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Aurora-C Kinase Deficiency Causes Cytokinesis Failure in Meiosis I and Production of Large Polyploid Oocytes in Mice

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We previously isolated Aurora-C/Aie1 in a screen for kinases expressed in mouse sperm and eggs. Here, we show the localization of endogenous Aurora-C and examine its roles during female mouse meiosis. Aurora-C was detected at the centromeres and along the chromosome arms in prometaphase I–metaphase I and was concentrated at centromeres at metaphase II, in which Aurora-C also was phosphorylated at Thr171. During the anaphase I–telophase I transition, Aurora-C was dephosphorylated and relocalized to the midzone and midbody. Microinjection of the kinase-deficient *Aurora-C (AurC-KD)* mRNA into mouse oocytes significantly inhibited Aurora-C activity and caused multiple defects, including chromosome misalignment, abnormal kinetochore–microtubule attachment, premature chromosome segregation, and cytokinesis failure in meiosis I. Furthermore, AurC-KD reduced Aurora-C and histone H3 phosphorylation and inhibited kinetochore localization of Bub1 and BubR1. Similar effects also were observed in the oocytes injected with *INCNEP-delIN* mRNAs, in which the Aurora-C binding motif was removed. The most dramatic effect observed in *AurC-KD*–injected oocytes is cytokinesis failure in meiosis I, resulting in producing large polyploid oocytes, a pattern similar to Aurora-C deficiency human spermatozoa. Surprisingly, we detected no Aurora-B protein in mouse oocytes. We propose that Aurora-C, but not Aurora-B, plays essential roles in female mouse meiosis.

INTRODUCTION

Sexually reproducing organisms rely on the precise reduction of chromosome number during meiosis, which generates haploid gametes from diploid precursors. During meiosis, a single round of DNA replication is followed by two rounds of meiotic division, termed meiosis I (MI) and meiosis II (MII) (Petronczki *et al.*, 2003; Hauf and Watanabe, 2004). In prophase of MI, homologous chromosomes pair with each other to form a bivalent chromosome in which at least one recombination event occurs between a paternal and a maternal chromatid. At metaphase I, the kinetochores of sister chromatids attach to microtubules emanating from the same spindle pole (co-orientation). However, the kinetochores of a homologous chromosome pair associate with microtubules emanating from opposite poles (biorientation). During anaphase and cytokinesis of MI, the homologous chromosomes are pulled to opposite spindle poles, resulting in two daughter cells. Resolution of chiasmata in meiosis I requires proteolytic cleavage of cohesin Rec8 along chromosome arms by separase, which is activated by the anaphase-promoting complex/cyclosome (APC/C) (Kudo *et al.*, 2006). However, the meiotic activation of the APC/C does not occur until chromosome biorientation has been completed. The key inhibitor responsible for APC/C-dependent anaphase onset is the spindle assembly checkpoint (SAC) (Musacchio and Salmon, 2007). In MII, segregation of chromatids to opposite spindle poles occurs through a process similar to mitosis and finally generates haploid gametes. In mammals, female meiosis usually produces a single, large, haploid oocyte and two small polar bodies via two successive meiotic divisions. Errors in the transmission of chromosomes during meiosis can produce aneuploidy, which is the leading cause of miscarriage and is also responsible for genetic disorders such as Down's syndrome (Hassold and Hunt, 2001). The molecular mechanisms that control chromosome segregation during both meiotic divisions have started to be delineated.

The Aurora kinases are a conserved family of serine/threonine kinases that are pivotal to the successful execution of cell division. In mammals, three Aurora kinases (Aurora-A, -B, and -C), which share sequence homology in their central catalytic kinase domains, have been identified previously (Nigg, 2001). Aurora-A and -B are known to play distinct roles in mitosis and localize to discrete mitotic structures (Andrews *et al.*, 2003; Carmena and Earnshaw, 2003; Ruchaud *et al.*, 2007). Aurora-A, which localizes to centrosomes and mitotic spindle poles, is required for centrosome maturation, bipolar spindle assembly, and chromosome segregation. Aurora-B is a member of chromosome passenger complex (CPC), which comprises Aurora-B itself and three nonenzymatic subunits: INCENP, survivin, and borealin. Aurora-B binds to a region called the IN box located at the C-terminal domain of INCENP and such binding activates Aurora-B in species from yeast to mammals. Aurora-B is essential for proper chromosome segregation, kinetochore–

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microtubule (K-MT) interaction, and cytokinesis (Carmena and Earnshaw, 2003; Ruchaud *et al*., 2007).

In contrast to Aurora-A and -B, characterization of Aurora-C has been rather limited. Aurora-C (originally named AIE1 in mouse or AIE2 in human) was identified in a screen for kinases expressed in sperm and eggs in our laboratory (Tseng *et al*., 1998). Unlike Aurora-A and -B, which are ubiquitously expressed in many tissues, particularly in mitotically dividing cells, Aurora-C is predominantly expressed in the testis (Bernard *et al*., 1998; Tseng *et al*., 1998) and is mainly restricted to meiotically dividing spermatocytes (Tang *et al*., 2006) and oocytes (this study). Immunofluorescence analysis revealed that Aurora-C localizes at the centromeres in MI and MII spermatocytes and relocates to the spindle midzone and midbody during the anaphase I–telophase I and anaphase II–telophase II transitions. Aurora-C is also associated with INCENP in male spermatocytes (Tang *et al*., 2006). Interestingly, no endogenous Aurora-C protein was detected in a number of normal mouse somatic tissues and mitotic spermatogonia, implying that Aurora-C is a member of meiotic CPC (Tang *et al*., 2006).

Studies of the function of CPC in meiosis in most species are commonly hindered by the fact that null mutations in CPC genes are embryonic-lethal (Cutts *et al*., 1999; Uren *et al*., 2000; Hanson *et al*., 2005; Chang *et al*., 2006). In *Caenorhabditis elegans*, depletion of the Aurora-B homologue AIR-2 by RNA interference causes a defect in separation of homologous chromosomes and release of chromosome cohesion during meiosis (Kaitna *et al*., 2002; Rogers *et al*., 2002). In fly, Aurora-B and INCENP contributes to the maintenance of meiotic chromosome cohesion through MEI-S332/Shugoshin (Resnick *et al*., 2006). Furthermore, Aurora/Ipl1 is not only required for correct Sgo1 localization (Monje-Casas *et al*., 2007; Yu and Koshland, 2007) but also maintains the centromeric localization of PP2A to protect cohesion during meiosis (Yu and Koshland, 2007).

In higher mammals, little is known about the function of Aurora-C kinase in meiosis. The slow progress in the characterization of Aurora-C in meiotic divisions has been due to lack of a specific antibody and the fact that multiple copies of *Aurora-C* (*Aie1*) genes are present in the mouse genome (Hu *et al*., 2000), which make it impractical to study Aurora-C's function using a knockout mouse approach. Recently, Dieterich *et al*. (2007) reported that a homozygous mutation (c.144delC) in the human *Aurora-C* gene led to the production of large-headed multiflagellar polyploid spermatozoa and that the same mutation also caused meiosis I arrest in male spermatocytes (Dieterich *et al*., 2009). However, the molecular basis of how the *Aurora-C* gene defect causes this phenotype is unclear. In this study, we have analyzed the subcellular localization of endogenous Aurora-C in mouse oocytes and analyzed the perturbing effects of Aurora-C kinase-deficient (AurC-KD) mutant as well as its binding partner INCENP mutant on meiotic divisions. Our results showed Aurora-C can be found at the chromosome axes and centromeres at meiotic metaphase I and is concentrated at centromeres at meiotic metaphase II. During the anaphase I–telophase I and anaphase II–telophase II transitions, Aurora-C relocalizes to the midzone and midbody. Furthermore, our results showed that inhibition of Aurora-C kinase activity induces abnormal kinetochore–microtubule attachment, premature chromosome separation, and cytokinesis failure in MI, which results in a polyploid oocyte at the end of meiosis. These findings may explain, at least in part, how homozygous mutation in the *Aurora-C* gene causes polyploid spermatozoa in humans. Interestingly, only Aurora-C kinase protein, but not Aurora-B, was detected in mouse oocytes, implying that Aurora-C may function as a meiotic chromosomal passenger protein during female mouse meiosis.

MATERIALS AND METHODS

Collection of Mouse Oocytes

Germinal vesicle (GV) stage oocytes were isolated from ovaries of 3-wk-old C57BL6/J female mice superovulated by intraperitoneal injection of 5 IU of pregnant mare's serum gonadotrophin for 48 h as described previously (Tang *et al*., 2004). For microinjection, denuded GV oocytes were cultured in M2 medium containing 100 μM 3-isobutyl-1-methylxanthine (IBMX) (Sigma-Aldrich, St. Louis, MO), a phosphodiesterase inhibitor that arrests oocytes in the prophase I stage. For oocytes maturation, injected oocytes were washed and cultured in IBMX-free M2 or M16 medium (Sigma-Aldrich) and incubated in a 5% CO_2 atmosphere at 37°C.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNAs were isolated from MI- or MII-stage oocytes and reverse transcripted first cDNA by using Cell-to-cDNA kit (Ambion, Austin, TX) according to manufacturer's instruction. Primers for mouse Aurora-C and Aurora-B were designed with no sequence overlap between them (Aurora-C sense primer, 5′-tgtacaactacttctatgatgacactcg-3′ and antisense primer, 5′-acagatcaaccatctcattgtacg-3′; Aurora-B sense primer, 5′-gaacacgttgtcccagagagtccta-3′ and antisense primer, 5′-gttgaaggatgttgggatgtttcag-3′). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (sense primer, 5′-gacccctcattgacctcaac-3′ and antisense primer, 5′-ggagatgatgacccttttggc-3′). PCR amplification was performed for 40 cycles (94°C for 30 s, 58°C for 30 s, and 72°C for 30 s) after initial heat at 94°C for 5 min by using a thermal cycler (Takara, Kyoto, Japan). The PCR-amplified products (Aurora-C, 351 base pairs; Aurora-B, 372 base pairs; and GAPDH, 264 base pairs) were electrophoresed in 1.5% agarose gel and visualized by ethidium bromide via UV light.

cDNA Construction and In Vitro mRNA Synthesis

cDNA clones for green fluorescent protein (GFP)-tagged mouse Aurora-C wild-type (WT) and KD mutant have been described previously (Chen *et al*., 2005). Full-length cDNAs encoding GFP, Aurora-B, INCENP, or a truncated INCENP mutant (INCENP-delIN) were obtained by RT-PCR using total RNA from mouse testis or TM4 cells (a Sertoli cell line). The PCR-amplified products were inserted into pEGFP-C1 vector (BD Biosciences, San Jose, CA) and completely sequenced. The cDNAs encoding GFP, GFP-tagged Aurora-B, Aurora-C, Aurora-C-KD, INCENP, INCENP-delIN, or mCherry-H2B also were subcloned into pCDNA3.1(+) vector (Invitrogen, Carlsbad, CA) for in vitro RNA synthesis, by using mMESSAGE mMACHINE kit (Ambion) according to the manufacturer's instructions. All resultant plasmids were sequence confirmed. The concentration of each synthesized mRNA was determined by NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA) before injection.

