MCAK is present at centromeres, mid spindle and chiasmata and involved in silencing of the spindle assembly checkpoint in mammalian oocytes

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ABSTRACT: Mitotic centromere-associated kinesin (MCAK) is an ATP-dependent microtubule (MT) depolymerase regulated by Aurora kinase (AURK) phosphorylation and implicated in resolution of improper MT attachments in mitosis. Distribution of MCAK was studied in oocyte maturation by anti-MCAK antibody, anti-tubulin antibody, anti-AURKB antibody and anti-centromere antibody (ACA) and by the expression of MCAK-enhanced green fluorescent protein fusion protein in maturing mouse oocytes. Function was assessed by knockdown of MCAK and Mad2, by inhibiting AURK or the proteasome, by live imaging with polarization microscope and by chromosomal analysis. The results show that MCAK is transiently recruited to the nucleus and transits to spindle poles, ACA-positive domains and chiasmata at pro-metaphase I. At metaphase I and II, it is present at centrosomes and centromeres next to AURKB and checkpoint proteins Mad2 and BubR1. It is retained at centromeres at telophase I and also at the midbody. Knockdown of MCAK causes a delay in chromosome congression but does not prevent bipolar spindle assembly. MCAK knockdown also induces a meiosis I arrest, which is overcome by knockdown of Mad2 resulting in chiasma resolution, chromosome separation, formation of aberrant meiosis II spindles and increased hypoploidy. In conclusion, MCAK appears to possess a unique distribution and function in oocyte maturation. It is required for meiotic progression from meiosis I to meiosis II associated with silencing of the spindle assembly checkpoint. Alterations in abundance and activity of MCAK, as implicated in aged oocytes, may therefore contribute to the loss of control of cell cycle and chromosome behaviour, thus increasing risk for errors in chromosome segregation and aneuploidy.

Key words: aneuploidy / meiosis / MCAK / oocyte / spindle

Introduction

Chromosome bi-orientation during metaphase and chromosome segregation during anaphase of mitosis and meiosis are mediated in part by motor proteins on the kinetochore. The process involves polymerization/depolymerization of kinetochore fibres (K-fibres) prior to anaphase and disassembly of fibres proximal to kinetochores for poleward chromosome movement during anaphase. Sequential assembly of centromeric proteins as in male meiosis (Parra et al., 2009) and the activity of motor proteins like kinesin-5 as well as progressive clustering of MTOCs (microtubule-organizing centres) appear to contribute to self-organization of a bipolar acentrosomal spindle in mammalian oocytes, which is a prerequisite to chromosome bi-orientation (Brunet and Maro, 2005; Schuh and Ellenberg, 2007). Proper attachment and bi-orientation of centromeres of replicated chromosomes before progression to anaphase are crucial for faithful chromosome segregation and genetic stability in all eukaryotic mitotically and meiotically dividing cells. This process is tightly regulated by the spindle assembly checkpoint (SAC) that monitors attachment and tension on kinetochores by spindle microtubules (MTs) and controls the timing of loss of chromosome cohesion and anaphase transition (recently reviewed by Nasmyth, 2001; Cleveland et al., 2003; Caudron et al., 2005; Musacchio and Salmon, 2007; Burke and Stukenberg, 2008). The integration of signals from the SAC helps to mediate cell cycle arrest and establish proper bipolar orientation and attachment of sister centromeres to spindle MTs on unaligned chromosomes. This involves differential regulation by phosphorylation/dephosphorylation of centromeric proteins and motor proteins as well as stage-specific changes in their localization.
and as a consequence, a tight regulation of spindle MT turnover and stability.

Several conserved motors localize to MTs at centromeres and to kinetochores including cytoplasmic dynein, together with the conserved RZZ complex (Rod/ZW10/Zwilch complex; Gassmann et al., 2008; Sivaram et al., 2009), and at least two kinesin-like proteins, centromere protein E (CENP-E) and mitotic centromere-associated kinesin (MCAK or kinesin-13, termed Kif1c in mouse; Wood et al., 1997; Maney et al., 1998; King et al., 2000; Kline-Smith and Walczak, 2002). Dynein signals to the SAC (Zhang et al., 2007b) and directly powers chromosome movement (Sharp et al., 2000; Vorozhko et al., 2008; Deng et al., 2009). CENP-E is also essential for chromosome congression to the spindle equator and in a complex way acts as sensor to maintain or silence the SAC (Cooke et al., 1997; Wood et al., 1997; Kim et al., 2008). MCAK does not function as a conventional kinesin motor protein but targets MT ends rapidly via diffusion where it initiates depolymerization (Helenius et al., 2006). MCAK is one of the three known ATP-dependent MT depolymerases of the kinesin-13 family of motor proteins, Kif2a, Kif2b and Kif2c (MCAK), with a central motor domain. It is active as a homodimer. Each member of this family has distinct functions in regulation of MT dynamics (Manning et al., 2007). At centromeres, MCAK promotes disassembly of K-fibres at plus-ends of MTs to facilitate chromosome movement and error correction (Wordeman et al., 2007). MCAK has been implicated in destabilizing improper kinetochore MT attachments during chromosome congression at prometaphase, metaphase and early anaphase to prevent chromosome segregation defects in mitosis (Kline-Smith et al., 2004; Cimini et al., 2006). However, MCAK’s role in oocyte maturation has not been investigated.

Depletion or inhibition of MCAK in mitosis or of its Xenopus orthologue XKCM1 in egg extracts leads to formation of abnormal spindles and/or chromosome misalignment in vitro but does not cause a mitotic arrest in vivo (Walczak et al., 1996; Maney et al., 1998; Walczak et al., 2002; Kline-Smith et al., 2004). Immunolabelling of MCAK in interphase cells localized MCAK within the nucleus, in the cytoplasm and at the plus-ends of MTs (Wordeman and Mitchison, 1995; Kline-Smith et al., 2004; Moore et al., 2005). At mitosis, MCAK is concentrated at centromeres of sister chromatids from promphase to telophase (Wordeman and Mitchison, 1995; Walczak et al., 1996). Thereafter, the majority of protein appears to become degraded. These data collectively demonstrate that MCAK is not a classical component of the SAC in mitosis.

In meiosis, the establishment of tension across centromeres is more complex than it is in mitosis. Meiosis I is immediately followed by progression to meiosis II, and the centromeres of sister chromatids, as opposed to homologues, are not under tension in meiosis I (Ishiguro and Watanabe, 2007; Lee et al., 2008). In contrast, monopolar attachment requires tight physical connection and cohesion at core centromeres (Sakuno and Watanabe, 2009). Because changes in the MCAK levels at mitotic centromeres influence the level of tension across centromeres (as measured by interkinetochore distance; Wordeman et al., 2007) and SAC silencing may require centromere stretching (Uchida et al., 2009), it is of interest to study the distribution of MCAK in spermatogenesis and oocyte maturation. MCAK forms a unique pericentromeric ring-like structure surrounding kinetochores of the sister chromatids of each homologue within the meiotic bivalents at meiosis I in spermatocytes (Parra et al., 2006). Although MCAK has been found at poles of in vitro polymerized acenriolar bipolar spindles in cytoplasmic mitotic extracts of Xenopus eggs lacking chromatin (Zhang et al., 2008), cell cycle-dependent distribution and function of MCAK have so far not been studied in mammalian oocytes, which possess acenriolar spindles during maturation. Therefore, we analysed MCAK distribution in in vitro maturing oocytes of the mouse by antibody and expression of an MCAK-EGFP (enhanced green fluorescent protein) fusion protein.

The activity of MCAK is regulated by several factors, for instance, by ICIS (an inner centromere protein) that stimulates MCAK activity (Ohi et al., 2004). Furthermore, accumulation of MCAK at spindle poles of anastal spindles in frog egg extracts requires phosphorylation by Aurora kinase A (AURKA; Zhang et al., 2008), whereas centromeric MCAK activity is influenced by AURKB-directed phosphorylation that appears involved in sequential phosphorylation of several residues of MCAK causing changes in localization, conformation and activity of MCAK (Ems-McClung et al., 2007; Zhang et al., 2007a). In addition, cytoplasmic and polar MCAK activity appears to be controlled by AURKA-induced phosphorylation controlling Ran-dependent formation of bipolar acenriolar spindles in frog eggs (Zhang et al., 2008). The molecular processes required for acenriolar meiotic spindle formation may involve specific proteins and mechanisms (e.g. Colombié et al., 2008; Groen et al., 2009). Therefore, it was of interest to study MCAK’s specific distribution and role in maturing mouse oocytes to detect common and specific modes of action/regulation, particularly with regard to cell cycle and spindle regulation, checkpoint control and chromosome segregation, for example, by using an inhibitor of AURK which at low concentrations mainly affects AURKB (Shuda et al., 2009; Vogt et al., 2009).

AURKB is a component of the chromosomal passenger complex (CPC) that is recruited to the centromeres at prometaphase of mitosis similar to MCAK and is involved in checkpoint regulation. Disruption of AURKB function decreases MCAK phosphorylation and also leads to a loss of centromeric MCAK in mitotic divisions (Andrews et al., 2004; Lan et al., 2004) suggesting complex mechanisms regulating acquisition, retention and activity of MCAK. In fact, Zhang et al. (2007a) defined several phosphorylation sites/residues of AURKB on MCAK, which may act sequentially to alter conformation, localization and activity of MCAK. In this way, AURKB and MCAK may act upstream of the SAC in response to lack of bi-orientation and tension (Kallio et al., 2002; Murata-Hori and Wang, 2002; Morrow et al., 2005; Wordeman et al., 2007). We and others showed that AURKB can be found on the kinetochore face as well as the centromeres of chromosomes in prometaphase and metaphase I mouse oocytes (Swain et al., 2008; Shuda et al., 2009; Vogt et al., 2009). AURKB appears to become spatially separated from MCAK at the centromere when chromosomes are fully saturated with MTs and tension is generated on centromeres attached to opposite spindle poles in mitosis (Andrews et al., 2004). A tension-dependent physical separation of MCAK from AURKB may regulate the phosphorylation state and activity of depolymerase. In meiosis, tension may be generated in bivalents by attachment of homologues to opposite spindle poles, whereas they remain physically connected by chiasmata. Sister centromeres within each homologue are monopolarly (syntetically) attached. How MCAK and AURKB affect error correction in these significantly more complex meiotic centromere configurations is mechanistically unclear.

