Differential regulation of maternal and paternal chromosome condensation in mitotic zygotes

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Summary

A-kinase anchoring protein AKAP95 is implicated in somatic mitotic chromosome condensation by recruiting the condensin complex. Here, we report a differential regulation of condensation of maternal and paternal chromosomes mediated by AKAP95 in mitotic mouse zygotes. AKAP95 is synthesized upon oocyte activation, targeted to the female pronucleus and specifically associates with maternal chromosomes at mitosis. AKAP95 mRNA is highly restricted to the vicinity of the meiotic spindle in metaphase II oocytes. In vivo displacement of endogenous AKAP95 in female pronuclei by microinjection of competitor peptides and rescue experiments show that AKPA95 is required for recruitment of the mCAP-D2 condensin subunit to, and condensation of, maternal chromosomes. In contrast, AKAP95 is dispensable for mCAP-D2 recruitment to, and condensation of, paternal chromosomes. Our results indicate that at first embryonic mitosis, paternal chromosomes target condensins and condense independently of AKAP95, whereas maternal chromosomes require AKAP95 for condensin recruitment and condensation. We propose a concept whereby condensation of chromosomes in gametes, zygotes and somatic cells involves related but distinct mechanisms.

Key words: AKAP95, Chromosome condensation, Condensin, Mitosis, Zygote

Introduction

Several lines of evidence indicate that the behavior of chromosomes during first embryonic mitosis relates to their parental origin. Fertilization is marked by extensive remodeling of the highly compacted, protamine-containing sperm chromatin into a large male pronucleus (MPN). The oocyte, arrested in metaphase II (MII) in most mammals, resumes meiosis and assembles a usually more compact female pronucleus (FPN) (Bouniol-Baly et al., 1997). In the mouse, both pronuclei migrate towards each other and at mitosis maternal and paternal chromosomes condense into a single metaphase plate. Nonetheless, both chromosome sets remain topologically separated, and this persists up to the four-cell stage (Mayer et al., 2000). Additionally, condensed maternal chromosomes appear shorter and more spiralized than paternal chromosomes (Donahue, 1972), but the significance of this finding remains unclear (Kaufman, 1973; Dyban and Sorokin, 1983). Similarly, male and female pronuclei respond differently to premature chromosome condensation induced by fusion of mouse zygotes with MII oocytes, with maternal chromosomes condensing faster than paternal chromosomes (Ciemerych and Czolowska, 1993). These observations suggest a different structural organization of maternal and paternal chromosomes and distinct mechanisms regulating the dynamics of each parental chromosome complement.

Condensation of somatic chromosomes at mitosis requires the highly conserved 13S condensin complex (Hirano, 2000). The *Xenopus* and human condensin complex is made of two structural maintenance of chromosomes (SMC) proteins [chromosome-associated protein (CAP)-C and -E] and three non-SMC regulatory proteins (CAP-D2, -G and -H) (Schmiesing et al., 1998; Schmiesing et al., 2000; Kimura et al., 2001). Condensins purified from *Xenopus* egg extracts display ATPase activity, which introduces positive writhes in the DNA and thereby may assist in condensation (Kimura and Hirano, 1997; Kimura et al., 1999). Targeting of condensins to chromatin at mitosis correlates with phosphorylation of the non-SMC subunits (Hirano et al., 1997) and phosphorylation of histone H3 (Kimura et al., 1998), and may involve additional factors (Kimura et al., 1998; Schmiesing et al., 2000; Giet and Glover, 2001).

The cAMP-dependent kinase (PKA or A-kinase) anchoring protein AKAP95 is also essential for mitotic chromosome condensation. Human AKAP95 is a 95 kDa zinc-finger protein of 692 amino acids, 89% identical to rat AKAP95 (Coghlan et al., 1994). AKAP95 is a component of the nuclear matrix-chromatin interface in interphase. Prior to nuclear envelope breakdown at mitosis, AKAP95 is recruited to the chromatin (Collas et al., 1999). Studies in mitotic HeLa cell extracts have shown that chromatin-bound AKAP95 acts as a targeting protein for the condensin complex (Steen et al., 2000). Chromatin- and condensin-binding domains of human AKAP95 have been mapped to the C-terminal half of the protein within residues 387-450 and 525-569, respectively (Eide et al., 2002). Chromatin-binding and chromosome condensation activities of AKAP95 do not require PKA anchoring nor PKA activity, whereas maintenance of condensed chromosomes throughout mitosis does (Collas et al., 1999).