Microinjection of mRNA and Live Imaging Microscopy

Denuded GV oocytes were microinjected with ~5–15 pl of ~0.5–1 μg/μl of the indicated mRNAs by using a micromanipulator (Narishige, Tokyo, Japan) as described previously (Tang *et al*., 2004). mRNA-injected oocytes were maintained in M2 medium containing IBMX at 37°C for 2 h to enrich protein expression. For resumption of maturation, injected oocytes were incubated as described above. Time-lapse movies were taken with an UltraView live cell imaging confocal scanner (PerkinElmer Life and Analytical Sciences, Boston, MA) equipped with a 37°C incubator and a 5% CO_2 supply.

Chromosome Spreads of Mouse Oocytes

For preparation of chromosome spreads of meiotic oocytes, we used the drying-down method as described previously (Peters *et al*., 1997). In brief, the zona pollucida of injected oocytes was removed in Tyrode's solution (Sigma-Aldrich), followed by incubation in a hypotonic solution (50% fetal bovine serum in water) at 37°C for ~10–20 min. The oocytes were then dispersed on a coverslip that contained the fixation solution (1% paraformaldehyde, 70 mM dithiothreitol, and 0.15% Triton X-100, pH 9.2), followed by slow drying of the coverslip in air for 2 h.

Antibodies

The Aurora-C antibodies (monoclonal and polyclonal) used in this study had been reported previously (Tang *et al*., 2006). For generation of phospho-Aurora-C antibody, a polypeptide corresponding to the surrounding sequence of Thr171 (NH_2-SLRRKpTMCGTL-COOH) was coupled to KLH and used for immunization of rabbit. The antibody was sequentially purified by using nonphospho- and phospho-peptide-affinity columns. The following

antibodies used in this study were purchased from the indicated commercial companies: rabbit polyclonal antibodies against phospho-histone H3 (Millipore, Billerica, MA), INCENP (Abcam, Cambridge, MA), Bub1 (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-centromere antibody (ACA; Antibodies, Davis, CA); mouse monoclonal antibodies against Aurora-B (AIM-1; BD Biosciences), α-tubulin (DM1α; Sigma-Aldrich), acetylated tubulin (Sigma-Aldrich), and β-actin (AC-74; Sigma-Aldrich); and goat polyclonal antibody against BubR1 (Santa Cruz Biotechnology).

Immunoblotting

For immunoblotting, the cellular proteins prepared from transfected cells or oocytes (~500 oocytes) were solubilized in the sample buffer, boiled, and resolved by SDS-polyacrylamide gel electrophoresis. Proteins were then transferred to polyvinylidene fluoride membranes and probed with the indicated antibodies as described previously (Tang *et al*., 2006).

Immunofluorescence Confocal Microscopy

Cultured cells were fixed with cold methanol for 10 min at −20°C. After fixation, the cells were incubated in phosphate-buffered saline (PBS) containing 10% normal goat serum (NGS) at room temperature (RT) for 30 min, followed by probing with the indicated primary antibodies in 10% NGS/PBS at RT for 60 min. After washing with PBS containing 0.1% Tween 20 (PBST), the cells were incubated with the appropriate secondary antibody conjugated with either Alexa 488, Alexa 568, or Alexa 647 (Invitrogen).

The oocytes were fixed in PHEM buffer [60 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 25 mM HEPES, 10 mM EGTA, and 4 mM $MgCl_2$, pH 6.9] containing 2% formaldehyde and 0.2% Triton X-100 at 37°C for 1 h. After fixation, oocytes were permeabilized with 0.1% Triton X-100/PBS containing 0.3% bovine serum albumin (BSA) at 37°C for 40 min, followed by incubation with a blocking solution (3% BSA in PBS) for 30 min. The whole-mount oocytes or chromosome spreads prepared as described above were probed with indicated primary antibodies in blocking solution at 4°C overnight as described previously (Tang *et al*., 2006). After washing with PBST buffer (0.01% Triton X-100 in PBS), oocytes were incubated with the appropriate secondary antibodies at 4°C overnight. DNA was counterstained with 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). After washing, cells or oocytes were mounted on coverslides with VECTASHIELD mounting solution (Vector Laboratories, Burlingame, CA) and observed with an LSM510 laser scanning confocal system (Carl Zeiss, Jena, Germany).

RESULTS

Aurora-C, but Not Aurora-B, Reveals a Dynamic Localization during Female Mouse Meiosis

We showed previously that Aurora-C shares a high sequence identity (77.6%) with Aurora-B (Tseng *et al*., 1998) and that both kinases are present in mouse meiotic spermatocytes, with a similar localization pattern during male meiosis (Tang *et al*., 2006). To investigate whether these two kinases are also present in mouse oocytes, we used confocal immunofluorescence to analyze endogenous Aurora-C and -B in whole-mount oocytes (Figure 1, A and B) and in chromosome spreads (Figure 1, C and D). ACA was used as a centromere and kinetochore marker for tracing the distribution of both kinases during various meiotic division stages.

As shown in Figure 1B, endogenous Aurora-C was detected at the centromeres and along the chromosome arms in both prometaphase I (a–d) and metaphase I oocytes (e–h). It relocalized to the midbody at telophase I (i–l) and then migrated to the centromeres again in metaphase II (m–p). Further fine resolution analysis using chromosome spreading techniques revealed that the association of Aurora-C to the chromosome arms was observed in metaphase I chromosomes (Figure 1D, a–d) but was lost from metaphase II chromosomes (Figure 1D, e–h). Surprisingly, we detected no endogenous Aurora-B protein either in the whole-mount oocytes at different meiotic stages (Figure 1A) or in the chromosome spreads of MI/MII chromosomes (Figure 1C). Immunoblot analysis further confirmed that only Aurora-C, but not Aurora-B, was detected in MI and MII oocytes (Figure 1F). The detected Aurora-C doublet bands in Figure 1F may possibly represent phosphorylated and unphosphorylated forms, because the presence of phospho-Thr171-Aurora-C in meiotic chromosomes was confirmed by immunostaining (see below). Interestingly, both Aurora-B and -C transcripts were detected in MI/MII oocytes by RT-PCR analysis (Figure 1E), whereas only Aurora-C protein was detected by immunostaining and Western blotting, implying that translation of Aurora-B transcripts into protein was inhibited in mouse oocytes.

To further confirm that our Aurora-C antibody (Tang *et al*., 2006) did not cross-react with Aurora-B and vice versa, although specificity of these antibodies had been demonstrated previously (Tang *et al*., 2006), we undertook more precise immunoblotting and immunostaining analyses. The GFP-tagged constructs that encode various portions of Aurora-B or Aurora-C cDNAs (Supplemental Figure S1A) were transfected into mouse N2a cells. As shown in Supplemental Figure S1B, anti-GFP antibody detected all GFP-tagged proteins, whereas anti-Aurora-B (AIM-1; BD Biosciences) or anti-Aurora-C antibody (Tang *et al*., 2006), which had been raised against the N-terminal portion (residues 1–124) of rat Aurora-B or the glutathione transferase-fused full-length mouse Aurora-C (residues 1–276), respectively, recognized only their own species and revealed no cross-reactivity. The specificity of these two antibodies was further strengthened by the observations that endogenous Aurora-B (Supplemental Figure S1C), but not Aurora-C (Supplemental Figure S1D), was detected in mitotic TM4 cells (a mouse testis Sertoli cell line); N2a cells (a mouse neuroblastoma cell line; data not shown); and several cancer cell lines, including HeLa (data not shown), whereas colocalization of endogenous Aurora-C with INCENP was only detected in meiotic chromosomes in mouse oocytes (Supplemental Figure S1E) as well as in spermatocytes (Tang *et al*., 2006). Furthermore, we also injected *GFP-AurB* (Supplemental Figure S1F) or *GFP-AurC* (Supplemental Figure S1G) mRNA into oocytes and examined these oocytes by immunostaining using each specific antibody. Both GFP-AurB and -C signals can be directly viewed by confocal fluorescence microscopy. Again, Aurora-B (Supplemental Figure S1F) and Aurora-C (Supplemental Figure S1G) antibodies specifically recognized their own GFP-tagged signal. Together, these facts show that Aurora-C, but not Aurora-B, serves as a unique chromosomal passenger protein in female mouse meiosis.