In meiosis, anaphase I progression involves the loss of cohesion between sister chromatid arms resulting in chiasma resolution,
AURK-mediated regulation of MCAK activity.

with well-aligned chromosomes under the influence of the cytostatic when they become constitutively arrested at the metaphase II stage oocytes (Pan et al., 2008; Wu and Komblut, 2008). Oocytes can thus be analysed in detail for localization and activity of MCAK throughout meiotic stages including transitions from meiosis I to meiosis II and in the constitutive metaphase II arrest.

MCAK mRNA has been reported to be reduced in aged mouse oocytes (Pan et al., 2008). We speculated that the depletion of MCAK together with other changes in the abundance of centromeric mRNAs/proteins is involved in the susceptibility of aged oocytes to meiotic errors. To investigate this hypothesis, we scored the consequences of MCAK depletion in young oocytes for induction of alterations in meiotic progression, spindle formation and chromosome segregation by knockdown of expression of MCAK without or with checkpoint protein Mad2 by specific small interfering RNAs (siRNAs). Furthermore, oocytes were matured in the presence of specific inhibitors of AURK (ZM44743; Ditchfield et al., 2003; Yang et al., 2005) and/or of the proteasome (MG132; Lee and Goldberg, 2003) and/or of the proteasome (MG132; Lee and Goldberg, 2003).

Expression of an MCAK-EGFP fusion protein in maturing mouse oocytes

Full-length human MCAK cDNA pCMV-SPORT6-Kif2c was obtained from RZPD (Clone IRATp970f111D, Berlin, Germany). Using primers (5′-ATGGCCATGGACCTGTCGCCTCAGG-3′ and 5′-TCACCTGGG CCGTTTCCTGTGCTTTAT-3′), the cDNA was amplified by PCR and subcloned into the BamHI/EcoRI sites of pGEX-Sx3 (GE Healthcare, Munich, Germany). Isolation of plasmid DNA containing full-length human MCAK from Escherichia coli DH5-alpha was purified by Wizard®Plus SV (Promega, Madison, USA) according to the standard protocols. MCAK was amplified by PCR using primers with restriction sites for Accl (primer forward 5′-AAAAATCCGGAATGGCCATGGACTCGT CGCT-3′) and SacII (primer reverse 5′-GCAAGATACGCCCAGCTG ACCTCCGAAA-3′). After purification by Wizard®SVgel and PCR Clean-up system (Promega, Germany), DNA was ligated by T4-DNA ligase (New England Biolabs, Ipswich, MA, USA) into the multiple cloning site of a modified pEGFP-C1 vector (pSP0-EGFP with Ampr, Tetr resistance and EGFP-actin-add-construct; BD Clontech, Heidelberg, Germany) containing an internal ribosomal entry site, an SP6 promoter and a short (30 nucleotides) poly(A)-tail for efficient in vitro transcription and in vivo translation (EGFP N-terminal). For in vitro transcription, DNA was linearized by EcoRI and the forward strand was cut 3 bp behind the poly(A)-tail, treated by proteinase K (Qiagen, Hilden, Germany) for 30 min at 50°C and cleaned by Wizard®SV (Promega) elution in RNase-free H2O (Qiagen). DNA concentration was determined by Nanodrop (Nanodrop products, Wilmington, USA). In vitro transcription (5 h 45 min at 37.4°C) was performed by MEGAscript® SP6 Kit (Ambion, Austin, USA) followed by incubation with Turbo-DNase (Ambion) treatment for 15 min at 37°C. mRNA was stored at −80°C. Before use, mRNA was in vitro 3′-polyadenylated by A-Plus T7poly(A)-polymerase tailing kit (Biozym, Oldendorf, Germany) for 60 min at 37°C to obtain a tail of ∼150 A in addition to the 30 A in the primary mRNA. Finally, mRNA was purified by ammoniumacetate/ethanol precipitation (5 M ammoniumacetate (end concentration 2.5 M); 20 min on ice followed by centrifugation for 15 min at 12 000g; uptake into 70% ethanol followed by 10 min centrifugation at 16 000g) and suspended in RNase-free H2O (Qiagen) to a concentration of 2–5 μg/μL. Before microinjection, mRNA was heated for 3 min at 65°C and then chilled on ice followed by centrifugation at 13 000g at 4°C. Message coding for EGFP alone was injected as a control. For microinjection, fully grown GV oocytes were isolated from large antral follicles of ovaries of unprimed MF1 mice at diestrus of the spontaneous estrous cycle. Oocytes were blocked in G2-phase (GV stage) by 0.2 or 1 μM cilostamide, a PDE3-specific inhibitor (Vogt et al., 2009), for 6–8 h. Oocytes released from the block were either directly viewed (after staining of chromosomes by Hoechst) or fixed (see below) and the distribution of fluorescence was compared between oocytes containing a nucleus with non-surrounded or surrounded nucleolus (NSN or SN), or oocytes were cultured in M2 medium and viewed non-invasively for EGFP expression and distribution of the EGFP (control) or MCAK-EGFP fusion protein by fluorescent microscope at various times after removal of cilostamide and resumption of maturation. Oocytes expressing MCAK-EGFP fusion protein were also

Materials and Methods

Animals, culture and microinjection of mouse oocytes

Inbred young female MF1 and NMRI mice (6–10 weeks old) were originally obtained from Harlan Winkelmann (Borchen, Germany) and breed in our local mouse house under a 12 h/12 h light/dark cycle with water and feed provided ad libitum. Mice at the diestrous or proestrous stage of the estrous cycle were selected for oocyte isolation as described previously (Eichenlaub-Ritter and Boll, 1989). For maturation, oocytes were placed in M2 medium (Sigma, Deisenhofen, Germany) and cultured in an unassed incubator under high humidity at 37°C for 12–16 h. For microinjection experiments, oocytes were maintained at the GV stage in M2 medium supplemented with 1 μM cilostamide (Sigma), a specific phosphodiesterase-3 (PDE3) inhibitor. They were microinjected using a semi-automatic injector with micromanipulators (Eppendorf, Germany) mounted to a Zeiss Axiovert 35 microscope (Zeiss, Jena, Germany). For immobilization of oocytes, a CellTram Air (Eppendorf) was used with holding pipette at 20° angle (BioMedical Instruments, Zolliinitz/Germany) followed by microinjection with the injection capillaries (Typ Barnow, 1.5 μm inner diameter; BioMedical Instruments). Oocytes were released into cilostamide-free M2 medium after 6 h following RNAi injection to undergo meiotic maturation.

Inhibitor treatment and checkpoint activation

For inhibition of AURKB activity, 1.5 μM ZM447439 (ZM inhibitor; Tocris, UK) or solvent (dimethylsulfoxide, DMSO, Sigma) was supplemented to M2 medium. This low concentration of the ZM inhibitor preferentially inhibits AURKB (and possibly, AURKC; for discussion and references, see Vogt et al., 2009). The proteasome was inhibited by exposure to 5 μM MG132 (Sigma) to inhibit progression of oocytes to anaphase I, although oocytes should possess active APC/C and have been released from the spindle assembly checkpoint (SAC). For meiotic arrest due to SAC activation, oocytes were cultured in M2 medium with 100 nM nocodazole (Sigma), as shown previously (Shen et al., 2005, 2008). Control oocytes were placed in a medium with solvent (DMSO).
fixed by formaldehyde (Albertini-protocol, see below), labelled with anti-tubulin antibody and viewed by confocal microscopy after reaction to TRITC-labelled secondary antibody and 4,6-diamidino-2-phenylindole (DAPI) staining to view chromosomes (see below).

**Knockdown of expression by siRNA**

siRNA duplexes were prepared with the Silencer siRNA Construction Kit according to the manufacturer’s protocol (Ambion) targeting the mouse *musculus* mad2l1 mRNA, nucleotides 234–255 (‘AAAGATATCTCAATAATGTTG-3′), and the kif2C mRNA (MCAK), nucleotides 108–129 (‘AAGGAGATGAGAAATAGAAG-3′). siRNA oligonucleotide templates were synthesized by Sigma-Genosys (Haverhill, UK). The following gene-specific sequences were successfully used: siRNA Mad2 sense (5′-AAATGTCAATCAGGGACACCTGTCCTC-3′) and antisense (5′-AATTCCGCATGATTAGAACCCTGTCCT-3′), siRNA MCAK sense (5′-AATATTCCTGTGCTTTACCTGTCCT-3′) and antisense (5′-AATAAAGGGAGGACAGAAATACCCCTGTCCT-3′). The unrelated non-specific siRNAs were designed as followed: siRNA Mad2 sense (5′-AAATGTCAATCAGGGACACCTGTCCTC-3′) and antisense (5′-AATTCCGCATGATTAGAACCCTGTCCT-3′), siRNA MCAK sense (5′-AATATTCCTGTGCTTTACCTGTCCT-3′) and antisense (5′-AATAAAGGGAGGACAGAAATACCCCTGTCCT-3′). siRNAs were eluted with 100 μl into nuclease-free water (Qiagen) to produce stock solutions of 400–800 μg/ml as quantified by absorbance at 260 nm. siRNA products were analysed by electrophoresis using a 1.2% formaldehyde agarose gel.

