n = 65) in controls. The abnormalities included 1-polar (22.2%), 3-polar (9.7%), broad polar (9.7%), and irregularly shaped (58.3%) spindles (Fig. 3*A*). To further investigate spindle assembly in vivo, we microinjected in vitro transcribed mRNAs encoding GFP-tagged β_5-tubulin and obtained time-lapse images to visualize the formation of spindles in *Filia* mutant embryos. Although we observed a high incidence of abnormal spindle formation [78.0% (n = 41), compared with 7.1% (n = 16) in normal embryos], the absence of maternal Filia did not affect spindle behavior. As with normal embryos (23), the spindles in embryos from *Filia* mutants did not change their orientation until cell cleavage, bipolar spindles elongated normally, and the spindles were anchored to the cortex (Fig. 3*B*). Spindle abnormalities often are accompanied by chromosome misalignment, which also can lead to aneuploidy. Therefore, we examined chromosome congression and consistently observed a high incidence of chromosome misalignment in embryos from *Filia* mutant females by immunofluorescent microscopy [64.3% (n = 98) vs 10.8% (n = 65) in control embryos] (Fig. 4*A* and *B*), as well as in time-lapse images [72.0% (n = 25) vs 18.8% (n = 16) in normal embryos].

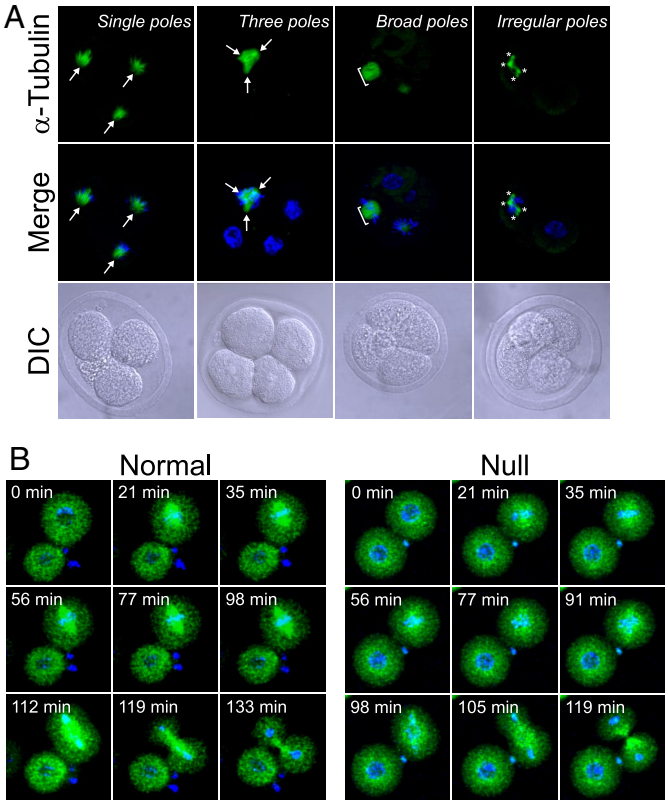

Fig. 3. Spindle dysmorphogenesis. (*A*) Immunofluorescence of delayed embryos recovered from *Filia* mutant females at E2.5 showing a high incidence of abnormal spindles, including 1-polar (arrows), 3-polar (arrows), broad spindles (brackets), and irregular shapes (asterisks). (*B*) Time-lapse images showing spindle formation and movement in embryos from normal and *Filia* mutant females after microinjection with GFP-β5 tubulin mRNA. The time points of the nuclear envelop breakdown were set at 0 min.

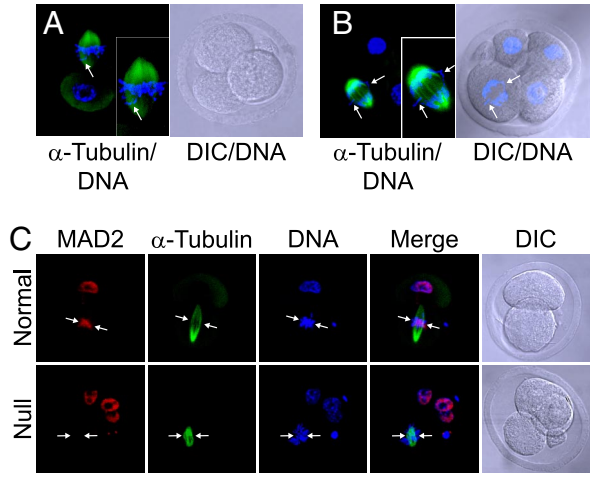

Fig. 4. Inactivation of the SAC. (*A*) Metaphase with misaligned chromosomes (arrow) in delayed embryos from *Filia* null females. (*B*) Same as (*A*) showing lagging chromosomes (arrows) at the onset of anaphase. (*C*) MAD2 required for the SAC was located on kinetochores (arrows) of normal embryos, but was not detected on kinetochores of delayed embryos from *Filia* mutant females.