Aurora-C Kinase-deficient Oocytes Fail to Complete Cytokinesis in Meiosis I

Previous reports showed that inhibition of Aurora-B kinase activity in mitosis (Kallio *et al*., 2002) or overexpression of Aurora-C kinase-deficient mutant (T171A/T175A) in somatic HeLa cells caused cytokinesis failure, resulting in a polyploid phenotype (Chen *et al*., 2005). Under normal conditions, oocytes proceed from the GV through meiosis I, meiosis II, and arrest in metaphase II of the second division until fertilization occurs. To address whether a functional Aurora-C kinase is required for meiotic divisions, denuded GV oocytes were coinjected with a mCherry-tagged histone H2B (*mCherry-H2B*) and a GFP-tagged Aurora-C kinase-deficient mutant transcript (*GFP-AurC-KD*), in which Thr171 and Thr175 were mutated to Ala (Chen and Tang, 2002) in the presence of IBMX (Figure 2A). The injected oocytes were incubated for another 2 h followed by washing out IBMX (a phosphodiesterase inhibitor that arrests oocytes at the prophase stage) to resume oocyte maturation. A schematic representation of the process of microinjection during oocyte maturation is shown in Figure 2A. To analyze the effects of injected mRNAs on oocyte maturation in vivo, the injected oocytes were directly monitored by an UltraView live cell imaging system (Figure 2, B–D). In all experiments, only postinjected oocytes with germinal vesicle

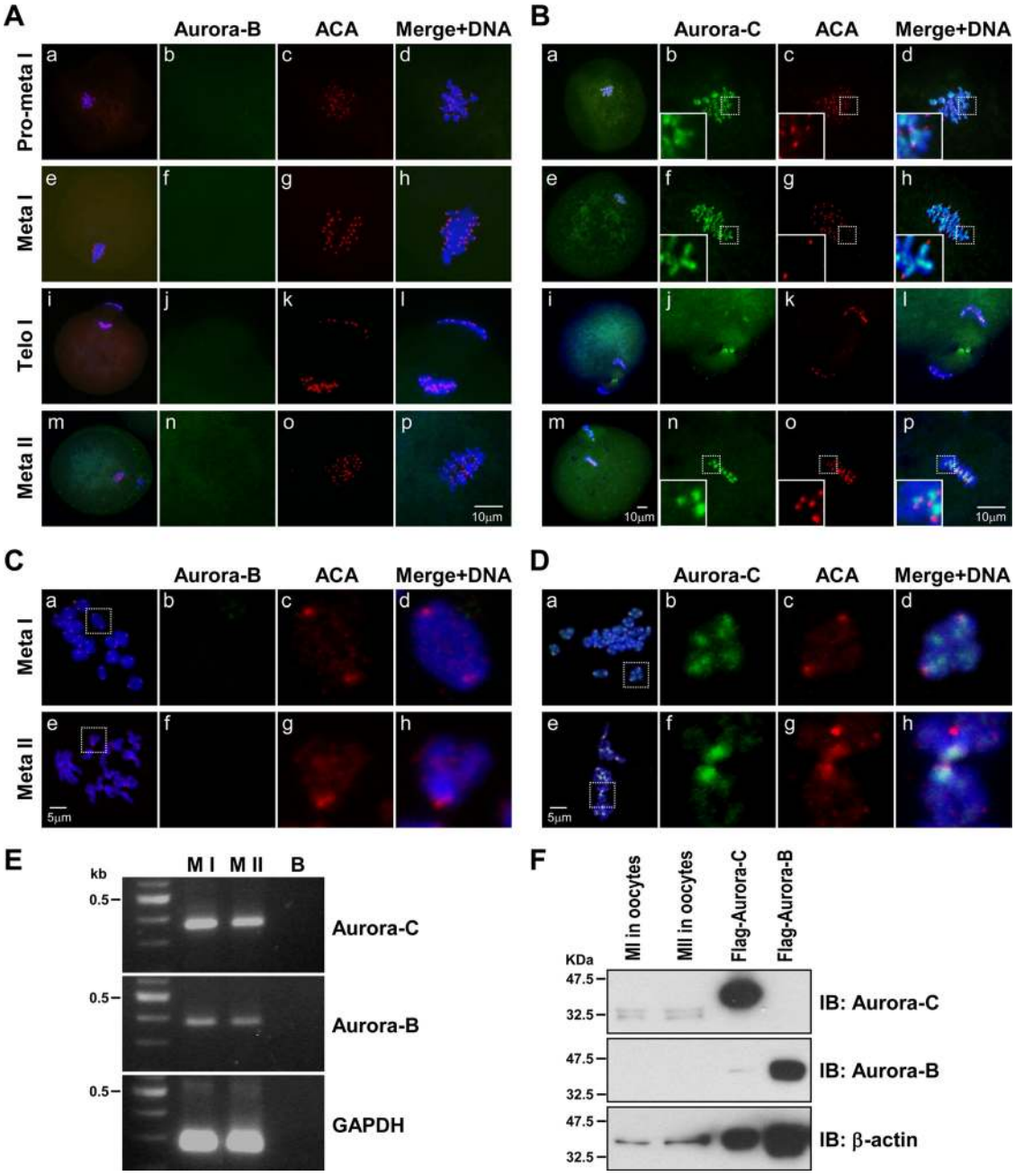


Figure 1. Expression and subcellular localization of Aurora-C, but not Aurora-B, in meiotic mouse oocytes. Confocal immunofluorescence analysis of endogenous Aurora-B (A and C) and Aurora-C (B and D) in whole-mount oocytes (A and B) or in chromosome spreads (C and D) during various stages of meiosis (prometaphase I ~ meta II) by using indicated antibodies (green, Aurora-B and -C; red, ACA). ACA was used as a marker of the centromeric region of meiotic chromosomes. DNA was counterstained with DAPI (blue). In A and B, the image in A, b–d, and B, b–d (prometa I), is an enlarged view derived from Aa and Ba, respectively. Similar enlarged views are derived from the images in meta I (f–h from e), telo I (j–l from i), and meta II (n–p from m). In B, the large square box in b, c, d, f, g, h, n, o, and p is a blowup of each corresponding smaller dotted box. (C, b–d) An enlarged view of the boxed region derived from Ca; (C, f–h) region from Ce; (D, b–d) region from Da; (D, f–h) region from De. The Merge + DNA image is a triple merge of Aurora, ACA, and DNA. (E) Total RNAs isolated from MI and MII oocytes were analyzed by RT-PCR. Both *Aurora-B* and *Aurora-C* transcripts were detected in MI and MII oocytes. B, blank control. (F) Immunoblot analysis of the cell lysates prepared from mouse MI and MII oocytes (500 oocytes/lane) or from Flag-Aurora-B– (10 μg/lane) and Flag-Aurora-C (5 μg/lane)–transfected HeLa cells. Aurora-C was detected as doublet bands, possibly due to phosphorylation. No endogenous Aurora-B signal was detected in MI or MII oocytes.

breakdown (GVBD) were calculated and counted. In other experiments (see Figures 3–7 and Supplemental Figures S1 and S2), chromosome spreads or whole-mount staining were prepared from injected oocytes at different time points after IBMX release and analyzed by confocal fluorescence microscopy. Control oocytes injected with *GFP* transcripts matured normally under these culture conditions. Control oocytes progressed to MI ~8 h after GVBD (~10 h after IBMX release), extruded the first polar body around 10 h post-GVBD (~13 h after IBMX release) and arrested in metaphase II.

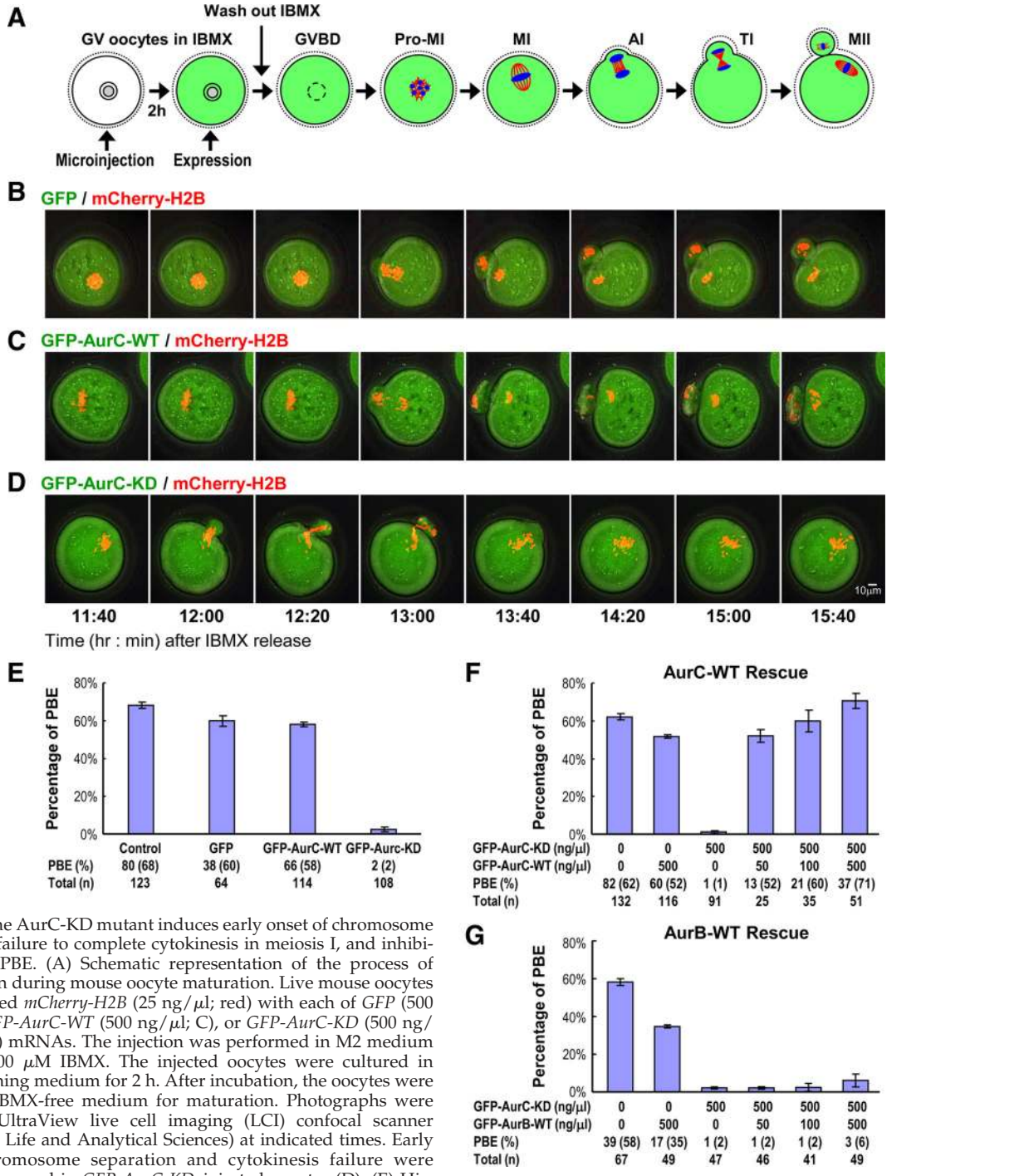
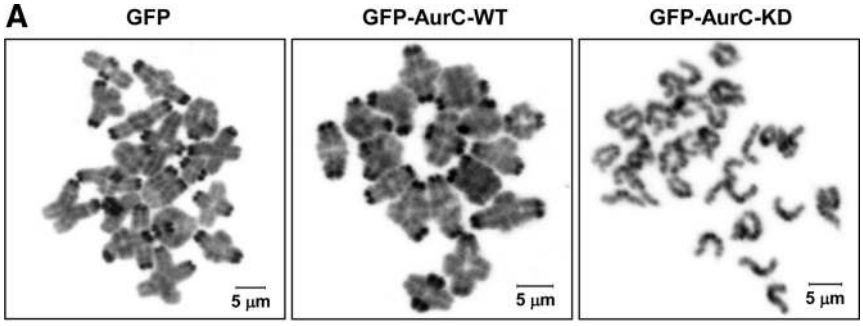
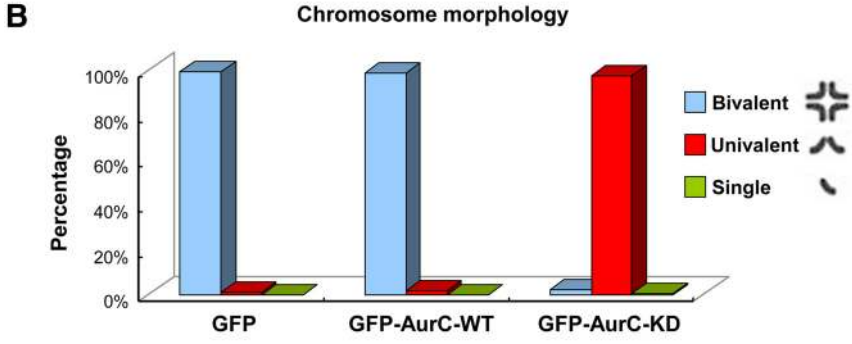