For microinjection, stock solutions were not diluted, when a single knockdown (Mad2 or MCAK) was performed. For double-knockdown experiments (Mad2 and MCAK), the siRNA with the higher stock solution concentration was diluted to the concentration of the siRNA with the lower stock solution concentration. The concentrations of siRNAs used in this study were in the range between 20 and 60 μM, which is higher than the siRNA concentrations utilized by Kim et al. (2002) and lower than the concentrations of dsRNA utilized by Lim et al. (2007). MCAK and Mad2 levels were normalized to β-actin expression levels. Experiments were repeated and analysed three times.

**Lysis and fixation of oocytes for analysis of centromeric proteins**

The zona pellucida was removed mechanically after brief exposure of oocytes to 7 mg/ml pronase (Roche Diagnostics). For the analysis of centromere and kinetochore proteins at various stages of maturation, oocytes were gently lysed and fixed on the slide to retain antigenicity of centromeric proteins with a slight modification of a method as described previously (Hodges and Hunt, 2002). In short, oocytes were initially subjected to a mild hypotonic treatment by immersion in 0.75% (w/v) trisodium citrate solution for 3 min before placing them in a small volume of hypotonic on slides dipped in 1% paraformaldehyde (pH 9.2) containing 0.15% Triton X-100 and 3 mM dithiothreitol for cell lysis. Further settling and flattening of samples was by placing the slides in a humid chamber for 2 h before washing briefly (10 s) with 0.4% Photoflo (Kodak) and further drying of slides at room temperature. Samples could be stored at −20°C. Finally, lysed oocytes on slides were briefly rinsed with PBS and blocked by PBS containing 1% BSA, 0.2% powdered milk, 2% normal goat serum and 0.1 M glycine for 30 min. A sheep anti-MCAK antibody (raised by Linda Wordemann, University of Washington, Seattle, WA, USA), mouse anti-Mad2 (a gift from Beth Weaver, UCSD, La Jolla, CA, USA) and mouse anti-BubR1 (a gift from Stephen Taylor, University of Manchester, UK) were used (1:50; 60 min). Following two washes in PBS, secondary antibodies were added to slides: an anti-sheep FITC-conjugated (Sigma), an anti-rabbit FITC-conjugated (Sigma) or an anti-mouse FITC-conjugated antibody (Sigma), respectively (1:50, 1 h). Amplification of signal was by rabbit anti-fluorescein and goat anti-rabbit Alexa Fluor 488 (Molecular Probes, Eugene, OR, USA). AURKB antibody (Transduction Laboratories, Lexington, KY, USA) labelling (1:50; 60 min) was localized after labelling with an anti-mouse TRITC-conjugated antibody (Sigma). Human CREST serum (HCT-100, Immunovision, Luxembourg) followed by anti-human IgG TRITC conjugate (Sigma) was used to localize the centromere domain (1:50). Finally, chromosomes were stained with DAPI (Sigma) and mounted with a coverslip using 0.2% 1,4-diaminobicyclo[2.2.2]octane (DABCO, Sigma) in 20% glycerol.

To quantify statistically the level of protein depletion following RNAi, images of 5–10 slides with lysed oocytes from each group were obtained by defined setting of the CCD camera and software. Subsequently, staining intensity was determined from the mean grey values of CREST, MCAK and Mad2 foci, respectively, using ImageJ software version 1.38s (National Institutes of Health, USA; http://rsb.info.nih.gov/ij). The mean grey values of MCAK and Mad2 were compared relative to CREST (anti-centromere antibody, ACA), whose staining was unaffected by RNAi, in each group to determine the magnitude of reduction in staining intensity as percentage following specific RNAi. Background for grey values of MCAK was determined in spreads stained by CREST/anti-human TRITC antibody and anti-sheep FITC antibody, omitting MCAK first antibody reaction.

**Analysis of spindle formation and distribution of MCAK-EGFP in fixed or extracted oocytes**

Extraction and fixation of oocytes for spindle immunofluorescence were done as described previously (Eichenlaub-Ritter and Betzendahl, 1995). In short, the zona pellucida was removed mechanically after brief exposure to pronase (Roche Diagnostics). Oocytes were then extracted in a pre-warmed MT-stabilizing solution containing glycerol, Triton X-100 and EGTA for 45–60 min at 37°C (25% (v/v) glycerol), 2% Triton, 50 mM KCl, 0.5 mM MgCl2, 25 mM HEPES, 20 μM phenyl-methyl-sulfonyl-fluoride,
5 mM EGTA, pH 6.75). Oocytes were attached to a slide coated with poly-L-lysine (Sigma) and fixed for 8 min in 100% methanol at −20°C. After rinsing with PBS labelling was with a monoclonal mouse anti-β-tubulin (Sigma; 1:400; 60 min, 37°C), followed by polyclonal anti-mouse FITC-conjugated antibody (1:60). Chromosomes were stained with 10 μg/ml DAPI or 1 μg/ml propidium iodide (Sigma). For staining of AURKB, an antibody (Transduction Laboratories, 1:60, 60 min) was used and telophase I stages were extracted, fixed and labelled as described previously (Vogt et al., 2009) following the above extraction protocol.

For confocal microscopy, EGFP or MCAK-EGFP expressing or lacking knockdown oocytes were also fixed according to a procedure by Messinger and Albertini (1991). In short, oocytes were fixed in a pre-warmed MT-stabilizing buffer (0.1 M Pipes, pH 6.9, 5 mM MgCl₂, H₂O, 2.5 mM EGTA) containing 2.0% formaldehyde, 0.5% Triton X-100, 1 μM taxol, 10 U/ml aprotinin and 50% deuterium oxide for 20 min at 37°C, followed by blocking (PBS, 1% BSA, 0.2% powdered milk, 2% normal goat serum, 0.1 M glycerine and 0.01% Triton X-100). Fixed oocytes were stored at 4°C in blocking solution until processed for indirect immunofluorescence. MTs of the spindles were labelled by a monoclonal mouse anti-β-tubulin antibody (1:200; 60 min, 37°C). Second antibody was an anti-mouse FITC-conjugated or TRITC-labelled antibody (1:50). Chromosomes were stained with propidium iodide or with Hoechst and oocytes were mounted on poly-L-lysine coated slides with DABCO (Sigma).

Microscopy and image acquisition
For fluorescence microscopy of chromosome spreads and spindle morphology, cells were viewed with a Zeiss Axiophot fluorescence microscope using a ×100 Neofluar (1.30 NA) oil objective (Zeiss) and captured with a sensitive charged coupled device camera (SensiCam, PCO CCD imaging, Kelheim, Germany). Spindles were also imaged on a confocal laser-scanning microscope using the Leica TCS SP2 (Leica Microsystems, Heidelberg, Germany). To obtain immunofluorescent images, Z-series optical sections were obtained at 0.5–0.6 μm steps and then 2D/3D reconstructed with Leica Confocal software (Leica Microsystems). Chromosome spread were further analysed and processed using ImageJ software. Final images were processed with Adobe Photoshop 7.0 software (Adobe System Inc., San Jose, CA, USA).

For live imaging, oocytes were analysed by polarization microscopy (OCTAX EyeWere™ MX, kindly provided by MTG, Altdorf, Germany) by placing them into preheated drops of 10 μl M2 medium covered with mineral oil (Sigma) in a WilCo Wells BV dish with glass bottom (Ref. No.: gWSt-5040, Amsterdam, Netherlands) on a heated stage of a Nikon microscope equipped with ×20 objective lens and warm plate (MTG), appropriate filters and LCD liquid crystal optics and hardware for imaging and recording for qualitative and quantitative polarization microscopy.

Time-lapse microscopy was performed by taking images at 2 min intervals from 420 min of maturation to 960 min (culture overnight) to assess the time of transition from M-phase to anaphase I, cytokinesis and first polar body (PB) formation and spindle length non-invasively in living oocytes. For the analysis of kinetics of PB formation, logarthmic plots and functions were generated for each experimental condition with Microsoft Excel software (Microsoft Corp., Redmond, WA, USA).

C-banding for chromosomal analysis
All oocytes were spread and C-banded as described previously (Eichenlaub-Ritter and Boll, 1989). Meiotic status of oocytes resuming maturation was analysed by counting numbers of oocytes with bivalent chromosomes or metaphase II chromosomes (dyads) or chromatids (monads), independent of ploidy. Polyploidy was considering oocytes with dyads in the diploid range (40 dyads). Hyperploid oocytes with >20 metaphase II chromosomes or the respective numbers of chromatids or hypoploids containing 16–19 metaphase II chromosomes or up to 19 chromatids plus a single chromatid or the respective numbers of chromatids were determined.

Statistical analysis
Statistical analysis was by the χ² test with Yates’ correction.

Results
Recruitment of MCAK to centromere domains after GVBD as visualized by antibody staining of lysed oocytes
The kinesin-13 MCAK is recruited to centromeres in prophase I spermatocytes where it remains until anaphase I (Parra et al., 2006, 2009).