Here, we present evidence that AKAP95 is implicated in a

differential regulation of condensin targeting and condensation of maternal and paternal chromosomes in mouse zygotes. Our results suggest a concept whereby condensation of chromosomes in gametes, zygotes and somatic cells involves related but distinct mechanisms.

Materials and Methods

Antibodies and peptides

Polyclonal anti-rat AKAP95 antibodies (Coghlan et al., 1994) and monoclonal antibody mAb47 against human AKAP95 (Collas et al., 1999) were from Upstate Biotechnology and Transduction Laboratories, respectively. Binding of these antibodies were mapped to residues 525-569 and 387-524 of human AKAP95, respectively (Eide et al., 2002). Affinity-purified polyclonal antibodies against hCAP-D2 have been described (Collas et al., 1999). Anti-histone antibodies were from Santa-Cruz Biotechnologies. Anti-protamine mAb Hup 1N was described previously (Stanker et al., 1993). Ht31 and Ht31-P peptides were described earlier (Carr et al., 1991). Expression of GST-AKAP95(387-692) was as described (Eide et al., 1998). GST-AKAP95(1-195), GST-AKAP95(387-450) and GST-AKAP95(387-450)ZF1CCSS peptides were generated by site-directed mutagenesis from a pGEX-AKAP95 expression vector (Eide et al., 1998; Eide et al., 2002). Constructs were sequenced and proteins expressed as described (Eide et al., 1998).

Gametes, embryos and cells

MII oocytes and pronuclear (PN) embryos were collected from superovulated B6D2 mice at 14 and 22 hours post-hCG injection, respectively. Cumulus cells were dispersed with 1 mg/ml hyaluronidase, washed and dissolved in SDS sample buffer or sedimented onto coverslips for immunofluorescence. Oocytes were washed in Flushing Holding Media (FHM; Specialty Media) prior to use. PN stage embryos were cultured in Potassium Simplex Optimized Media (KSOM; Specialty Media).

Mature sperm were collected in phosphate buffered saline (PBS) from the epididymis of C57 males, washed and stored in liquid nitrogen without cryoprotectant. After thawing on ice, sperm were washed in FHM and held on ice until use. Sperm were also dissolved in SDS sample buffer or settled on coverslips for immunofluorescence. To prepare sperm nuclei, frozen-thawed sperm (without mid-piece and tail) were permeabilized for 15 minutes in 1% Triton X-100, washed in PBS and held on ice until use.

Oocyte activation

MII oocytes were parthenogenetically activated at 15 hours post-hCG in KSOM containing 10 mM $SrCl_2$ (Sigma) for 4 hours at 37°C. To inhibit RNA polymerase II-mediated transcription, oocytes were activated with 10 mM $SrCl_2$ together with 5 μg/ml actinomycin D (Sigma) for 4 hours. To inhibit protein synthesis, oocytes were activated for 4 hours with 10 μg/ml cycloheximide (Sigma). Activation was monitored by appearance of the FPN at the end of the activation treatment. Activated oocytes were washed in KSOM and processed for immunofluorescence or immunoblotting.

Micromanipulation

Intracytoplasmic sperm injection (ICSI)

MII oocytes were maintained in FHM at 37°C during ICSI. Sperm heads were recovered from liquid nitrogen, thawed at 25°C, centrifuged at 2000 ***g*** and resuspended in 7% polyvinyl-pyrrolidone in PBS to decrease stickiness. When required, sperm nuclei were labeled with 1 μg/ml Hoechst 33342 prior to injection. Injections were performed at 19°C in FHM containing 3 mg/ml BSA using a piezo-drill. Oocytes were injected at 15 hours post-hCG, washed and cultured in KSOM. PN formation was detected by phase-contrast microscopy 4-5 hours after ICSI.

Enucleation of the FPN

PN stage embryos collected from B6D2 females mated to CD1 males were enucleated in FHM containing 3 mg/ml BSA and 5 μg/ml cytochalasin B. The zona pellucida was penetrated with a piezo drill and the FPN (smaller than the MPN) removed by aspiration. Embryos were washed and cultured in KSOM.