Figure 2. The AurC-KD mutant induces early onset of chromosome segregation, failure to complete cytokinesis in meiosis I, and inhibition of first PBE. (A) Schematic representation of the process of microinjection during mouse oocyte maturation. Live mouse oocytes were coinjected *mCherry-H2B* (25 ng/μl; red) with each of *GFP* (500 ng/μl; B), *GFP-AurC-WT* (500 ng/μl; C), or *GFP-AurC-KD* (500 ng/μl; D) (green) mRNAs. The injection was performed in M2 medium containing 100 μM IBMX. The injected oocytes were cultured in IBMX-containing medium for 2 h. After incubation, the oocytes were cultured in IBMX-free medium for maturation. Photographs were taken with UltraView live cell imaging (LCI) confocal scanner (PerkinElmer Life and Analytical Sciences) at indicated times. Early onset of chromosome separation and cytokinesis failure were frequently observed in *GFP-AurC-KD*–injected oocytes (D). (E) Histogram showing percentages of first PBE in postinjected oocytes. Only postinjected oocytes with GVBD were calculated and counted. Number (n) of postinjected GVBD oocytes is shown. Grouped data were collected from three (*GFP*) to five (control, *GFP-AurC-WT*, and *GFP-AurC-KD*) independent experiments. Rescue of cytokinesis failure by *AurC-WT* (F) or *AurB-WT* (G) mRNA. GV oocytes were coinjected with a fixed concentration of *GFP-AurC-KD* transcripts (500 ng/μl) with increasing amounts of *GFP-AurC-WT* or *GFP-AurB-WT* mRNA (0–500 ng/μl). *AurC-WT*, but not *AurB-WT*, can rescue cytokinesis failure in *GFP-AurC-KD*–injected oocytes in a dose-dependent manner. Number (n) of postinjected GVBD oocytes is shown. Grouped data were collected from at least three independent experiments. Data are shown as mean ± SD.

Figure 2E illustrates that the majority of oocytes injected with either *GFP* (60%; n = 64) or *GFP-AurC-WT* (58%; n = 114) transcripts successfully underwent maturation and extrusion of the first polar body extrusion (PBE). Examples of GV oocytes injected with *GFP* (Figure 2B and Supplemental Movie S1), *GFP-AurC-WT* (Figure 2C and Supplemental Movie S2), or *GFP-AurC-KD* (Figure 2D and Supplemental Movie S3) mRNAs as indicated were shown in Figure 2. Surprisingly, we found that *GFP-AurC-KD*–injected oocytes (Figure 2D and Supplemental Movie S3) revealed cytokinesis failure in MI and failed to extrude the polar body (2% PBE; n = 108). The meiotic I division in *GFP-AurC-KD*–injected oocytes





	GFP	GFP-AurC-WT	GFP-AurC-KD
Bivalent (%)	1000 (98.8)	1024 (98.5)	20 (2.1)
Univalent (%)	12 (1.2)	16 (1.5)	944 (97.3)
Single (%)	0 (0)	0 (0)	6 (0.6)
Total (n)	1012	1040	970

Figure 3. Aurora-C deficiency induces premature chromosome segregation, resulting in univalent chromosomes in MI. (A) Chromosome spreads prepared from MI oocytes injected with *GFP*, *GFP-AurC*, or *GFP-AurC-KD* mRNAs 8 h post-GVBD. The oocytes were fixed and stained with DAPI as described in *Materials and Methods*. For a better view of chromosome morphology (bivalent vs. univalent), the DAPI image was converted to pseudocolor (black and white). (B) Histogram showing the percentage of bivalent (4N), univalent (2N), and singlet (1N) in oocytes injected with different mRNAs. Most chromosomes in *GFP-AurC-KD*–injected oocytes were univalent (~97.3%). Chromatid number: n. Grouped data from four independent experiments.

seemed to proceed to telophase I and attempted to organize a contractile furrow, which is evidenced by a clear chromosome bridge between the dividing polar body and the large oocyte (12:20; Figure 2D) but finally failed to complete cytokinesis and were unable to extrude the polar body (13:40; Figure 2D). We also noticed that onset of chromosome separation in MI occurred earlier in oocytes injected with *GFP-AurC-KD* (~12:00; Figure 2D) than in oocytes with *GFP* or *GFP-AurC-WT* (~13:00; Figure 2, B and C), implying that the SAC activity may be perturbed in *GFP-AurC-KD*–injected oocytes.

Aurora-C Kinase Deficiency Induced Premature Chromosome Separation in MI, Resulting in Producing Univalent Chromosomes

Precise reduction of chromosome numbers in germ cells is important for sexual reproductive organisms. Homologous chromosomes pair with each other and form a bivalent chromosome in MI and resolve into univalent chromosomes in MII. Our current results showed cytokinesis failure in MI and failure of PBE in *GFP-AurC-KD*–injected oocytes (Figure 2D), raising the interesting question of whether bivalents were still maintained in those oocytes. To resolve this question, we used chromosome spreading techniques to visualize chromosome morphology in more detail. Chromosome spreads were prepared from the MI oocytes injected with *GFP*, *GFP-AurC-WT*, or *GFP-AurC-KD* mRNAs 8 h post-GVBD. The oocytes were fixed and stained with DAPI as described in *Materials and Methods*. As shown in Figure 3A, bivalent chromosomes with paired homologues were frequently observed in spread preparations of *GFP*-or *GFP-AurC-WT*–injected oocytes. In contrast, univalent chromosomes were the predominant type (~97.3% in 13 oocytes; Figure 3B) found in the spreads of *GFP-AurC-KD*–injected oocytes, implying a premature resolution of chiasmata in meiosis I, possibly by proteolytic cleavage of cohesion proteins along the chromosome arms. The total number of univalents present on the spreads was 40, consistent with a cytokinesis failure in MI.