Initially, we were unable to obtain immunostaining of MCAK in conventionally fixed mouse oocytes (extraction protocol or Messinger and Albertini-protocol, see M&M). To overcome this problem and detect MCAK at centromeres, a protocol involving gentle lysis of oocytes in the moment of fixation was used that has been especially developed to identify centromeric proteins in large mammalian oocytes with highly contracted chromosomes buried within the spindle (Hodges and Hunt, 2002). The present study, using antibody to MCAK in gently lysed mouse oocytes, shows that MCAK is initially enriched between the arms of sister chromatids (arrows in Fig. 1A) and may also be seen on chromosomes after GVBD in in vitro maturing mouse oocytes (Supplementary Fig. SA–A′”). By later prometaphase I stage, MCAK is consistently localized at centromeric sites from mid-prometaphase I to metaphase I as well as at metaphase II stage (Fig. 1B and C). Double immunolabelling of MCAK and human CREST serum (ACA) that recognizes centromere proteins (CENP-A and CENP-C) reveals that MCAK occupies foci overlapping with ACA at anaphase I (Fig. 1E and E′), prometaphase II (Fig. 1D) and metaphase II (Fig. 1L–L″″″″, insets), similar to the distribution of AURKB (Fig. 1F–F′, M–M″″″″, insets), and checkpoint proteins BubR1 and Mad2 (Fig. 1G and H). In telophase I, MCAK is retained at centromeres in the lysed oocytes (Fig. 1I), whereas there is no staining of AURKB at the centromeres (Fig. 1K), in contrast to earlier anaphase I stage (Fig. 1F and F′).

MCAK appears as a pericentromeric ring below kinetochores at metaphase II of male meiosis in the mouse (Parra et al., 2006). In oocytes, it is either concentrated at distinct foci at centromere domains of chromosomes of gently spread metaphase II-arrested mouse oocytes (Fig. 1L″″″″) or in a cone-like structure, presumably beneath the kinetochore proper (Fig. 1C, inset). Since chromosomes usually become deposited with their long axis and chromosome arms on the surface of the slide during the lysis procedure, it is not possible with this lysis protocol to unambiguously determine whether the cone of MCAK staining actually depicts a ring-like structure beneath the kinetochore as is recognized in the top view of squashed spermatocytes. Although it has not been possible to detect MCAK by antibody staining in conventionally fixed oocytes, the observations in gently lysed oocytes suggest that MCAK becomes stably recruited to a position within the centromere region shortly after GVBD from mid-prometaphase I until metaphase II of oocyte maturation, similar to its localization at metaphase I and II in male meiosis.
To see if MCAK shifts its localization at the centromere when oocytes progress from metaphase I to anaphase I and the SAC is silenced, oocytes were either fixed at late metaphase I stage (after 7 h of culture) or kept for 4 h in the presence of a proteasome inhibitor (5 µM MG132) to prevent degradation of cyclin B and proteolysis of cohesin protein (Fig. 2A and B). MCAK remains co-localized with ACA-positive centromeres when oocytes are blocked from progression into anaphase I by exposure to the proteasome inhibitor.
Figure 2 (A–B′) Distribution of MCAK and AURKB in gently lysed oocytes at a late metaphase I (A and B) stage after maturation without the proteasome inhibitor MG132 (−MG): mCAK (green) (A) and AURKB (green) (B) are enriched at ACA-positive sites (CREST, red). Inhibition of anaphase I progression with the proteasome inhibitor MG132 (+MG) does not consistently shift the localization of MCAK (green; A′) and AURKB (green; B′) relative to the ACA-positive sites (red). (C–I′′′′′′′′′′ and K–L′′′′′′′′′′) Distribution of MCAK as assessed by the analysis of MCAK-EGFP fusion protein in maturing oocytes fixed for immunofluorescence and viewed by a confocal microscopy. EGFP (green) alone is randomly distributed in control oocytes with SN and some microtubular fibres (red) in the cytoplasm (C). Similarly, the MCAK-EGFP fusion protein is also not enriched in the nucleus of incompetent oocytes with non-surrounded nucleus (NSN) (D) but is abundant within the nucleolus and at chromatin in the nucleus of developmentally competent oocytes with SN (E–E′). After GVBD (4 h of culture), fusion protein is within the cytoplasm but not at spindle poles of oocytes in early prometaphase I just establishing a bipolar spindle (F), whereas it is associated with the spindle periphery in more advanced prometaphase I stages (G). A sphere of MCAK-EGFP is present at later prometaphase I and metaphase I stage at centromeres (H–I′′′′′′′′′′ and K–L′′′′′′′′′′) of homologous chromosomes within bivalents (7 h of culture) (white arrows) as well as at chiasmata and at centrosomes at spindle poles (K, K′). Green arrows in I′–I′′, K′, L′ and L′′ show enrichment of MCAK-EGFP at interstitial chiasmata and possibly distal chiasmata or telomeres. Some MT fibres are attached to what might represent distal exchanges/telomeres or centromeres of bivalents at the periphery of the spindle of a metaphase I stage (green solid arrow in H′, H′′, H′′′ and H′′′′). Green arrows in upper part of L, L′ and L′′′ depict MCAK-EGFP at distal chiasma or telomere and in L′′′′ some staining of chromosome arms next to a centromere. For further explanation, see text. Bar in A′ for A–B′ and in C–D, E′, F and G: 10 μm, in H′′ and H′′′: 5 μm, and in I, K and L′′′′: 10 μm.
MG132 from late prometaphase I stage (7 h) for 4 h, consistent with an extended metaphase I arrest with chromosomes under high tension and a release from the SAC (Fig. 2A, inset), comparable to the control (Fig. 2A). AURKB, which is capable of directly inactivating MCAK by phosphorylation, also preferentially localizes to the ACA-positive centromere domain during late metaphase I of meiosis (Fig. 2B, inset) or in oocytes blocked in late meiosis I by the proteasome inhibitor (Fig. 2B, inset) consistent with a co-localization of MCAK and AURKB throughout metaphase I to transition to anaphase I. Comparison of localization of MCAK with respect to ACA or AURKB in late metaphase I oocytes and those blocked by the proteasome inhibitor did not reveal characteristic alterations (Fig. 2A–B′, respectively).

**MCAK distribution throughout maturation studied by overexpression of MCAK-EGFP fusion protein: presence in nucleoli of competent oocytes and on centrosomes, centromeres, as well as chiasmata and the midbody during maturation**

The information on the localization of MCAK immunofluorescence in fixed and lysed oocytes is confirmed and extended by analysis of the distribution of MCAK-EGFP fusion protein in living and fixed mouse oocytes expressing the fusion protein from injected mRNA. Control GV oocytes or such from later stages of maturation after GVBD consistently exhibit homogenous EGFP staining within the cytoplasm (Fig. 2C). This is similar in immature GV-stage oocytes with NSN expressing the MCAK-EGFP fusion protein within the entire ooplasm (Fig. 2D). However, the MCAK-EGFP fusion protein transits to the nucleus and becomes enriched within the GV and in the nucleolus in meiotically competent, transcriptionally quiescent oocytes with SN (Fig. 2E″–E‴). Upon GVBD, initially some fluorescence is observed in diffuse aggregates throughout the cytoplasm (Fig. 2F), whereas it subsequently accumulates in the ooplasm surrounding the spindle at mid-prometaphase I, when bipolar, barrel-shaped spindles are formed (Fig. 2G). Later, MCAK-EGFP is found at distinct foci at centromeres of the homologous chromosomes (Fig. 2H″–I‴ and K–L‴, white arrows) as well as at the centromeres at the poles of the bipolar spindle forming a characteristic ring-like structure (Fig. 2K and K′). Unexpectedly, there are also distinct foci of MCAK-EGFP fusion protein at distal chiasmata at the telomeres of bivalents (Fig. 2L–L‴) and at interstitial chiasmata, particularly the first proximal chiasma of bivalents (green arrows in Fig. 2I″–I‴, K‴, L, L‴ and L‴″) of late prometaphase I and metaphase I stages, which can be visualized in optical sections of some but not all oocytes. The latter presumably depends on the longitudinal orientation of bivalents within the optical plane and the relative abundance of the MCAK-EGFP fusion protein (Fig. 2G–I‴ and K–K‴). The association of MCAK-EGFP is particularly obvious in bivalents with more than one chiasma (Fig. 2L″), which are located at the spindle periphery with the centromeres and the first chiasma buried in the spindle and the arms of homologues extending out of the spindle body with their telomeres (Fig. 2L″, L‴ and L‴″, green arrows). In some longitudinally sectioned bivalents, it appears that some fusion protein is still present at the chromosome arms where sister chromatids are attached to each other, in the vicinity of centromeres at later prometaphase I/metaphase I stages (Fig. 2L‴″, green arrows). In some cases, it is unclear whether centromeres or telomeric regions that are associated with MCAK-EGFP of a chromosome are attached to spindle MTs (Fig. 2H″–H‴, H‴ and H‴″, green solid arrow). Since MCAK is known to interact with the plus-ends of MTs (Wordeman and Mitchison, 1995; Jiang et al., 2009), it is possible that plus-ends of non-kinetochore MTs in the central spindle are enriched in the vicinity of chiasmata or telomeres of chromosomes (e.g. Fig. 2H‴″). However, we did not detect MTs at all sites at telomeres or distal chiasmata where there is an enrichment of MCAK-EGFP on the chromosomes in distinct foci (e.g. Fig. 2L‴″).

At anaphase I (Fig. 3A–A‴‴) and telophase I (Fig. 3B–C), some MCAK-EGFP is retained in the central part of the spindle, consistent with the presence of MCAK at plus-ends of MTs in the central part of the spindle. This region forming the midbody at telophase I also contains AURKB (Fig. 3D and D‴), at a site where constriction occurs at cytokinesis (Fuller et al., 2008) when the first PB forms. At meiosis II, there is a distinct spot of MCAK-EGFP fluorescence retained at this site where the first PB and the oocyte are still connected (Fig. 3E, lower inset and Fig. 3E‴–E‴‴), a position which marks the midspindle region/midbody and the furrow from cytokinesis. This is evident from remnants of the spindle, which are also sometimes still present at meiosis II in the first PB and the midbody (Fig. 3E‴‴, white arrow). This spot of staining was retained in 100% of oocytes at 16 h of maturation analysed by confocal microscopy by serial optical sectioning of this part of the oocyte connected to the first PB (n=17). At metaphase II, there is predominantly strong staining for MCAK-EGFP at the spindle poles (Fig. 3E, upper inset) and at the centromeres of metaphase II chromosomes at the spindle equator (Fig. 3E‴, inset), similar to meiosis I. Similar to GV oocytes, staining of EGFP in control oocytes is retained in a homogenous pattern all over the cytoplasm (Fig. 3F).