Peptide injection

PN stage embryos in FHM were microinjected into fully formed FPN or MPN with 250 pg GST-AKAP95 peptide using a pulled glass capillary. Injections took place at 8 hours post-ICSI, or 23 hours post-hCG when using normally fertilized embryos. Embryos were washed and cultured in KSOM. Nuclei of two-cell stage blastomeres were injected similarly except that the peptide-containing injection solution contained 10 μg/ml of a 150 kDa FITC-dextran (Sigma) as tracer.

Immunological procedures

Immunoblotting analysis of cumulus cells (30 μg protein), oocytes (n=200), embryos (n=200) and sperm or sperm nuclei (n=10^6) was performed essentially as described (Collas et al., 1999) using anti-AKAP95 polyclonal antibodies (1:250 dilution), anti-AKAP95 mAb47 (1:250), anti-protamine Hup1N (1:500) or anti-hCAP-D2 antibodies (1:5000). For immunofluorescence, oocytes and embryos were washed in PBS, fixed with 3% paraformaldehyde for 15 minutes, washed in PBS, permeabilized with 0.1% Triton X-100 for 15 minutes, washed in PBS/0.01% Tween-20 (TBST) and proteins blocked in PBST/2% BSA. Primary and secondary antibodies were used at a 1:100 dilution in PBST/BSA and incubated for 30 minutes. DNA was counterstained with 0.1 μg/ml Hoechst 33342 or 0.1 μg/ml propidium iodide (PI) as indicated. Immunofluorescence analysis of methanol-fixed cumulus cells sedimented on poly-L-lysine-coated coverslips was performed as described earlier (Steen and Collas, 2001). Sperm were processed as described for oocytes after sedimentation onto poly-L-lysine-coated glass coverslips. Observations were made on an Olympus BX60 microscope and photographs taken with a JVC CCD camera and AnalySIS software (Soft Imaging Systems).

In situ extraction of nuclear matrices was carried out as described (Martins et al., 2000) after affixing cumulus cells onto glass coverslips. Briefly, cells were extracted with 0.1% Triton X-100 for 5 minutes, incubated for 5 minutes with ice-cold cytoskeleton stabilization (CSK) buffer containing 0.1% Triton X-100 and washed in CSK buffer. DNA was digested for 30 minutes with 1 mg/ml DNase I and washed twice for 5 minutes in PBS. Resulting matrices were fixed with –20°C methanol and processed for immunofluorescence.

RNA in situ hybridization

An anti-rat AKAP95 cDNA probe was generated using as a template a 732 bp PCR product amplified from rat genomic DNA using the primers 5′-AGGTTGGCTGCTGAACAATTC-3′ and 5′-GATGGCT-ATGACAGGTACTGG-3′. PCR conditions were denaturation at 94°C for 5 minutes, and 35 cycles of 94°C denaturation (30 seconds), 58°C annealing (30 seconds) and 72°C extension (1 minute). The probe was biotinylated using a random-priming biotin labeling kit containing biotin-16-dUTP (Amersham) (Collas and Aleström, 1998).

Oocytes were fixed with 3% paraformaldehyde in PBS for 15 minutes, washed and post-fixed with methanol:acetic acid (3:1) for 10 minutes on ice. Fixed oocytes were permeabilized with 1% Triton X-100 for 15 minutes at room temperature. Samples were denatured at

70°C in 70% formamide/2× SSC for 5 minutes, dehydrated in ethanol series and air dried. The labeled DNA probe was allowed to hybridize overnight at 37°C. Post-hybridization washes were performed in 50% formamide/2× SSC (3 times, 5 minutes), all at 45°C, and twice for 3 minutes in PN buffer (0.2 M Na_2HPO_4, 6 mM NaH_2PO_4, pH 8.0, and 0.05% NP-40). Proteins were blocked for 5 minutes in PN buffer containing 5% dry milk (PNM buffer). The probe was revealed by successive 30-minute incubations at 37°C in avidin-TRITC (1:400 dilution in PNM buffer), biotin-conjugated anti-avidin antibodies (1:100 dilution in PNM buffer) and another layer of avidin-TRITC. Slides were washed in PN buffer for 5 minutes and mounted in antifade (1 mg/ml *p*-phenylenediamine dihydrochloride, 10% PBS, 90% glycerol) containing 0.2 μg/ml Hoechst 33342. When indicated, samples were treated with 1 mg/ml DNAse I (Sigma) or 100 μg/ml RNAse A (Amersham) for 30 minutes prior to hybridization.