Aurora-C, but Not Aurora-B, Can Rescue Cytokinesis Failure in GFP-AurC-KD–injected Oocytes

Our results showed that *GFP-AurC-KD*–injected oocytes revealed a failure of cytokinesis (Figure 2D) and produced univalent chromosomes (Figure 3). We next examined whether these phenotypes could be rescued by increasing the expression level of exogenous *Aurora-C-WT* or *Aurora-B-WT*, which shares a high sequence homology with *Aurora-C-WT*. To test this, we coinjected oocytes with a fixed concentration of *GFP-AurC-KD* transcripts (500 ng/μl) and with increasing amounts of *GFP-AurC-WT* or *GFP-AurB-WT mRNA* (0–500 ng/μl). As shown in Figure 2F, *GFP-AurC-WT* did rescue cytokinesis failure in *GFP-AurC-KD*–injected oocytes in a dose-dependent manner. We found that the frequency of PBE recovered from 1% (n = 91; *GFP-AurC-KD*, 500 ng/μl) to 52% (n = 25) with a dose of *GFP-AurC-WT* mRNA as low as 50 ng/μl. The recovery rate for PBE is gradually increased to 60% (n = 35; *GFP-AurC-WT*, 100 ng/μl) and 71% (n = 51; *GFP-AurC-WT*, 500 ng/μl), respectively. Coinjection of mRNA for *GFP-AurC-KD* with a high dose of *GFP-AurC-WT* transcripts (>1 μg/μl) seemed to decrease the recovery efficiency (data not shown), possibly due to a high-dose interference effect. Furthermore, recovery of successful extrusion of the first polar body by wild-type *Aurora-C* transcript was always accompanied by dose-dependent recovery of a normal bivalent phenotype (data not shown). In contrast, no significant rescue was observed in *GFP-AurB-WT*–injected oocytes, even the concentration of *GFP-AurB-WT* was increased to 500 ng/μl (Figure 2G). We

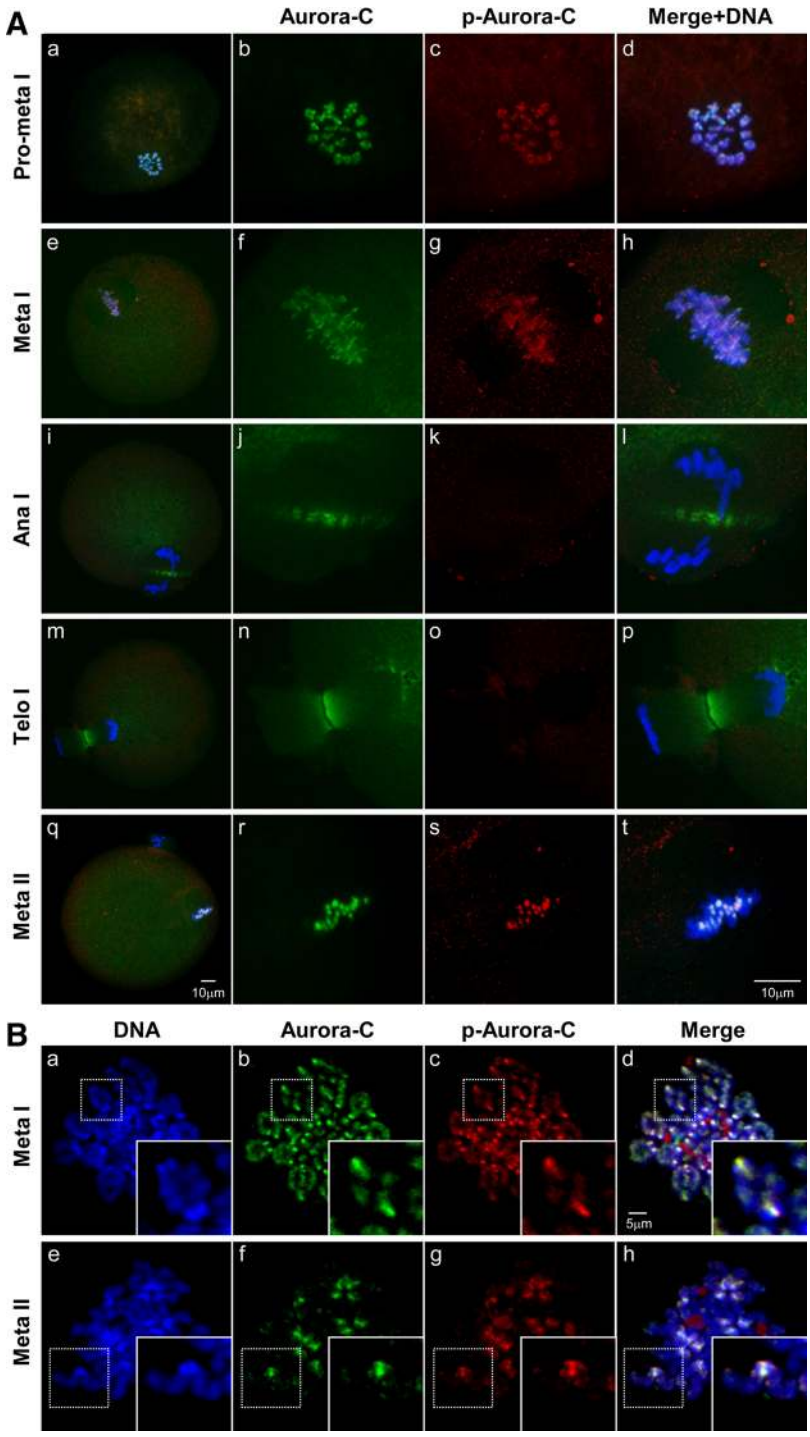


Figure 4. Subcellular localization of endogenous Aurora-C and p-Aurora-C during meiotic divisions in mouse oocytes. Confocal immunofluorescence analysis of endogenous Aurora-C and p-Aurora-C in whole-mount oocytes (A) or in chromosome spreads (B) by using indicated antibodies (green for Aurora-C; red for p-Aurora-C). DNA was counterstained with DAPI (blue). In A, the image in A, b, c, and d (prometa I), is an enlarged view derived from Aa. Similar enlarged views are derived from the images in meta I (f–h from e), ana I (j–l from i), telo I (n–p from m), and meta II (r–t from q). The Merge + DNA image is a triple merge of Aurora-C, p-Aurora-C, and DNA. In B, the large square box in a–d (meta I) and e–h (meta II) is a blowup of each corresponding smaller dotted box.

thus conclude that *Aurora-C*, but not Aurora-B, is a key player that regulates meiotic divisions in mouse oocytes.

AurC-KD Mutant Inhibits Aurora-C (Thr171) and Histone H3 (Ser10) Phosphorylation, and Disrupts Bub1 and BubR1 Targeting to Kinetochores

We showed previously that Thr171 of Aurora-C can be phosphorylated in vitro and the double mutation T171A/T175A impaired its kinase activity (Chen and Tang, 2002). We then generated a specific antibody against p-Thr171-Aurora-C and examined its localization during female mouse meiosis. As shown in Figure 4, both Aurora-C and p-Aurora-C were localized along the chromosome arms and on the centromeric regions in both prometaphase I (Figure 4A, a–d) and metaphase I (Figure 4A, e–h). However, during the anaphase I–telophase I transition, only Aurora-C, but not p-Aurora-C, was detected at the spindle midzone and midbody (Figure 4A, i–l and m–p), suggesting that dephosphorylation of Aurora-C at Thr171 occurred during this transition. In metaphase II, Aurora-C was rephosphorylated at Thr171 and migrated to the centromeres again (Figure 4A, q–t). Further analysis of chromosome spreads revealed that

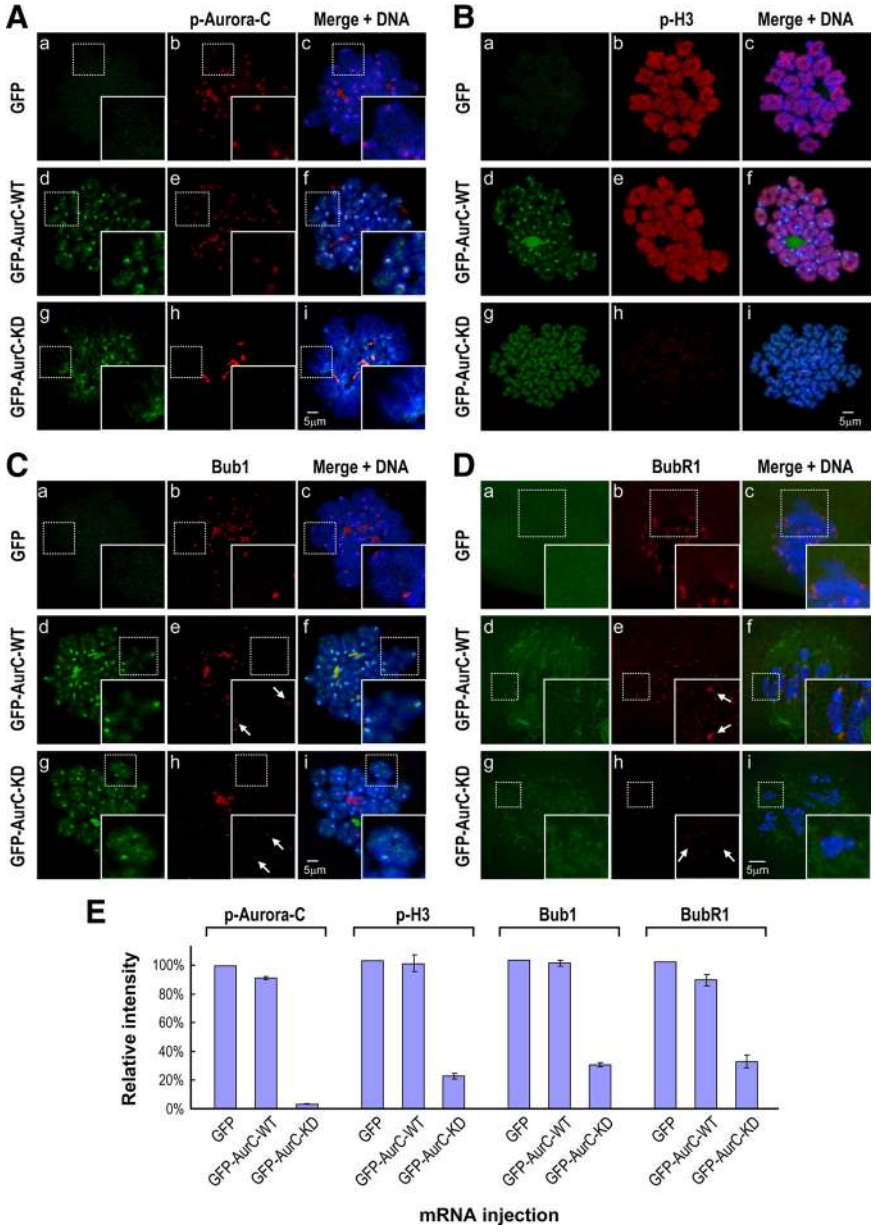


Figure 5. AurC-KD inhibits kinetochore localization of Bub1 and BubR1 and reduces Aurora-C and histone H3 phosphorylation. The chromosome spreads (A–C) or whole-mount oocytes (D) were prepared either from prometaphase I oocytes (C and D; 6 h post-GVBD) or metaphase I oocytes (A and B; 8 h post-GVBD) injected with indicated mRNAs, followed by immunostaining with specific antibodies against phospho-Thr171-AurC (A), p-H3 (B), Bub1 (C), and BubR1 (D). DNA was counterstained with DAPI. The large square box in A, C, and D is a blowup of each corresponding smaller dotted box. The white arrow in C, e and h, and D, e, and h, indicates the position of kinetochore. The strong dotted p-Aurora-C signals (red) detected in A–H seem to be nonspecific, because these signals do not target to the centromeric regions. During normal oocyte maturation, Bub1 and BubR1 (spindle checkpoint proteins) signals were easily detected at the kinetochores on prometaphase I chromosomes (6 h post-GVBD) but frequently lost in metaphase-I chromosomes (8 h post-GVBD). The relative intensity of each injected mRNA compared with *GFP* (used as a control) is shown in E. Data are shown as mean ± SD.

the association of Aurora-C and p-Aurora-C at the centromeric region of MI (Figure 4B, a–d) and MII (Figure 4B, e–h) chromosomes was clearly evidenced.