A second critical event governing proper chromosome segregation during mitosis is the spindle assembly checkpoint (SAC). To investigate whether the SAC played a role in the aneuploidy observed in embryos from *Filia* mutants, we examined chromosomes in anaphase. In both immunofluorescent and time-lapse images, we frequently observed lagging chromosomes at the onset of anaphase in embryos from *Filia* mutant females (Fig. 4*B*), indicating failure of the SAC in the absence of Filia. The SAC operates in mouse oocytes and early cleavage embryos, and its activation requires the presence of MAD2 (official symbol, *Mad2l1*) at kinetochores (24, 25). In normal embryos, MAD2 was located on kinetochores at prophase, prometaphase, and metaphase (100%; n = 21); however, in affected embryos from *Filia* mutant females, MAD2 was absent from kinetochores (100%; n = 33) (Fig. 4*C*). Furthermore, in embryos treated with colchicine to inhibit the microtubule polymerization and leave kinetochores unattached, MAD2 was detected at kinetochores of normal embryos but not at those of *Filia* mutants (Fig. S4*D*).

Dysregulation of AURKA, PLK1, and γ-Tubulin in Embryos from *Filia* null Females. Centrosomes play key roles in regulating cell cycle progression and spindle assembly in somatic cells (26). We examined whether the centrosome-associated spindle assembly regulators aurora kinase A (official symbol, *Aurka*) and polo-like kinase 1 (official symbol, *Plk1*) were affected by depletion of maternal Filia and might mediate the function of *Filia* on spindle morphogenesis. As reported previously (27), AURKA was highly concentrated in nuclei at interphase and on spindle apparatus at mitosis in normal embryos (Fig. 5*A*). Strikingly, AURKA was completely absent on mitotic spindles of virtually all affected embryos from *Filia* null

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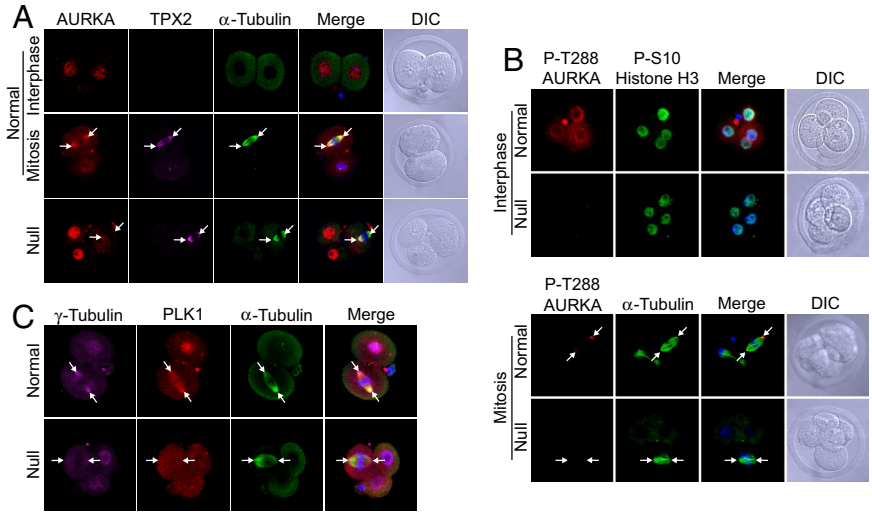

Fig. 5. Dysregulation of mitotic kinases in embryos from *Filia* null females. (*A*) AURKA was highly concentrated in interphase nuclei of embryos from both normal and *Filia* null females. At mitosis, AURKA (arrows) was translocated in spindle poles of normal embryos but was absent in spindles of the delayed embryos from mutant females. TPX2 appeared normal. (*B*) P-T288 AURKA (activated AURKA) was initially detected in the cytoplasm surrounding nuclei at G2/M phase (P-S10 histone 3–positive) (interphase panel) and translocated to the spindle poles at mitosis (mitosis panel, arrows) in normal embryos. P-T288 AURKA was not detected at the G2/M transition or at mitosis in delayed embryos from mutant females. (*C*) PLK1 and γ-tubulin were observed in metaphase spindle poles of normal embryos (arrows), but not in the spindle poles of delayed embryos derived from *Filia* null females.

females [94.6% (n = 37) vs 0% (n = 58) in normal embryos], whereas its regulator TPX2 (microtubule-associated protein homolog of *Xenopus* laevis), which targets it to the spindle (28, 29), appeared to be unperturbed.