Results

Mouse AKAP95 expression is developmentally regulated

The distribution of AKAP95 in mouse somatic cells, gametes and preimplantation embryos was examined using an affinity-purified polyclonal antibody against rat AKAP95. Consistent with labeling of HeLa cells (Eide et al., 1998), AKAP95 staining was restricted to interphase nuclei in mouse cumulus cells (Fig. 1A). In situ extraction of cells with 1 mg/ml DNAse I and 1% Triton X-100 to produce 'nuclear matrices' indicated that a fraction of AKAP95 decorated the matrix and co-localized with DNAse I-resistant DNA (Fig. 1A). Immunoblotting analysis of cumulus cells confirmed the presence of a single anti-AKAP95-reactive antigen of 95 kDa (Fig. 1B). Antigen detection was prevented by pre-adsorbing the antibody to a 1000× molar excess of glutathione-S-transferase (GST)-AKAP95(387-692) peptide, suggesting that the antibody detected a mouse homologue of rat AKAP95 (Fig. 1B).

Immunoblotting analysis of mouse epididymal sperm nuclei revealed no AKAP95, even after reducing disulfide bonds with 100 mM DTT (resulting in decondensed nuclear 'halos') prior to SDS-PAGE (Fig. 1C). Metaphase II (MII) oocytes contained traces of AKAP95, as seen on an immunoblot of 200 oocytes (Fig. 1C) and by overexposure of immunofluorescently labeled oocytes (Fig. 1D, MII, inset, arrow). After fertilization, AKAP95 was strongly upregulated at the pronuclear (PN) stage (Fig. 1C,D). Notably, AKAP95 was restricted to the FPN – usually smaller than the MPN – and was absent from polar bodies (Fig. 1D, PN). In subsequent stages of development in vitro, AKAP95 was localized in the nucleus of each blastomere and on condensed chromosomes in mitotic cells (Fig. 1D, arrows), reminiscent of earlier findings in somatic cells (Collas et al., 1999).

Synthesis of AKAP95 correlated with activation of the oocyte. Parthenogenetic activation of MII oocytes with 10 mM $SrCl_2$ elicited FPN formation and pronuclear accumulation of AKAP95 (Fig. 1E, +$SrCl_2$). $SrCl_2$-induced AKAP95 synthesis was verified by western blot (Fig. 1E, insert). AKAP95 labeling was essentially absent from oocytes activated with 10 μg/ml of the protein synthesis inhibitor, cycloheximide (Fig. 1E, +CHX), indicating that accumulation of AKAP95 in the FPN results from protein synthesis. However, AKAP95 was detected after activation with 10 mM $SrCl_2$ together with 5 μg/ml of the RNA polymerase II inhibitor, actinomycin D (Fig. 1E, +$SrCl_2$ +Act.D). Therefore, oocyte activation induces strong upregulation of AKAP95 translation from a maternal store of mRNA.

AKAP95 mRNA is localized near the meiotic spindle

To demonstrate the presence of AKAP95 mRNA in the MII oocyte and to provide some explanation to account for targeting of AKAP95 to the FPN after fertilization (Fig. 1D), we visualized AKAP95 mRNA in MII oocytes by in situ RNA hybridization. A 730 bp fragment of the AKAP95 coding region amplified by PCR was used to generate a biotinylated oligonucleotide probe. Hybridization of MII oocytes and detection of the probe with TRITC-conjugated avidin revealed a concentration of AKAP95 mRNA around the meiotic spindle, as judged by DNA staining with Hoechst 33342 (Fig. 2A). The signal disappeared when oocyte mounts were treated with RNAse A prior to hybridization (Fig. 2B). DNAse treatment did not eliminate the RNA hybridization signal, despite the disappearance of most of the MII chromosome Hoechst labeling (Fig. 2C). Thus, MII oocytes contain AKAP95 mRNA, arguing that AKAP95 protein synthesis upon oocyte activation is translationally regulated. Moreover, RNA hybridization data reveal AKAP95 mRNA exclusively in the vicinity of the meiotic spindle. To our knowledge, this is the first report of a highly restricted mRNA localization in a mammalian oocyte. By analogy to eggs of non-mammalian species (Alarcon and Elison, 2001) (see Discussion), this may enable efficient targeting of the AKAP95 protein to the FPN.