Interestingly, microinjection of *GFP-AurC-KD* mRNA into GV oocytes significantly reduced both p-Aurora-C (Figure 5A, g–i) and phospho-H3 (Figure 5B, g–i), bound to MI chromosomes, suggesting that meiotic phosphorylation of these two proteins were inhibited by the AurC-KD mutant. In contrast, only minor reductions of the signals derived from these two phosphorylated proteins were observed in *GFP* (Figure 5A, a–c; and B, a–c) or *GFP-AurC-WT*–injected oocytes (Figure 5A, d–f; and B, d–f). A quantitative analysis revealed that the relative intensities of p-Aurora-C and phospho-H3 in the chromosome spreads of MI oocytes were reduced to 3.5% and 21.9%, respectively (Figure 5E), of those of *GFP*-injected oocytes.

Previous studies showed that the SAC in mitosis is regulated by Aurora-B (Kallio *et al*., 2002; Mistry *et al*., 2008) and that overexpression of exogenous GFP-AurC-KD inhibits kinetochore localization of two SAC members Bub1 and BubR1 in transfected HeLa cells (Chen *et al*., 2005). We thus examined whether AurC-KD mutant affects Bub1 and BubR1 targeting to kinetochore in meiosis. As shown in Figure 5, AurC-KD did reduce the amount of Bub1 (Figure 5C) and BubR1 (Figure 5D) bound to kinetochores. The relative intensities of Bub1 and BubR1 signals were significantly reduced to 29.5% and 32.3% (Figure 5E), respectively, compared with *GFP*-control. Together, these results suggest that GFP-AurC-KD prevented the localization of two SAC proteins (Bub1 and BubR1) to the kinetochores and inhibited meiotic phosphorylation of histone H3 and Aurora-C itself.

Aurora-C Kinase Is Required for Correct Kinetochore–Microtubule Attachment and Chromosome Alignment in Meiosis I

Aurora-B can promote amphitelic or bipolar attachments by destabilizing syntelic attachments and merotelic attachments (Kelly and Funabiki, 2009) in mitotic cells. Cells use a

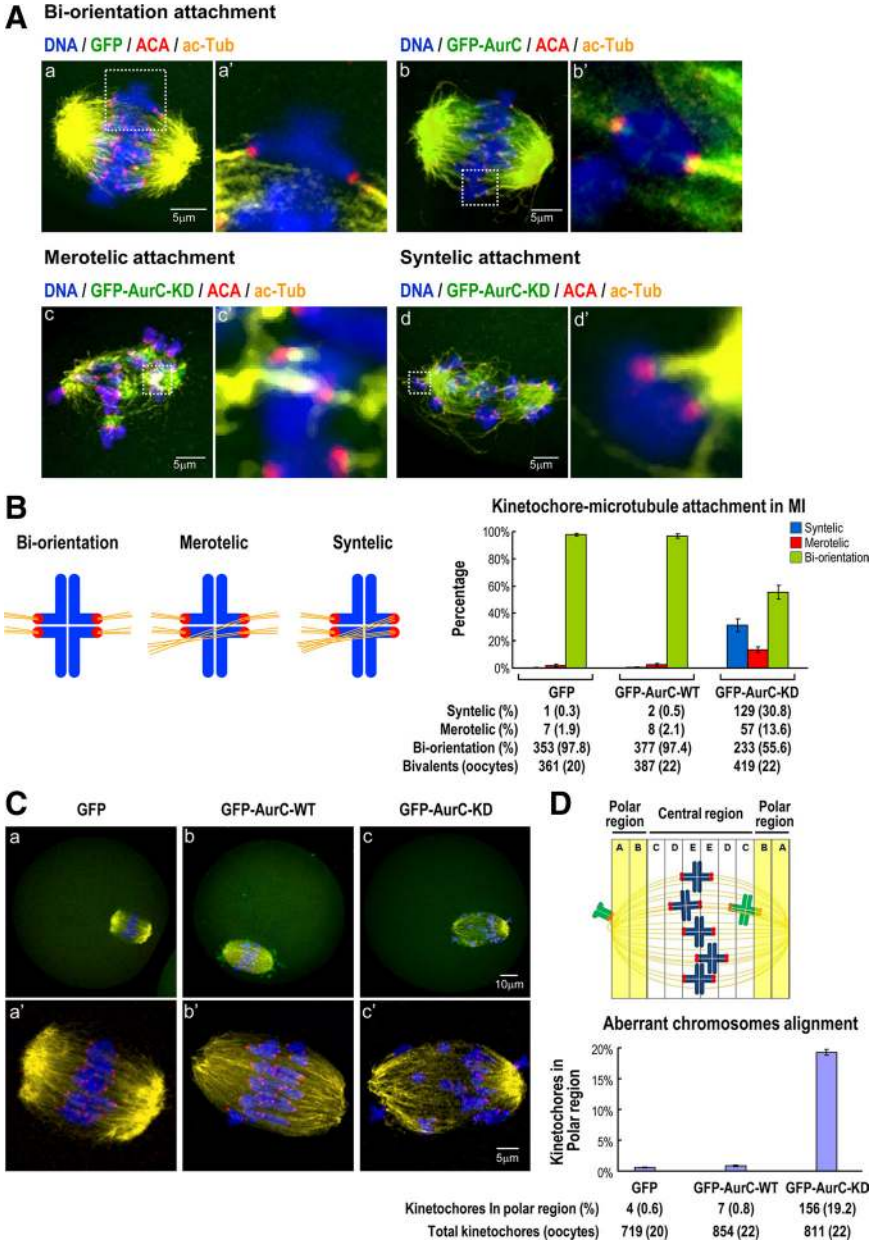


Figure 6. AurC-KD induces aberrant K-MT attachment and chromosome misalignment in meiosis I. (A) Images were prepared from intact oocytes injected with *GFP* (a and a′, 8 h post-GVBD), *GFP-AurC-WT* (b and b′, 8 h post-GVBD), or *GFP-AurC-KD* (c and c′ and d and d′, 7 h post-GVBD) mRNAs. Under normal conditions, oocytes progress to metaphase I around 8 h after GVBD. Because, in *GFP-AurC-KD*–injected oocytes, the premature separation of bivalent chromosomes to univalent chromosomes was commonly occurred between 7 and 8 h post-GVBD, we thus examined the K-MT attachments in *GFP-AurC-KD*–injected oocytes at 7 h post-GVBD. The fixed oocytes were immunostained with antibodies against ACA and acetyl tubulin and analyzed by LSM510 laser confocal microscopy. The images shown in a–d are complied from five to eight confocal optical sections (0.8 μm/section). Enlarged views of each representative image (boxed) are shown on the right (a′–d′). The images shown in a′–d′ are complied from two optical sections (0.8 μm/section). (B) Statistical analysis of the percentage of aberrant kinetochore–microtubule attachments in postinjected oocytes. In *GFP*- and *GFP-AurC-WT*–injected oocytes, most kinetochore–microtubule attachments were in a biorientation arrangement, whereas merotelic and syntelic attachments were significantly increased in *GFP-AurC-KD*–injected oocytes. (C) The images were prepared as described in A. Most chromosomes were located at the central region in *GFP*- (C, a and a′) and *GFP-AurC-WT* (C, b and b′)–injected oocytes, whereas chromosome spread into the polar region was significantly increased in *GFP-AurC-KD*–injected oocytes (C, c and c′). (D, top) A cartoon showing the possible chromosome position during meiosis. (D, bottom) Statistical analysis of the percentage of aberrant chromosome alignment in postinjected oocytes. One bivalent carries two separated kinetochores. The pair of kinetochores on each sister chromatid fuses and functions as a unit during meiosis I. DNA was counterstained with DAPI. Grouped data were collected from at least three independent experiments. Data are shown as mean ± SD.

similar mechanism to segregate sister chromatids in mitosis and meiosis II. However, chromosome segregation in meiosis I is more complex, as sister kinetochores must be co-oriented and homologues bioriented. To examine whether Aurora-C kinase is required for correct K-MT attachments in MI, we examined the effects of K-MT attachment in *GFP-AurC-KD*–injected oocytes. Figure 6 shows that *GFP* or *GFP-AruC-WT*–injected oocytes revealed a normal K-MT attachment, known as biorientation of bivalent, in which the kinetochores of sister chromatids attach to microtubules emanating from the same pole (co-orientation), whereas the kinetochores of homologous chromosomes attach to microtubules emanating from opposite poles (biorientation; Figure 6A–Aa′ for *GFP*; Figure 6A–Bb′ for *GFP-AurC-WT*). In contrast, abnormal K-MT attachments were frequently observed in *GFP-AurC-KD*–injected oocytes, including a "meiosis–merotelic" attachment (attachment of sister chromatids of a homologue to opposite poles; Figure 6A–Cc′) and a "meiosis–syntelic" attachment (attachment of both homologues of a bivalent to the same pole; Figure 6A–Dd′). Quantitative analysis of a total of 419 bivalents in 22 oocytes revealed that both syntelic (~30%) and merotelic attachments (~13%) in *GFP-AurC-KD*–injected oocytes are significantly higher than those in *GFP* or *GFP-AurC-WT*–injected oocytes (Figure 6B).