AURKA localization requires its kinase activity (30); to test whether the absence of Filia affected AURKA activation, we stained with an antibody specific for active (phosphorylated) AURKA. In normal embryos, activated AURKA was first detected in the cytoplasm surrounding the nucleus at the G2/M transition (Fig. 5*B*, *Upper Panel*) when histone H3 is phosphorylated on serine 10 (77.1%; n = 48). Thereafter, active AURKA was enriched at spindle poles (100%; n = 40) (Fig. 5*B*, *Lower Panel*). In contrast, no signal was detected in affected embryos from *Filia* null females at either interphase (92.8%; n = 111) or metaphase (100%; n = 24). Like AURKA, PLK1 was enriched at spindle apparatus in metaphase of normal embryos (Fig. 5*C*). Whereas PLK1 staining was not detected at metaphase in nearly all delayed embryos from *Filia* null females (94.1%; n = 34) (Fig. 5*C*), it was present in the midbody of spindles during telophase, as it was in normal controls (100%; n = 12; data not shown). In somatic cells, AURKA and PLK1 recruit γ-tubulin, another spindle assembly regulator, to centrosomes (31, 32). Concomitant with the absence of AURKA and PLK1 at the spindle poles, γ-tubulin was not detected at spindle poles in delayed embryos from *Filia* null females (Fig. 5*C*).

RhoA GTPase Signaling Mediates the Regulatory Role of *Filia* on Spindle Assemblies. The subcortical localization of Filia (19) makes it unlikely that Filia has a direct role in regulating AURKA and PLK1 present on the microtubule-organizing center (MTOC) of early blastomeres. Therefore, we have tested known AURKA activators, including RhoA, cyclinB1/CDK1, HEF1 (official symbol, *Nedd9*), and Ajuba (official symbol, *Jub*), which shuttle within the cytoplasm (28, 33–37). We found that RhoA, but not the other AURKA regulators, was affected by depletion of maternal Filia (Figs. 6*A* and S4*A*-*C*). RhoA cycles between active (GTP-bound) and inactive (GDP-bound) conformations. Because the presence of RhoA protein does not reflect the distribution of active RhoA, we used GFP-rGBD (the RhoA binding domain of rhotekin) as a sensor for the active form of RhoA (38, 39). mRNA encoding GFP-rGBD was injected into the cytoplasm of embryos, and time-lapse images were obtained. In normal embryos (Fig. 6*A*), active RhoA initially appeared in the area surrounding chromosomes at the time of nuclear envelope breakdown and thereafter

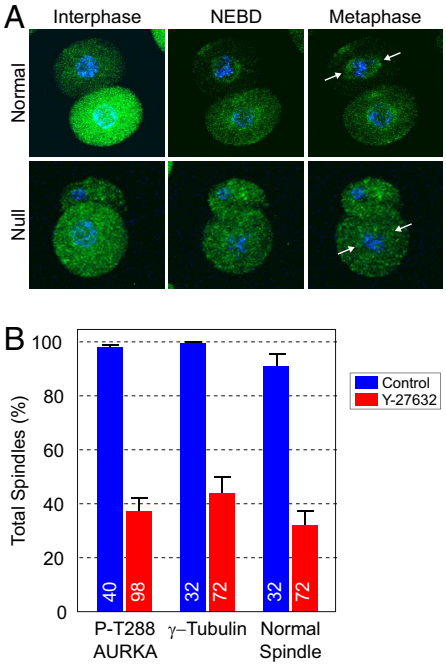

Fig. 6. Dysregulation of RhoA signaling leading to spindle dysmorphogenesis in embryos from *Filia* null females. (*A*) In normal embryos, GFP-rGBD was distributed uniformly in the cytoplasm at interphase. On NEBD, GFP-rGBD was detected in the area surrounding chromosomes and thereafter formed a spindle-like structure (arrows) in embryos from normal, but not mutant, females. (*B*) P-T288 AURKA (active), γ-tubulin, and spindle morphology were examined in normal embryos treated with or without Y-27632 (control). Y-27632 treatment significantly decreased the percentages of P-T288 AURKA, γ-tubulin at the MTOC, and normal spindles compared with untreated controls. The numbers on the bars reflect the number of spindles analyzed. Data are the average of 4 experiments ± SEM.

formed a spindle-like structure (20 of 21 mitoses). No such localization was observed in embryos from *Filia* null females (13 of 13 mitoses), indicating abnormal RhoA signaling in these embryos.