Localization of mCAP-D2, a putative subunit of the mouse condensin complex, in mouse gametes

Distribution of the condensin complex in mouse gametes was examined using an affinity-purified antibody against hCAP-D2/CNAP1, a non-SMC subunit of the human condensin complex. Immunoblotting analysis of mouse cumulus cells revealed a single anti-hCAP-D2-reactive band of 150 kDa, the expected apparent M_r of hCAP-D2. Anti-hCAP-D2 reactivity also occurred with MII oocytes and in PN stage embryos, but not with sperm, even after enhancing antigen accessibility in decondensed halos with DTT (Fig. 3A). In unfertilized oocytes, anti-hCAP-D2 labeling was restricted to the MII chromosomes (Fig. 3B, arrow) and co-localized with the faint AKAP95 staining (not shown). Thus, the antibody crossreacted with a putative homologue of hCAP-D2/CNAP1 in mouse cumulus cells and oocytes. The mouse protein was designated mCAP-D2.

AKAP95 is targeted to the female pronucleus and to maternal chromosomes at first mitosis

To unequivocally demonstrate targeting of AKAP95 to the FPN in mouse zygotes, sperm was pre-labeled with the DNA stain, Hoechst 33342, and introduced into MII oocytes by ICSI. Resulting PN stage zygotes were analyzed by immunofluorescence using the anti-AKAP95 polyclonal antibody. Total (maternal and paternal) DNA was counterstained with PI. Dual DNA staining unequivocally discriminated the FPN (labeled red only) and the MPN (labeled blue), and indicated that AKAP95 was localized exclusively in the FPN (Fig. 4A, green and yellow labeling). Examination of similar Hoechst- and PI-labeled embryos at mitosis showed that anti-AKAP95 antibodies decorated a set of chromosomes not labeled with Hoechst (Fig. 4B), illustrating the restriction of AKAP95 to maternal chromosomes. At the two-cell stage, AKAP95 decorated both