We also observed a high frequency of chromosome misalignment in *GFP-AurC-KD* injected oocytes (Figure 6C, c–c′). In those oocytes (n = 22), nearly 20% chromosomes were found in the polar regions (Figure 6D). In contrast, in *GFP*- (Figure 6C, a–a′) or *GFP-AurC-WT* (Figure 6C, b–b′)–injected oocytes, most MI chromosomes were aligned at the equatorial plate and very few of them (<1%; Figure 6D) were found in the polar region.

Furthermore, GV oocytes treated with ZM447439, a small-molecule pan-Aurora kinase inhibitor, had meiotic defects in a dose-dependent manner. ZM447439 treatment induced

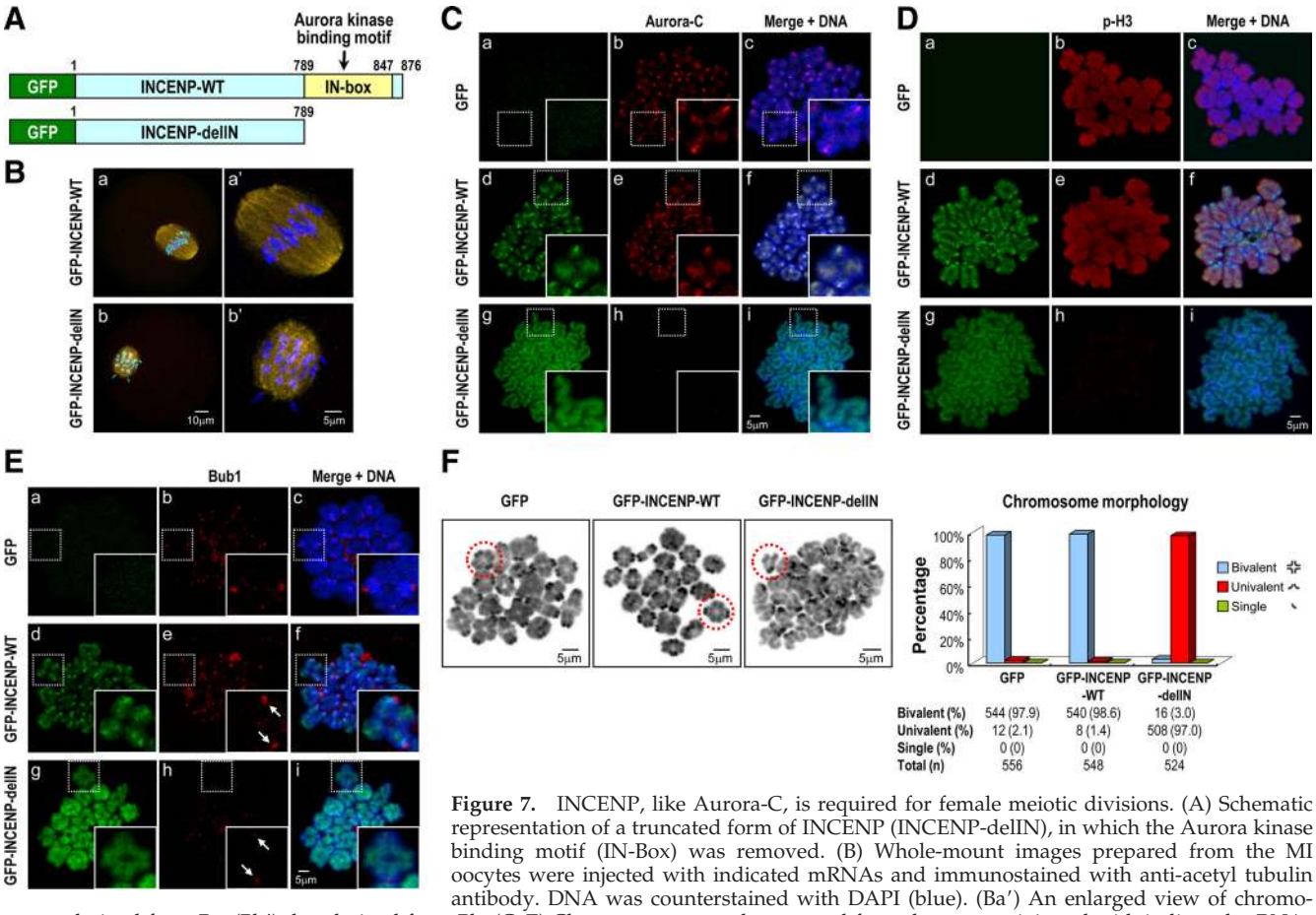


Figure 7. INCENP, like Aurora-C, is required for female meiotic divisions. (A) Schematic representation of a truncated form of INCENP (INCENP-delIN), in which the Aurora kinase binding motif (IN-Box) was removed. (B) Whole-mount images prepared from the MI oocytes were injected with indicated mRNAs and immunostained with anti-acetyl tubulin antibody. DNA was counterstained with DAPI (blue). (Ba′) An enlarged view of chromosomes derived from Ba; (Bb′) that derived from Bb. (C–E) Chromosome spreads prepared from the oocytes injected with indicated mRNAs were immunostained with anti-Aurora-C (C; 8 h post-GVBD), anti-phospho-H3 (D; 8 h post-GVBD), or anti-Bub1 antibodies (E; 6 h post-GVBD) and analyzed by LSM510 laser confocal microscopy. The large square box in C and E is a blowup of each corresponding smaller dotted box. The white arrow in Ee and Eh indicates the position of kinetochore. (F) Left, chromosome spreads prepared from the MI oocytes (8 h post-GVBD) injected with indicated mRNAs were fixed and stained with DAPI. For a better view of chromosome morphology (bivalent vs. univalent) in F, the DAPI image was converted to pseudocolor (black and white). Right, percentage of bivalent (4N), univalent (2N), and singlet (1N) in oocytes injected with different mRNAs. Most chromosomes in *GFP-INCENP-delIN*–injected oocytes were univalents (97%). Bottom, grouped data from four independent experiments. Chromatid number, n.

chromosome misalignment (Supplemental Figure S2A) and univalent chromosomes in MI (Supplemental Figure S2B) and inhibited Aurora-C phosphorylation at Thr171 (Supplemental Figure S2C) and PBE (Supplemental Figure S2D), a similar pattern to that seen in *GFP-AurC-KD*–injected oocytes. Our findings that ZM447439 induces chromosome misalignment and inhibits PBE are consistent with a previous report (Swain *et al*., 2008). Together, our results imply that Aurora-C is required for correct kinetochore–microtubule attachment and chromosome alignment during the first meiotic division.

INCENP, Like Aurora-C, Is Required for Accurate Chromosome Segregation and Cytokinesis during Female Meiosis

We showed previously that INCENP is associated with Aurora-C in male spermatocytes (Tang *et al*., 2006) and its carboxy-terminal motif (IN-box) was responsible for binding Aurora-C (Chen *et al*., *2005*). To address whether an INCENP defect also would prevent normal meiotic maturation of mouse oocytes, we used a similar approach to that used with *GFP-AurC-KD* and injected GV oocytes with *GFP-INCENP-delIN* transcript (Figure 7A), from which the Aurora-C binding box (the IN-box) sequence had been removed, and then we examined its effects on oocyte maturation. The *GFP-INCENP-delIN* mutant, like *GFP-AurC-KD*, significantly inhibited localization of endogenous Aurora-C (Figure 7C, g–i) and Bub1 (Figure 7E, g–i) to centromeres and kinetochores and reduced histone H3 phosphorylation (Figure 7D). Furthermore, the *GFP-INCENP-delIN* mutant also induced a high frequency of chromosome misalignment (Figure 7B, b and b′), and the formation of univalent chromosomes in MI (Figure 7F), and failed to complete cytokinesis. In contrast, minimal or no effects were observed in the oocytes injected with *GFP* or *GFP-INCENP-WT* transcripts with concentration of 500 ng/μl (Figure 7, B–F). Thus, the INCENP-delIN mutant seems to perturb meiotic division, possibly by inhibiting endogenous Aurora-C kinase targeting to centromeric regions.

DISCUSSION

Recent studies on the subcellular location and function of Aurora-C and -B during female mouse meiotic divisions are mostly derived from the results obtained with pan-Aurora kinase inhibitors and ectopically expressed GFP-tagged con-

structs (Swain *et al*., 2008; Shuda *et al*., 2009). Direct immunofluorescence of endogenous Aurora-C has never been reported in female mouse oocytes. We reported previously a dynamic localization of three CPC members (Aurora-C, Aurora-B, and INCENP) during male mouse meiosis and hypothesized that INCENP may recruit both Aurora-C and -B to meiotic chromosomes (Tang *et al*., 2006). In this study, we have analyzed the subcellular localization of Aurora-C during female mouse meiosis (Figure 1, B and D). Aurora-C was first detected at the chromosome arms and centromeres at prometaphase I–metaphase I and was concentrated at centromeres at metaphase II, in which Aurora-C was also phosphorylated at Thr171. During the anaphase I–telophase I transition, Aurora-C was dephosphorylated and relocalized to the midzone and midbody (Figure 4A, i–l and m–p). The distribution pattern of Aurora-C is similar to that reported in mouse spermatocytes (Tang *et al*., 2006). Surprisingly, we detected no endogenous Aurora-B protein in meiotic chromosomes of mouse oocytes by Western blot (Figure 1F) or immunofluorescence analyses (Figure 1, A and C), implying a distinct function of Aurora-C in female mouse meiosis. Consistent with our findings, Shuda *et al*. (2009) also failed to detect endogenous Aurora-B protein by immunocytochemistry despite using several different antibodies and fixation conditions. Furthermore, only *Aurora-B* transcript, but not its corresponding protein, was detected in meiotic oocytes, suggesting a translational control of *Aurora-B* mRNA is present in mouse oocytes.