To determine whether RhoA signaling is required for AURKA function and spindle assembly, we treated normal embryos with Y-27632, which blocks the activity of ROCK (Rho-associated kinase, a major downstream target of RhoA) and thus specifically interferes with RhoA function. Treatment of early 2-cell embryos with the inhibitor significantly postponed mitotic entry (data not shown), reminiscent of the mitotic delay observed in embryos from *Filia* null females (Fig. 1*F*). Notably, blockage of the RhoA/ROCK pathway caused spindle dysmorphogenesis and affected the presence of active AURKA and γ-tubulin, but not PLK1, at the spindle poles (Fig. 6*B*). Consistent with these observations, when RhoA function was disrupted by the dominant-negative mutant RhoAN19, AURKA was not detected at the spindle poles of microinjected embryos (97.6%; $n = 41$), whereas PLK1 was unperturbed (100%; $n = 41$), suggesting an RhoA-independent pathway in the regulation of PLK1.

Discussion

The first 3 cell divisions of mouse embryogenesis host the transition of developmental control from the maternal to the embryonic genome. As in simpler model systems, it has become clear that this changeover is dependent on proteins stored in the mouse egg and encoded by maternal effect genes (8). Ensuring faithful chromosome segregation during initial cell divisions is of critical importance in early development, where aneuploidy in even a few cells can have a devastating effect and lead to embryonic lethality. Although Filia is abundant in growing oocytes, its absence does not affect meiosis I and II, which have normal spindle morphogenesis replete with spindle assembly regulators, including γ-tubulin, AURKA, and PLK1 (data not shown). We now report that after fertilization, maternal Filia plays a role in maintaining euploidy in cleavage-stage embryogenesis by integrating proper spindle formation and the SAC. In the absence of maternal Filia, spindle morphogenesis, as well as the SAC, are impaired, and affected embryos display aneuploidy, as indicated by lagging chromosomes, micronuclei, and hyperploidy. Filia is conserved in humans (217 aa; 41% identity), and mutation in the cognate gene may result in a similar phenotype and account for early embryonic loss in clinic populations (40).

Our observations suggest that Filia ensures proper spindle assembly, at least in part through RhoA signaling, which regulates AURKA function. RhoA belongs to the Rho subfamily of rho GTPase, which has long been thought to regulate actin organization. However, there is growing evidence that Rho GTPases also coordinate mitotic events, including activation of AURKA at the G2/M transition as well as spindle assembly at mitosis (36, 37). The requirement of RhoA activity in the normal spindle assembly is cell type–specific; for example, Rho signaling has a critical role in spindle assembly in Rat-2, Xlk-1, MDCK, and Ptk-1 but not in HeLa cell lines (36). Our data also indicate that RhoA/ROCK signaling is required for proper spindle organization in early cleavage mouse embryos. Blockage of RhoA/ROCK signaling led to abnormal spindle formations, which could be attributed to the absence of spindle assembly regulators γ-tubulin and AURKA at the MTOC. Blockage of RhoA/ROCK signaling also postponed cell cycle progression into mitosis and mimicked the delay in G2/M transition observed in embryos derived from *Filia* null females.

AURKA is a key regulator of bipolar spindle formation, and its localized kinase activity is required to target factors involved in microtubule nucleation and stabilization to the centrosome (e.g., γ-tubulin) (28). The roles of AURKA in spindle assembly and chromosome alignment have been well documented in somatic cells by genetics (41), RNA interference (42), and small-molecule inhibitors (43). The in vivo physiological role of AURKA in regulating early mammalian embryo development also has been investigated by targeted disruption of the gene in mice (44). Homozygous *Aurka* null embryos exhibit defects similar to those in embryos derived from *Filia* null female mice, including growth retardation, leading to the noticeably smaller embryos with fewer cells at E3.5, abnormal mitotic spindle assembly, and chromosome misalignment in preimplantation embryos (44). Based on these observations, we suggest that RhoA and AURKA are mediators of *Filia*'s functions in regulating cell cycle progression into mitosis and spindle morphogenesis.