The stage-specific distribution pattern observed for MCAK-EGFP fusion protein and MCAK recognized by antibody in lysed oocytes (indicated by asterisk) are shown schematically in Fig. 3G. Our observations suggest that there is a transition of MCAK from cytoplasm in immature, developmentally incompetent oocytes with NSN to the nucleus and nucleolus in competent oocytes with SN, a dispersal of protein into the cytoplasm upon GVBD and the subsequent enrichment of MCAK around and within the spindle. At prometaphase I stage, MCAK appears to become recruited to centrosomes at spindle poles, and it is found at the poles of the acentriolar spindles in metaphase I as well as in metaphase II. It is associated briefly with chromosome arms and cohesion sites but then consistently with both centromeres and chiasma from late prometaphase I until anaphase I. With resolution of chiasmata at anaphase I MCAK appears retained in the central part of the spindle and at the midbody at telophase I and finally may be still found in the cytokinesis furrow. In metaphase II, it consistently occupies a centromeric and centrosomal position (Fig. 3G).

**Meiotic arrest by knockdown of MCAK**

To analyse the function of MCAK in spindle formation, chromosome congression, chiasma resolution and cell cycle regulation, we knocked down MCAK by microinjecting siRNAs specific to this kinesin-13 member. Real-time RT–PCR revealed that MCAK mRNA was
significantly reduced (94.1%, P < 0.001) relative to β-actin mRNA after microinjection of specific message into GV-stage oocytes (green striped bar in Fig. 4B), followed by a 6-h meiotic arrest of oocytes at the GV stage by the specific PDE3 inhibitor cilostamid. Meiotic arrest of the oocytes in G2-phase was overcome by washing and transfer to cilostamid-free medium, such that over 80%
Figure 4  Resumption of maturation of oocytes following the knockdown of MCAK or double knockdown of MCAK and checkpoint protein Mad2. (A) Depletion of MCAK by specific RNAi (RNAi MCAK) leads to a meiosis I arrest (red bars) with significant increase in GVBD oocytes (left panel) compared with controls (left panel, black bar); the arrest is released by double knockdown of MCAK and Mad2 (grey bars), followed by first PB formation and progression to meiosis II to numbers comparable to controls (black bars); asterisk denotes significantly different from control ($P < 0.001$). (B) Oocytes were analysed by real-time RT–PCR for MCAK and Mad2. Expression ratios of MCAK and Mad2 mRNAs in oocytes injected with specific siRNAs relative to negative control siRNAs were calculated and normalized to $\beta$-actin with REST software and converted to percentage of gene knockdown. Mad2 (green bar) and MCAK expression (green striped bar) was significantly reduced following knockdown by specific RNAi ($P < 0.001$). (C–D) Detection of MCAK and Mad2 in meiosis I arrested (MI) or meiosis II (MII) knockout oocytes. MCAK is present in meiosis II oocytes (RNAi control, green dots in C, inset) but absent in meiosis I arrested MCAK-depleted oocytes (RNAi MCAK, C’, inset) cultured for 16 h following microinjection. Similarly, Mad2 (RNAi control, green dots in C”, inset) is expressed at centromeres of untreated metaphase II oocytes of the control but not at the centromeres of meiosis II oocyte depleted of Mad2 (RNAi Mad2, C”’, inset). (D) Protein expression following RNAi was examined by analysing mean grey values of CREST (red bars), MCAK (green striped bars) and Mad2 (green bars) foci. MCAK and Mad2 were significantly reduced following specific RNAi in contrast to CREST. Asterisk denotes significantly different from control ($P < 0.001$). (E) Chromosomal constitution of spread, C-banded metaphase II oocytes without knockdown of MCAK or double knockdown of MCAK and Mad2. RNAi control meiosis II oocytes usually possess 20 metaphase chromosomes (E”, control). Oocyte arrested at meiosis I following RNAi MCAK containing 20 homologous chromosomes (bivalents) (E’, RNAi MCAK). Many RNAi MCAK + Mad2 knockdown oocytes are euploid containing 20 dyads (E”’), but few are also hypoploids (E), for instance, one oocyte (Oo) possesses 19 dyads and one chromatid (19 1/2), whereas the PB is hyperploid and contains the other chromatid (20 1/2). Numbers of chromosomes in spreads do not reflect chromosome identity. Bar in C and E: 10 μm.
of oocytes of the control microinjected with unspecific RNAi emit a PB and mature to meiosis II (black bars in Fig. 4A, Table I). Immunostaining of siRNA-treated and spread mouse oocytes verified that MCAK protein was depleted from centromeres at 16 h of in vitro maturation while ACA signal was normal (Fig. 4C, inset), in contrast to oocytes microinjected with unspecific RNAi (Fig. 4C). Quantitative analysis by determining the mean greyscale value in immunofluorescent signal in comparison to signal obtained for ACA-reactive epitopes at centromeres in oocytes injected with specific or unspecific RNAi to MCAK revealed that depletion of MCAK message resulted in a significant reduction in MCAK protein as assessed by reduced staining intensity by 82.7% relative to CREST staining following injection with specific RNAi (*P < 0.001) compared with 3.0% following microinjection of unspecific RNAi in the controls (Fig. 4D; left panel, red bars and green striped bar). This suggests that little MCAK protein was retained at centromeres. Importantly, the depletion of MCAK mRNA and protein at centromeres was associated with a significant increase in meiotic arrest at meiosis I after 16 h of maturation in oocytes injected with specific or unspecific RNAi to MCAK (Fig. 4A, red bars, n = 194).

The next set of experiments was designed to assess the effect of MCAK knockdown on earlier meiotic stages (Fig. 5A). In controls injected with unspecific RNAi, most oocytes (86.7%) had already progressed to anaphase/telophase I at 10 h of culture (Fig. 4A) and 77.8% of the oocytes, significantly more than in the control (black bars), arrested at meiosis I (Fig. 4A, n = 194 and 255, respectively; *P < 0.001; compare also decreased maturation rate in oocytes used for cytogenetic analysis, Table I). These MCAK-depleted oocytes arrested in GVBD possessed bivalent chromosomes after extended meiosis I arrest at 16 h past resumption of maturation (Fig. 4C′ and E′), unlike the PB control oocytes with 20 metaphase II chromosomes (Fig. 4E′). However, unlike at 10 h of culture, the bivalent chromosomes of arrested, MCAK-depleted meiosis I oocytes were by then nearly all (87%, n = 63) aligned on the bipolar spindle equator at 16 h of culture (Fig. 5A′ and D) and the majority of the oocytes had a fairly normal appearing spindle (87%; Fig. 5A and D) indicating that MCAK may be dispensable for chromosome congression with sufficient time in prometaphase I (Fig. 5D). Spindles appeared similar to oocytes prevented from progressing into anaphase I by proteasome inhibitor from 7 h of maturation to 10 h of culture (Fig. 5E). However, further quantitative evaluation needs to be performed to determine whether spindles had comparable length, width and MT density. Our data suggest that full expression of MCAK was not required to achieve spindle bipolarity at 10 and 16 h of meiotic maturation but that MCAK depletion significantly delayed but did not abolish chromosome congression.

Interestingly, MCAK appears to be indispensable for the metaphase I to anaphase I transition in spite of the presence of aligned chromosomes. The prolonged activity of the SAC was implicated by the persistence of checkpoint proteins Mad2 (Fig. 5D) and BubR1 (Fig. 5D′) at centromeres of bivalents in MCAK-depleted oocytes cultured for 16 h, despite the fact that MCAK was depleted (Fig. 4C). One would not expect to see checkpoint proteins at centromeres if MCAK depletion primarily caused problems with chiasma resolution.

### Table I Chromosomal constitution of spread and C-banded oocytes following microinjection of unspecific (RNAi control MCAK + Mad2) and specific siRNAs (RNAi Mad2 or MCAK or MCAK + Mad2).

<table>
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<tr>
<th>Nuclear maturation</th>
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<tr>
<td>n</td>
<td>GV (%)</td>
</tr>
<tr>
<td>RNAi control (MCAK + Mad2)</td>
<td>100</td>
</tr>
<tr>
<td>RNAi Mad2</td>
<td>334</td>
</tr>
<tr>
<td>RNAi MCAK</td>
<td>96</td>
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<tr>
<td>RNAi MCAK + Mad2</td>
<td>101</td>
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*Statistical difference to control: P = 0.34.