The absolute requirement of Aurora-C in meiosis comes from the findings that homozygous mutation of *Aurora-C* gene produces large-headed polyploid spermatozoa (Dieterich *et al*., 2007, 2009), by an as-yet unclear mechanism. Previous studies of spermatogenesis in *AurC* $^{-}/^{-}$ knockout mice showed minor effects on sperm morphology (Kimmins *et al*., 2007), but the interpretation was complicated by the presence of multiple *Aurora-C* copies in the mouse genome (Hu *et al*., 2000). Here, we introduced an Aurora-C kinase-deficient mutant (*AurC-KD*) into mouse oocytes, in which both Thr171 and Thr175 were mutated to Ala (Chen and Tang, 2002), to characterize the function of Aurora-C during female meiosis. Aurora-C and Aurora-B share a high degree of amino acid sequence similarity and reveal similar localization. Thr171 in mouse Aurora-C can be phosphorylated in vitro (Chen and Tang, 2002) and is equivalent to Thr248 in xAurora-B, which is located at the activation loop of the xAurora–B/INCENP complex and is required for its kinase activity (Sessa *et al*., 2005).

The most dramatic effect in *AurC-KD*–injected oocytes was cytokinesis failure in meiosis I, resulting in production of large polyploid oocytes, a pattern similar to that observed in *Aurora-C*–deficient males who produce large-headed polyploid spermatozoa (Dieterich *et al*., 2007). However, this pattern was not found in homozygous *AurC* mutant women (Dieterich *et al*., 2009). Perhaps, these women might have a yet-unknown compensatory activity, possibly with Aurora-B compensating for the lack of Aurora-C. Indeed, both Aurora-B transcripts and protein were detected in bovine oocytes during meiotic maturation (Uzbekova *et al*., 2008). Future analysis of Aurora-C knockout rats, which contain only a single copy of *Aurora-C*, may resolve this puzzle.

In eukaryotes, the SAC maintains genome stability by inhibiting APC/C^{Cdc20} activity until all chromosomes are correctly aligned on the microtubule spindle apparatus via their kinetochores (Musacchio and Salmon, 2007; Nezi and Musacchio, 2009). The SAC device is composed of mitotic checkpoint complex (BubR1, Mad2, and Bub3) and core

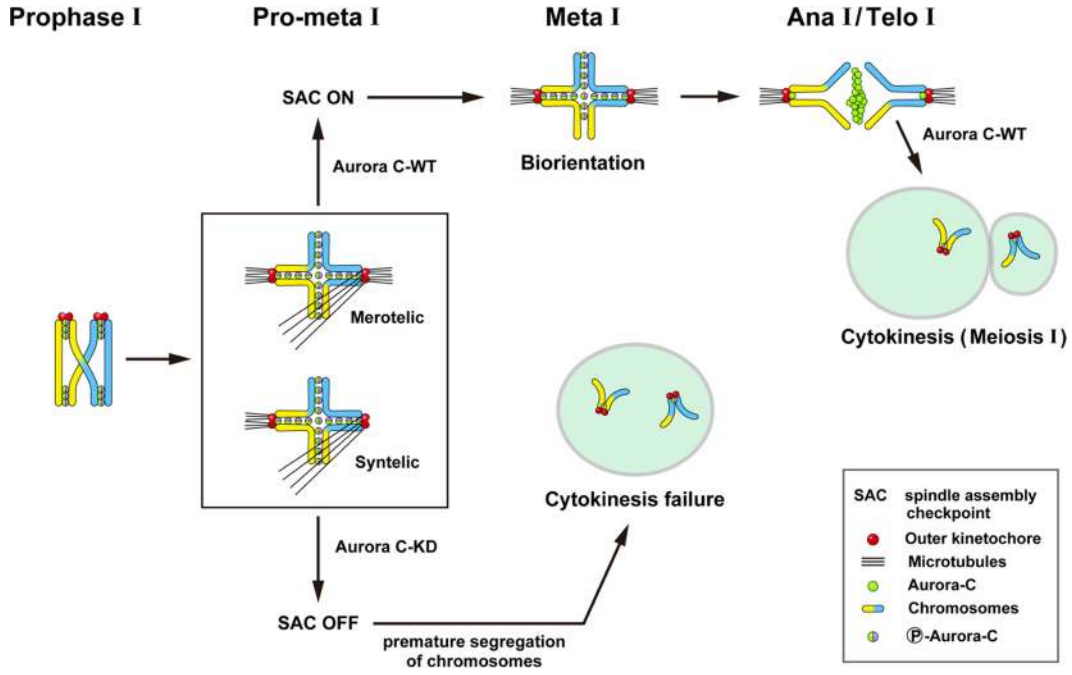


Figure 8. Schematic representation of the subcellular localization of Aurora-C and its possible functions in female meiosis. During late prophase I–metaphase I, Aurora-C is phosphorylated at Thr171 and located at the centromeric regions as well as along the chromosome arms (see Figure 4). Aurora-C is dephosphorylated and relocalized to the midzone and midbody (see Figure 4) during anaphase I–telophase I transition and then rephosphorylated and concentrated again to the centromeres at metaphase II (see Figure 1, B and D). During prometaphase-metaphase I, Aurora-C is involved in promoting chromosome biorientation by correcting aberrant kinetochore-microtubule attachments in meiosis I, whereas its deficiency causes early onset of chromosome segregation in MI and failure to complete cytokinesis. Aurora-C-KD, Aurora-C kinase-deficient mutant.

components, including Bub1, Mad1, Mps1, and Aurora-B (Musacchio and Salmon, 2007). SAC activity is turned on when an unattached kinetochore is sensed and turned off as biorientation attachment is achieved. Previous reports have shown that BubR1 can bind to and inhibit the APC/C^{Cdc20} activity (Tang *et al*., 2001; Fang, 2002). Interestingly, this BubR1-mediated inhibition of APC/C^{Cdc20} has to be maintained by the cooperation between Aurora-B and Bub1 (Morrow *et al*., 2005), thus providing a molecular link between kinetochore–microtubule interactions and the proteolytic machinery that regulates mitosis.

In this study, only Aurora-C, but not Aurora-B kinase, was detected in mouse meiotic oocytes (Figure 1). Furthermore, we observed that the AurC-KD mutant cannot only inhibit localization of Bub1 and BubR1 to the kinetochore (Figure 5, C and D) but also induce premature chromosome segregation (Figure 3) in female meiosis. In mouse oocytes, homologue disjunction during meiosis I requires APC-mediated activation of separase to resolve chiasmata through the removal of meiotic specific cohesin subunit Rec8 from sister chromatid arms (Terret *et al*., 2003; Kudo *et al*., 2006). Thus, a possible mechanism yet to be tested is that AurC-KD mutant may cause an early relief of BubR1-mediated inhibition of APC/C^{Cdc20} during meiosis I. This event may thus promote APC/C activity to release separase, which is required to remove Rec8 cohesin in sister chromatid arms.

Furthermore, two meiosis-types of aberrant kinetochore-microtubule attachments (merotelic and syntelic) were commonly found in Aurora-C kinase-deficient oocytes, implying an essential role of Aurora-C in correcting erroneous microtubule attachments at kinetochores during meiosis (Figure 6A). Indeed, the Aurora-B/Ipl1 in yeast has been demonstrated to regulate kinetochore–microtubule attachment during meiotic divisions and the monopolin complex helps Aurora-B to co-orient sister chromatids during meiosis I (Monje-Casas *et al*., 2007). Thus, whether a monopolin-like complex or its functional equivalent is present in mouse oocytes that may help Aurora-C to co-orient sister chromatids during meiosis I will be an interesting issue for future analysis.

On the basis of the above-mentioned observations, we propose a possible model of how Aurora-C functions in meiosis I (Figure 8). During prometaphase I–metaphase I transition, Aurora-C is involved in promoting chromosome biorientation by correcting aberrant kinetochore–microtubule attachments in meiosis I. In contrast to mitosis, abnormal K-MT attachments such as attachment of sister chromatids of a homologues to opposite poles (meiosis-merotelic) or attachment of both homologues of a bivalent to the same pole (meiosis-syntelic), have to be corrected into a biorientation attachment. Once chromosome biorientation is complete and the SAC is satisfied, resolution of chiasmata in meiosis I require proteolytic cleavage along chromosome arms of cohesin Rec8 by separase, which is activated by APC/C (Kudo *et al*., 2006). INCENP recruits Aurora-C via its IN-box motif (Chen *et al*., 2005) and forms a complex in meiotic chromosomes (Tang *et al*., 2006). Aurora-C/INCENP may thus be involved in the control of APC/C-mediated activation of the separase pathway, possibly through the regulation of SAC members such as Bub1 and BubR1 that control APC/C activity (a hypothesis yet to be tested). Nevertheless, the findings that AurC-KD and INCENP-delIN mutants inhibited kinetochore localization of Bub1 and BubR1 (Figures 5, C and D; and 7E) and induced premature chromosome separation (Figures 3 and 7F) in meiosis I and that the APC/C activity could be prematurely turned on in the absence of Bub1 (McGuinness *et al*., 2009) and BubR1 (Tang *et al*., 2001; Fang, 2002) are consistent with this hypothesis.

We also attempted to knockdown endogenous Aurora-C by microinjection or transfection of *siRNA-AurC* into mouse oocytes, but this approach usually revealed low efficiency and only partial effects. Thus, microinjection of a dominant-negative Aurora-C kinase-deficient mutant to interfere with its normal counterpart seems to be an efficient way to study Aurora-C function during female meiosis. In summary, we have analyzed the subcellular localization and functions of Aurora-C kinase during female mouse meiosis. Surprisingly, we did not detect Aurora-B protein in mouse oocytes. We thus propose that Aurora-C is a unique chromosomal passenger protein that regulates chromosome segregation, kinetochore–microtubule attachment, and cytokinesis in mouse oocytes.

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