PLK1 is a multifunctional mitotic serine/threonine kinase that plays major roles in spindle assembly and chromosome alignment (45, 46). The activity of PLK1 is modulated by phosphorylation, protein degradation, and, most strikingly, changes in subcellular localization during mitotic progression. Initially, PLK1 is located at the MTOC, where it functions in the assembly of bipolar spindles, partially by recruiting γ-tubulin. Later in the cell cycle, PLK1 accumulates in the midbody of the spindle during telophase (45). In embryos derived from *Filia* null females, PLK1 is not detected at spindle poles during metaphase, which could account for the concomitant absence of γ-tubulin and spindle abnormalities; however, PLK1 still accumulates at the midbody during telophase, even in the absence of maternal Filia. What caused the failure of PLK1 docking onto spindle poles at metaphase is not clear, but inhibition of RhoA signaling did not perturb localization of PLK1 to the spindle at metaphase, suggesting RhoA independence of PLK1 regulation.

Along with governing proper spindle assembly, Filia appears to ensure euploidy by regulating the SAC, a surveillance system that senses failure of kinetochore attachment and generates signals to hold the cell cycle at metaphase until all chromosomes are attached to the spindle and congress to the metaphase plate. In the absence of this "wait" signal, premature anaphase can occur, resulting in missegregation of chromosomes and aneuploidy (25). MAD2 is an important component of this surveillance system; however, in the absence of Filia, MAD2 is not properly located on kinetochores. Thus, despite the presence of unattached kinetochores, the SAC failed in embryos derived from *Filia* null females, contributing to the observed embryopathy.

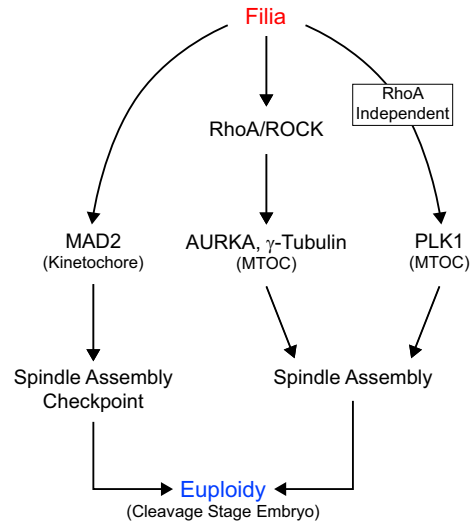

Fig. 7. Model of Filia's role in ensuring euploidy of early cleavage embryos. *Filia* regulates the proper localization of AURKA and γ-tubulin to the MTOC via RhoA/ROCK signaling, as well as PLK1 via a RhoA-independent pathway. Concomitantly, *Filia* is required for the placement of MAD2 to kinetochores, to enable the function of the SAC. Thus, *Filia* serves as an upstream spatiotemporal regulator of mitotic kinases and the SAC to ensure fidelity of chromosome segregation and genomic stability in cleavage-stage mouse embryogenesis.

In summary, we have provided evidence that a maternal protein, Filia, helps ensure chromosome stability and euploidy during cleavage-stage mouse embryogenesis by regulating 2 mitotic kinases (AURKA and PLK1) and MAD2 (Fig. 7). Filia (≈50 kDa) is well conserved in mammals but has no distinguishing motifs except for a 10-fold tandem, 23-aa repeat that could play a role in interactions with other maternal proteins (19) and contribute to the phenotype. The scarcity of early cleavage-stage embryos presents a challenge for biochemical analyses to dissect the mechanistic links between Filia and downstream effectors, although RhoA signaling participates in at least 1 of the pathways. Partners of Filia (i.e., FLOPED, MATER, and TLE6) interact in a SCMC complex that forms a regulatory network critical for progression beyond the 2-cell stage (16). The severity of the phenotype (2-cell arrest) observed in embryos lacking FLOPED or MATER makes their role in these processes indeterminate; however, the rescue of *Floped* and *Mater* null embryos with hypomorphic mutant alleles should provide more comprehensive insight into their interactions with Filia, as well as into the role of these maternal proteins in successful cell cycle progression during preimplantation development.

Materials and Methods

Filia null mouse lines were established by DNA recombineering and embryonic stem cell technology (22). Embryos were analyzed by immunofluorescence and confocal microscopy on fixed tissue or by time-lapse imaging after microinjection of mRNA encoding GFP-β5 tubulin or GFP-rGBD. Metaphase chromosome spreads were prepared (47) for karyotyping. See SI *Materials and Methods* for more details.

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