Release of metaphase I arrest by RNAi double knockdown of MCAK and Mad2

To further assess whether the meiotic arrest by MCAK depletion depends on the prolongation of the SAC, oocytes were depleted of both Mad2 and MCAK by injection of specific siRNAs. Following knockdown of MCAK and Mad2, Mad2 mRNA levels were significantly reduced by 87.3% relative to β-actin in controls (P < 0.001, Fig. 4B, green bar). Immunostaining confirmed that Mad2 protein...
Figure 5  Spindles and chromosomes in meiosis I and II oocytes with knockdown of MCAK and/or checkpoint protein Mad2. Spindles and chromosomes of the control oocytes injected with unspecific siRNA, which have not progressed to anaphase I and are still in metaphase I are all bipolar and chromosomes are align at the spindle pole (A, A' and C) similar to oocytes inhibited to separate chromosomes by proteasome inhibitor (+MG, E) given from 8 to 12 h of culture. In contrast, the majority of meiosis I-arrested oocytes after knockdown of MCAK (A, A' and B) that are still in meiosis I fail to congress chromosomes at 10 h of culture, although spindles are bipolar (middle bars). Most meiosis I-arrested MCAK-depleted oocytes eventually possess bipolar spindles and aligned chromosomes at 16 h of culture (right bars) (A, A' and D). Metaphase I-arrested oocytes depleted of MCAK (RNAi MCAK) express Mad2 (green dots in D, inset) and BubR1 (green dots in D', inset) at centromeres. At metaphase II after 16 h of culture, spindles are bipolar and possess aligned chromosomes in most RNAi control oocytes (E, I and I'), whereas overall bipolarity may be retained in some oocytes depleted of Mad2 (F) developing to metaphase II, although chromosomes fail to align at the equator (I and I'); aberrant spindles of MCAK-depleted oocytes fail to form focused poles (G) and there are no aligned chromosomes in one plane. A large percentage of oocytes in the double-depleted oocytes have totally unorganized spindles, lack focused poles and possess dispersed chromosomes (H, I and I'). For further explanation, see text. B–C and E–H: tubulin-immunofluorescence images of spindle, green; propidium iodide stained chromosomes, red. Bars: 10 μm.
was depleted from kinetochores of metaphase II chromosomes (Fig. 4C′′′′), in contrast to control oocytes injected with unspecific siRNAs (Fig. 4C′′′, inset). Quantitative analysis by determination of greyscale values showed that immunostaining intensity for Mad2 protein was significantly reduced by 83.0% relative to ACA following specific RNAi (P < 0.001), whereas Mad2 and ACA levels were nearly the same following microinjection of unspecific RNAi in the control (Fig. 4D, red and green bars). The majority (82.9%) of double-depleted oocytes progressed to anaphase I and subsequently to metaphase II (Fig. 4A, grey bars; n = 217, Table I), comparable to controls (uninjected oocytes and oocytes injected with unspecific RNAi, black bars in Fig. 4A, Table I) implying that the meiotic arrest following SAC depletion is dependent on the SAC and that inactivation of the SAC by knockdown of Mad2 releases oocytes from meiosis I arrest. Accordingly, the double-depleted metaphase II oocytes possess metaphase II chromosomes (Fig. 4E′′′′ and E′′′′′).

**Spindle morphology, chromosome congression and chromosomal constitution of MCAK-depleted metaphase II oocytes**

When Mad2 alone is ablated in mouse oocytes, they progress to meiosis II despite severe spindle aberrations induced by an exposure to nocodazole (Homer et al., 2005a,b,c, and our unpublished results). Most oocytes depleted from both Mad2 and MCAK by RNAi double knockdown emitted a first PB in the present study, comparable to controls (Fig. 4A, grey bars). Therefore, we analysed the spindle morphology and chromosome alignment of those oocytes, which progressed to metaphase II after double knockdown of MCAK and Mad2 or single knockdown of MCAK or Mad2. The percentage of oocytes showing aberrant spindles (no bipolar spindle formed) after 16 h of maturation, when they had reached metaphase II increased from 5.0% in the control-injected oocytes (n = 58) to 34.0% and 43.0% in Mad2 and MCAK single-depleted oocytes, respectively (n = 32 and 7, respectively; Fig. 5I and I′). Numbers of metaphase II oocytes in the MCAK group were low due to the efficient meiotic arrest, which was overcome in only few oocytes in this group. There was a reduction in oocytes with normal metaphase II spindles in the double-depleted group (n = 58; Fig. 5I and I′) compared with the single Mad2 knockdown oocytes. A total of 53.8% failed to establish bipolarity and had highly aberrant spindles (Fig. 5H), significantly different from the control (P < 0.001; Fig. 5E, and left bars in I and I′). In single-depleted Mad2 oocytes spindles appeared to frequently possess focused poles (Fig. 5F), whereas there were more astral MTs found in MCAK-depleted meiosis II oocytes (Fig. 5G). Double-depleted oocytes exhibited a total loss of bipolarity and focusing of polar/centrosomal fibres (Fig. 5H) but the phenotypes were not compared quantitatively. In the double-depleted group, oocytes tended to possess extremely disordered meiosis II spindles without astral arrays around centrosomes (Fig. 5H). Further quantitative analysis will be performed in a separate study. The quantitative analysis performed so far showed that chromosome congression failure (Fig. 5I, right bar) was increased from 9% in the RNAi control to 56% and 43% in the Mad2 and MCAK RNAi single-depleted oocytes, respectively, and it was 41% in the double-depleted meiosis II oocytes (P < 0.001). Increases in spindle aberrations and chromosome congression defects in both single- or double-depletion experiments indicate therefore that knockdown of MCAK perturbs spindle formation at metaphase II (Fig. 5G), particularly, when the SAC is overcome by Mad2 depletion (Fig. 5H). There appears to be no synergistic effect of depletion of MCAK and Mad2 compared with Mad2 alone in failures of chromosome congression but the numbers of metaphase II oocytes in the MCAK-depleted group are too low to draw any firm conclusions on relative impact of MCAK depletion on metaphase II (Fig. 5I, right bars). Although MCAK does not appear to be essential for eventual first meiotic spindle formation and Mad2 depletion alone causes some metaphase II spindle aberrations, the especially severe spindle abnormalities observed in double-knockdown oocytes may reflect problems in resolving merotelic chromosome attachments as well as organizing MTs at centrosomes at prometaphase II due to the MCAK depletion throughout meiosis I up to metaphase II (Zhang et al., 2008).

To determine whether spindle abnormalities in double-knockdown oocytes are related to chromosome segregation errors during meiosis I, cytogenetic analysis was performed. There were no hyperploid oocytes in uninjected controls (Table I) or in the unspecific RNAi controls (Table I; Fig. 4E′′′′). Also, we did not find significant increases in hyperploidy in young mouse oocytes progressing to meiosis II after Mad2 depletion by RNAi (1.6%; Table I), possibly due to the existence of multiple feedback controls in cell cycle control in healthy, young and largely unstressed oocytes from natural cycles (group RNAI Mad2 in Table I). Hyperploidy was also not increased in Mad2 and MCAK double-knockdown oocytes, although numbers of oocytes with 20 dyads (Fig. 4E′′′′′) dropped to 84.5% (Table I). It was not possible to obtain meaningful values of numerical aberrations for the MCAK-depleted oocytes escaping the meiotic block since most failed to mature to meiosis II under our currently employed conditions.

In contrast to hyperploidy, hypoploidy was low in uninjected controls (5.6%) and in the control group microinjected with unspecific RNAi for MCAK and Mad2 (6.8%) but was increased to 10.1% in Mad2-alone-depleted oocytes and 13.8% in the double-depleted MCAK and Mad2 oocytes, respectively (Table I; Fig. 4E′′′′′′). The increase in hypoploidy was apparently not reliant on spreading artefact but could be verified in some oocytes by analysis of supernumerary chromosomes/chromatids in the first PB (Fig. 4E′). The highly aberrant spindles in the double-depleted group could therefore possibly be related to the chromosome segregation defects inducing hyperploidy.

**Maturation kinetics and anaphase lagging after inhibition of AURK from prometaphase I**

In order to interfere with AURKB-mediated regulation of MCAK activity, oocytes were matured in the presence of ZM447439 inhibitor from late prometaphase I stage (from 7 h of maturation; ZM t = 7) to 12 or 17 h of culture. This did not prevent first PB formation, and the majority of oocytes matured to metaphase II as shown also in a previous study (Vogt et al., 2009). However, polarization microscopy revealed that oocytes exposed to ZM from 7 h of culture emitted the first PB with a delay in comparison to controls (Fig. 6A). Polynomial regression analysis of PB formation in 80 min time intervals suggests that PB formation was delayed in ZM-exposed oocytes since the majority of controls emitted their first PB at 560 min of
maturation whereas ZM-exposed oocytes mainly formed the first PB at 637 min in response to AURK inhibition from late stages of meiosis I (Fig. 6A). Although MCAK’s MT depolymerizing activity is negatively affected by phosphorylation by AURKB, its localization to centromeres is specifically disrupted by AURK inhibition (Andrews et al., 2004). Therefore, it is not surprising that both MCAK depletion by RNAi and AURKB inhibition caused meiotic delay. Both treatments would eliminate MCAK activity from centromeres.

Figure 6 Maturation of oocytes exposed to AURK inhibitor ZM from 7 h of in vitro maturation, prior to the metaphase I/anaphase I transition. (A) Selected images by polarization microscopy (OctaxEyeWear) of spindles of in vitro maturing control and ZM-exposed oocytes cultured from 7 to 10 h in the presence of ZM (1.5 μM ZM t = 7). (A') The line for polynomial regression curves of PB formation in 80 min time intervals from 480 to 960 min was generated by fitting with Microsoft Excel software. $R^2$ values for each fit are reported at the right side of the graph. Maximum of anaphase I progression and PB formation are indicated by arrows showing substantial delay in cell cycle progression and PB formation in the ZM-exposed group. (B) Most control oocytes contain metaphase II spindles that are bipolar with aligned chromosomes (B and C), whereas ZM-exposed oocytes show spindle aberrations and unaligned chromosomes (B and D). (E) Control meiosis II oocytes usually possess 20 metaphase chromosomes (left upper panel), as well as ZM-exposed oocytes (right upper panel). However, many ZM-exposed oocytes are hypoloid (<20) and some contain sticky chromosomes (right lower panel in E) or condensed bivalents or dyads attached to each other at their telomeres (arrows in E, left lower panel) as well as metaphase II chromosomes and are therefore aneuploid (E). Numbers do not reflect identity of chromosomes in E. (G) ZM-exposed oocytes contain more single chromosomes, which are lagging behind at anaphase I (MG + ZM; G) in comparison to controls (Low MG; F). Tubulin-immunofluorescence images of spindle, green; propidium iodide stained chromosomes, red. Bar in A: 50 μm. Bar in C–E and G: 10 μm.
In addition, we can show here that about one-third of the metaphase II oocytes exposed to ZM447439 from 7 h of culture, which progressed to meiosis II upon culture to 12 h, failed to form normal bipolar spindles (Fig. 6B and D), and in about half of these oocytes, chromosomes did not congress at the spindle equator (Fig. 6B), in contrast to controls cultured for the same time without inhibitor (Fig. 6C).

As shown already by a previous study (Vogt et al., 2009), the present study confirmed that ZM disturbs chiasma resolution and loss of chromosome cohesion not only in oocytes exposed to ZM throughout maturation but also in oocytes exposed to ZM only from 7 h of maturation when they had already reached prometaphase I stage (Fig. 6E). Although some oocytes that progressed to meiosis II under these conditions had a normal chromosomal constitution (Fig. 6E, right upper image) as in the controls (left upper image), other ZM-exposed oocytes had less than 20 dyads and what appeared to be ‘sticky’ chromosomes (Fig. 6E, right lower image). Still, others were hypoploids and contained bivalent-like chromosomes (Fig. 6E, arrows in lower left image).

In order to analyse chromosome behaviour at anaphase I when AURK was inhibited, oocytes were also matured from 7 h up to late prometaphase I in normal culture medium before continuing culture in the presence of 1 mM MG132 proteasome inhibitor, thus preventing or slowing anaphase I progression. In addition, one group of oocytes matured in 1.5 μM ZM AURK inhibitor plus 1 mM MG132 from 7 h of maturation. Both groups of oocytes were fixed 2 h later for tubulin-immunofluorescence. The concentration of MG132 was kept purposefully low to allow some oocytes to progress to anaphase I during the 2 h time window without or with the ZM inhibitor. Although most control oocytes in anaphase I of the MG132 group (more than 80%) showed no signs of lagging chromosomes at anaphase I (Fig. 6F), the group exposed to both the ZM447439 and MG132 inhibitors contained frequently single chromosomes, which were lagging behind (14% versus 36% in the MG control and ZM/MG group, respectively; Fig. 6F and G). Although numbers of anaphase I oocytes in both groups were low, the data imply that lagging may thus relate to loss of centromere-associated MCAK activity by the inhibition of AURKB-mediated phosphorylation. This will have to be confirmed by further studies.

**Discussion**

**Dynamic distribution of MCAK in oocyte maturation**

The control of MT dynamics involving members of the kinesin protein family such as MCAK is essential in order to achieve a balance between stable chromosome anchoring to the spindle and resolution of improper or hyperstable MT attachments in genomic stability of eukaryotic cells (Mayr et al., 2007; Bakhoun et al., 2009; Cai et al., 2009). This process is controlled by AURKB kinases which regulate MCAK and other components of the kinetochore to facilitate error correction. In first meiosis of spematocytes, INCENP and AURKB load first on chromocentres prior to nuclear envelope breakdown followed by Shugosin-like 2 protein and MCAK accumulation at centromeres at late diakinesis/early diplonete (Parra et al., 2009). Expression of the MCAK-EGFP fusion protein in mouse oocytes suggests that uptake of MCAK into the nucleus is taking place exclusively in developmentally competent G2-phase oocytes possessing an SN, designating it as a new marker of oocyte competence. The protein appears particularly enriched at the nucleolus around which centromeres and pericentromeric heterochromatin accumulate at this stage (Longo et al., 2003). MCAK first transits to cytoplasm before its recruitment to the region around the spindle and to chromosomes and centromeres at mid to late prometaphase I. In contrast, MCAK is retained at the centromeres upon nuclear membrane breakdown in spermatocytes (Parra et al., 2009). This difference might relate to the specific requirements for acentriolar spindle formation in oocytes, in which a gradient of targeting protein (TPX-2) at chromosomes is involved in initiation of spindle formation after GVBD (Brunet et al., 2008).

It is possible that chromosome-bound MCAK at sites of cohesion between sister chromatids at subsequent prometaphase I stage plays a role in preventing stable attachment of MTs to sister chromosome arms which would promote their untimely separation and chiasma resolution. The retention of MCAK at chiasmata could serve this role also at later stages of meiosis I until anaphase I when chromosomes come under tension. We did not detect MCAK-EGFP protein at chiasmata in all prometaphase I or metaphase I oocytes or in gently lysed oocytes using MCAK antibodies. This might relate to the compaction and contraction of chromosomes at prometaphase I/metaphase I and to the relative concentration of the fusion protein in ooplasm, which determines the intensity of signal at a distinct time window with more or less accumulation of protein, e.g., at late prometaphase I or before transition from metaphase I to anaphase I. Unfortunately, it is not possible to unambiguously define sites of exchanges on meiotic chromosomes by staining with antibodies to meiotic proteins like MLH3 mismatch repair protein as in meiotic pachytenne stages in maturing mouse oocytes. Such proteins are no longer present on chromosomes once oocytes have progressed beyond the prophase I stage in the fetal ovary and have reached dictyate stage shortly before resumption of maturation or at M-phase (e.g. Kan et al., 2008). Therefore, it has not been possible to unambiguously determine whether MCAK is consistently retained at sites of exchanges throughout the chromosome, at sites with attached MTs at telomeric sites or along the sister chromatid interface where tension is high (e.g. the most proximal chiasma). However, the unexpected detection of MCAK at both sites on bivalent chromosomes connecting homologous chromosomes and also at distal sites close to telomeres where chromosomes are physically connected is indicative of a unique role of MCAK at sites of exchanges and chiasmata in female meiosis.

The presence of MCAK at the central spindle and the midbody, a site where the most proximal exchanges/chiasmata become positioned during metaphase I might result from a release of the protein from chiasmata upon anaphase I transition. This needs to be further explored to decide whether there is a cell cycle-dependent ‘shedding’ regulated by changes in activity and distribution of other proteins or a recruitment of MCAK to the plus-ends of depolymerizing MTs in the central spindle and the midbody (Jiang et al., 2009). MCAK in the central spindle may play a role in local regulation of MT dynamics (Glotzer, 2009).

Here, it is shown for the first time that MCAK is a major protein of centrosomes in acentriolar spindles at meiosis I and II in vivo and is associated with the centrosomal MTOCs forming a string-of-bead ring at the anastral spindle poles in oocyte maturation, consistent with a role of MCAK in focusing of spindle poles (De Luca et al., 2008; Zhang et al., 2008). Aberrant metaphase II oocytes depleted of MCAK and Mad2 throughout maturation characteristically exhibit...
unordered spindles without focused spindle poles but further studies are required to prove this quantitatively. The activity of MCAK at spindle poles may be regulated primarily by AURKA-mediated phosphorylation of MCAK at early M-phase as shown in cytoplasmic extracts of Xenopus eggs (Zhang et al., 2008). The distribution of the fusion protein in oocytes resembles the assembly of foci of MTOCs recognized by pericentrin, which are also forming a ring-like assembly of foci at the flat poles of the meiosis I spindle during in vitro maturation and at meiosis II (Carabatsos et al., 2000; Can et al., 2003; Eichenlaub-Ritter et al., 2007). Overall, the observations suggest that MCAK might have a role in mediating integrity of spindle poles in acellulor spindles of mammalian oocytes. MCAK activity is involved in regulation of MT dynamics at centromeres in dependence of inactivation/activation by differential AURKB phosphorylation or ICIS, respectively, at later prometaphase and metaphase stages of mitotic cells (Huang et al., 2007; Zhang et al., 2008). In fact, we were initially unable to recognize MCAK at spindle poles and centromeres by antibodies in Triton X-100 permeabilized and formaldehyde or methanol-fixed oocytes, possibly because there is a loss of protein after extensive exposure of oocytes to the non-ionic detergent or because epitopes cannot be recognized by antibody because the interactions of MCAK with other proteins of the spindle and centromeres, respectively. We were unable to detect the MCAK-EGFP fusion protein in Triton X-100 permeabilized and methanol-fixed oocytes (unpublished observations); therefore, the loss upon cell permeabilization is more likely. In contrast, in gently lysed oocytes with a protocol developed to retain centromeric proteins and their antigenicity (Hodges and Hunt, 2002), in which chromosomes become dispersed on the slide, centromeres become exposed and reactive to MCAK antibody. In such spread preparations, we were able to clearly localize MCAK to sites next to and overlapping with centromere proteins recognized by ACA. In addition, it became possible for the first time to recognize MCAK in living oocytes by expression of an EGFP-tagged MCAK fusion protein confirming the presence and the enrichment of MCAK at centromeres in meiosis I and II. A distinct role of MCAK specific to female meiosis can be postulated from the differences in MCAK distribution between oocytes and spermatocytes at the transition from meiosis I to meiosis II. Although this transition is characterized by a brief interphase in male meiosis before spermatocytes progress to prometaphase II, oocytes transit continuously without interphase from telophase I to prometaphase II and retain condensed chromosomes. In contrast to spermatocytes, in which MCAK disappears from centromeres at telophase I, MCAK is retained at centromeres throughout first and second meiosis in oocytes. MCAK remains continuously enriched at centromeres from prometaphase I to telophase I up to prometaphase II and metaphase II in mouse oocytes. In contrast, AURKB translocates to the midspindle at telophase I and is absent from centromeres briefly at this stage but associates immediately again with chromosomes and centromeres even before completion of cytokinesis when oocytes enter prometaphase II (Vogt et al., 2009). Unphosphorylated MCAK could therefore help to depolymerise MTs at centromeres efficiently at telophase I stage of meiosis I in oocytes and thus prevent chromosome lagging. Interestingly, at this stage and later, we observe consistently also a distinct spot of MCAK-EGFP fusion protein at the site marking the remnant of the midspindle, where cytokinesis occurred. At the midzone, plus-ends of MTs interdigitate (Glotzer, 2009).

Since MCAK associates with the plus-ends of MTs (Jiang et al., 2009), one may speculate that MCAK could have a role in the efficient disassembly of interpolar spindle MTs at the completion of first meiosis in oocytes and that the large pool of MCAK at this site might also facilitate MCAK’s transit together or after the CPC and AURKB to the centromeres of sister chromatids at the prometaphase II stage (Sampath et al., 2004; Vagnarelli et al., 2004; Parra et al., 2009).

The differences in localization and activity of MCAK might also contribute to the sexual dimorphism in chromosome segregation mechanisms, (Bond and Chandley, 1983; Hunt and Hassold, 2002; Morelli and Cohen, 2005; Adler et al., 2007; Pacchierotti et al., 2007) for instance, the processing of dicentric chromosomes or lagging chromosomes in male and female meiosis. Unlike in male meiosis, segregation of a dicentric chromatid thus frequently results in precocious separation of the sister centromeres of at least one homologue in oocytes and embryos rather than in chromosome breakage, stretching or loss as prevalent in spermatogenesis (Koehler et al., 2002). This could relate to the presence of MCAK in the central part of the spindle of dividing oocytes to facilitate depolymerization of MTs attached to both centromeres of dicentric chromosomes or to merotelically attached lagging chromosomes.

**Function of MCAK in meiotic spindles**

RNAi depletion of MCAK in mouse oocytes does not prevent establishment of a bipolar spindle but it appears to increase disturbances in spindle formation by knockdown of Mad2 in double-depleted oocytes. This does not appear to be related to inefficient reduction in MCAK mRNA or protein. The quantitative RT–PCR shows that mRNA is dramatically reduced relative to β-actin mRNA. Furthermore, the absence of distinct signals for MCAK in immunofluorescence when compared with the strong staining by centromere-specific antibodies suggests a significant reduction in concentration/abundance of MCAK protein. In fact, MCAK-EGFP is initially found at the periphery rather than within the spindles at early prometaphase when spindle bipolarity is first established. Similarly, MCAK has not been previously correlated with establishment of bipolar spindles during mitosis (Zhu et al., 2005). This role has rather been attributed to Kif2A kinesin (Manning et al., 2007). However, recent studies suggest that Kif15, and not Kif2A, participates in the establishment of bipolar spindles when Eg5 (kinesin-5 motor protein) is weakened (Tanenbaum et al., 2009). In mouse oocytes, there was no evidence for dramatic increases in aberrant spindles in prometaphase I/metaphase I oocytes after knockdown of MCAK and culture for 10 h while forced progression into meiosis II by depletion of Mad2 interfered with normal spindle formation and caused failure in chromosome congression at metaphase II.

Importantly, we demonstrate here that RNAi-mediated single knockdown of MCAK in mouse oocytes induces a much delayed congression of chromosomes at the spindle equator at prometaphase I and a prolonged meiosis I arrest. Although knockdown of MCAK does not prevent eventual alignment of chromosomes on a bipolar meiosis I spindle at 16 h of culture, this is associated with a substantial maturation arrest. Therefore, our data clarify why the role of MCAK in congression in mitosis has been somewhat equivocal. Some studies report a congression failure but others do not. The oocyte system clearly shows that congression is delayed but not prevented by depletion of MCAK.
Regulation of MCAK activity by AURKB

In mitosis, centromere tension may be sensed by increased spatial separation of AURKB from kinetochore substrates, which reduces protein phosphorylation and stabilizes kinetochore MTs. Repositioning AURKB closer to the kinetochore prevented stabilization of bi-oriented attachments and activated the SAC suggesting that AURKB’s position correlates with MT turnover (Liu et al., 2009). AURKB is also required to localize MCAK to centromeres (Andrews et al., 2004). We exposed mouse oocytes to an inhibitor to AURKs, ZM447439, which preferentially inactivates AURKB at low concentrations, in order to interfere with the level of MCAK activity on centromeres. Shuda et al. (2009) showed that overexpression of AURKB, but not AURKA or AURKC, rescued the chromosome alignment defects induced by the ZM kinase inhibitor suggesting that AURKB is the primary AURK responsible for regulating chromosome dynamics during meiosis in mouse oocytes (Shuda et al., 2009). A previous study by our group revealed that continuous exposure to low ZM447439 caused spindle aberrations, chromosome congression failure, inhibition of cytokinesis and alterations of post-translational modifications of histones at pericentromeric heterochromatin in mouse oocytes (Vogt et al., 2009). Under these circumstances, there was also a maturation arrest, and many oocytes failed to form a first PB and to progress to anaphase I/telophase I, consistent with a role of AURKB and its regulation of MCAK in control of the SAC. However, the AURKB inhibitor caused a leaky meiotic arrest at meiosis I and affected loss of sister chromatid cohesion in oocytes since we observed inhibitor-treated oocytes without first PB that contained a polyploid number of metaphase II chromosomes, or bivalents and metaphase II chromosomes within the same oocyte (Vogt et al., 2009). Since AURKB has so many targets, these observations are unlikely to be restricted to its role in regulating MCAK. Inhibition of AURKs only from late prometaphase/metaphase I onwards by ZM447439 in the present study permitted oocytes to progress to anaphase I suggesting that once chromatin at centromeres attained a ‘mature’ constitution with chromosomes aligned, inhibition of AURKB no longer blocks anaphase progression. A delay in anaphase I progression of ZM447439-treated oocytes was evident by polarization microscopy. This delay might correlate with the loss of centromeric MCAK in ZM447439-treated cells.

In a previous study, consequences of transient inactivation of AURKB on the chromosomal constitution were analysed in metaphase II oocytes showing that there was no increase in hyperploidy (>20 dyads; zero hyperploidy in control and ZM-exposed oocyte, Vogt et al., 2009) but, similar to the MCAK and Mad2 knockout situation (Fig. 5E and E'”, Table I), hyperploidy rate (<20 dyads) was increased from 5.6% in controls to 15.4% in ZM-exposed oocytes (Vogt et al., 2009). This suggests that deregulation of MCAK activity, by either depletion or inhibition of AURKB, may disturb chromosome segregation at anaphase I (Wang et al., 2006) and may cause a meiotic drive for segregation of a supernumerary number of chromosomes to the first PB.

MCAK in checkpoint control and protection from errors in chromosome segregation

Several studies in human and mouse oocytes from aged females have lead to the concept that alterations in gene expression contribute to the high risk for errors in chromosome segregation with advanced maternal age. In particular, such studies suggest that products of genes involved in spindle formation, checkpoint control and protein stability may be altered in abundance in aged oocytes, e.g. Mad2, MCAK and AURK (Steuerwald et al., 2001, 2007; Hamatani et al., 2004; Pan et al., 2008). Messenger RNA for human Mad2 (MAD2L1) was more abundant in individual aged compared with young human metaphase II oocytes from stimulated cycles (Grøndahl et al., 2010), and this was discussed in increased risks for chromosome non-disjunction (Sotillo et al., 2007). Aneuploidy is increased in somatic cells of homozygous Mad2 knockout mice or haplo-insufficient Mad2 animals (Dobles et al., 2000; Michel et al., 2001). Metaphase II oocytes of heterozygous Mad2+/- mice have also increased levels of aneuploidy (Niault et al., 2007). However, we did not find significant increases in hyperploidy in Mad2 knockout oocytes, which came from adult females and spontaneous cycles, although hypoploidy was increased, unlike in previous studies involving superovulation (Homer et al., 2005b). In one study in mice (Pan et al., 2008), MCAK mRNA, and mRNAs coding for checkpoint proteins and for components of the CPC like Incenp (inner centromere protein), appeared reduced, whereas AURKB message was increased in aged compared with young oocytes (Pan et al., 2008). Unexpectedly, the efficient depletion of MCAK in the present study does not cause aneuploidy since it leads to a meiosis I arrest. Activation of the SAC by MCAK depletion has not been previously reported in mitosis. In fact, MCAK depletion is commonly assumed to evade checkpoint surveillance because aberrant merotelic connections that form in the absence of MCAK are under tension (Cimini et al., 2006). This suggests that in meiosis MCAK is part of the checkpoint surveillance machinery.

Double depletion of MCAK and Mad2 caused the release from the meiotic arrest and a number of oocytes matured to metaphase II. Chromosomal analysis failed to show increases in hyperploidy in these oocytes, whereas hypoploidy was nearly two times as high as in the unspecific RNAi-injected controls and also higher than in Mad2 single knockdown oocytes. The absence of MCAK likely leads to a deficiency in solving improper attachments of MTs. This may prolong, but not eliminate, chromosome congression and also lead to lagging chromosomes in oocytes progressing to anaphase I. Interestingly, such chromosomes appear to have a greater probability to end up in the first PB rather than the oocyte. This would explain the increase in hypoploidy in our model with reduced MCAK and Mad2. Since spindles are extremely aberrant and chromosomes fail to congress on the spindle in many meiosis II double-depleted oocytes, the probability that second meiotic errors occur upon fertilization of such oocytes is extremely high. Therefore, this study suggests that critical reductions in both mRNAs/proteins regulating spindle dynamics, chromosome behaviour and also checkpoint control, like MCAK and Mad2, are predisposing to first and second meiotic errors.

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