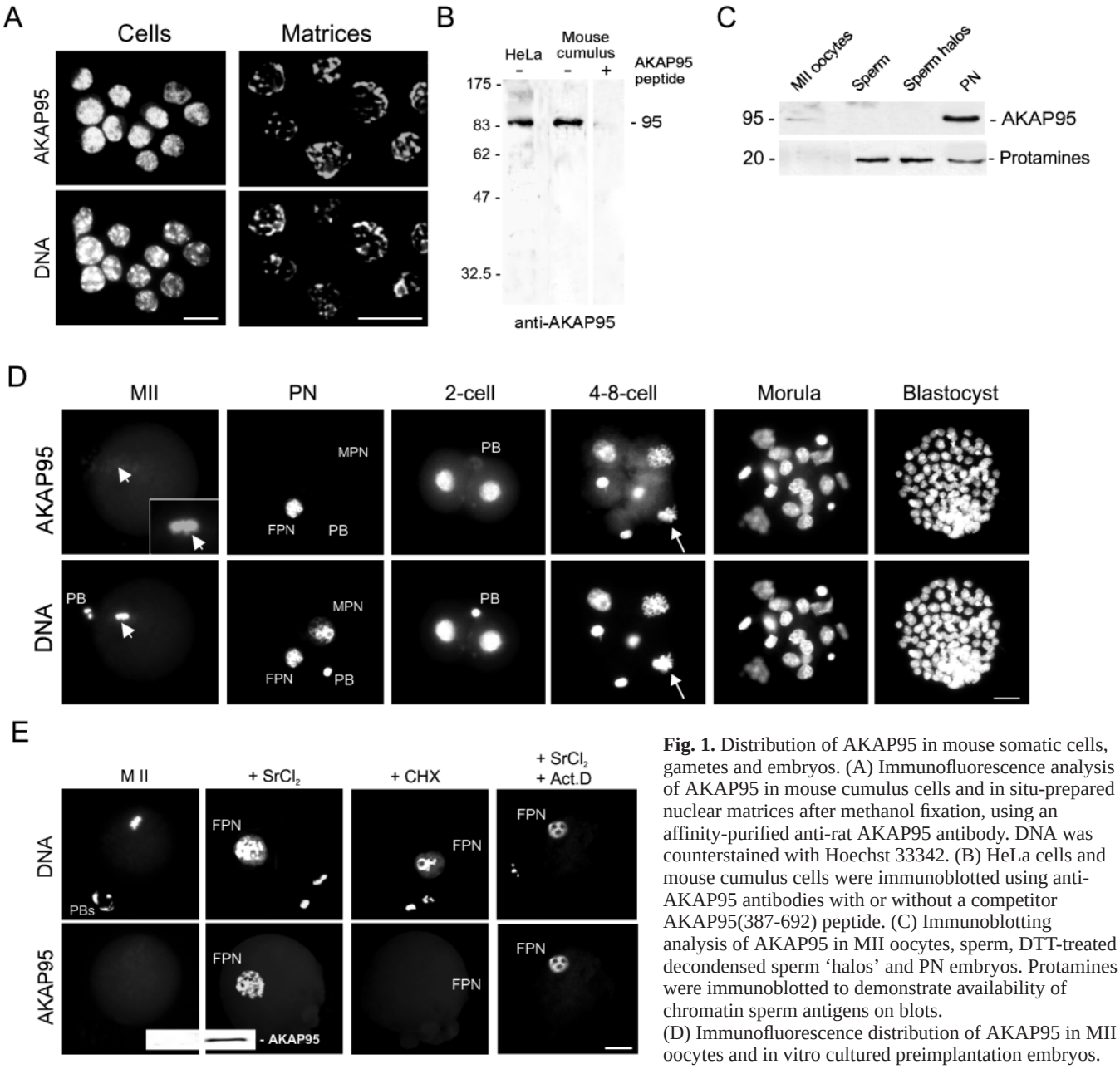

Fig. 1. Distribution of AKAP95 in mouse somatic cells, gametes and embryos. (A) Immunofluorescence analysis of AKAP95 in mouse cumulus cells and in situ-prepared nuclear matrices after methanol fixation, using an affinity-purified anti-rat AKAP95 antibody. DNA was counterstained with Hoechst 33342. (B) HeLa cells and mouse cumulus cells were immunoblotted using anti-AKAP95 antibodies with or without a competitor AKAP95(387-692) peptide. (C) Immunoblotting analysis of AKAP95 in MII oocytes, sperm, DTT-treated decondensed sperm 'halos' and PN embryos. Protamines were immunoblotted to demonstrate availability of chromatin sperm antigens on blots.
(D) Immunofluorescence distribution of AKAP95 in MII oocytes and in vitro cultured preimplantation embryos. Arrowheads point to MII chromosomes. Arrows point to a metaphase blastomere. (E) MII oocytes were activated with either 10 mM $SrCl_2$, 10 μg/ml cycloheximide (CHX) or 10 mM $SrCl_2$ together with 5 μg/ml actinomycin D. Activated oocytes were fixed at the PN stage and labeled using anti-AKAP95 antibodies. Insets, MII and $SrCl_2$-activated oocytes were immunoblotted using anti-AKAP95 antibodies. DNA was stained with Hoechst 33342. FPN and MPN, female and male pronucleus, respectively. PB, polar body. Bars, 10 μm (A); 20 μm (D,E).

chromosome complements (not shown). Together, these results indicate that AKAP95 is specifically targeted to the FPN and to condensed maternal chromosomes at first mitosis.

Both maternal and paternal chromosomes recruit mCAP-D2

To assess the presence of mCAP-D2 on condensed maternal and paternal chromosomes at first mitosis, mitotic zygotes produced by ISCI of Hoechst-labeled sperm were examined by total DNA staining with PI and by immunofluorescence using the anti-hCAP-D2 antibody. Fig. 4C shows that mCAP-D2 was detected on both parental chromosome sets. Double immunolabeling with anti-AKAP95 mAb47 demonstrated mCAP-D2 and AKAP95 co-localization on female chromosomes (Fig. 4D, yellow label), and mCAP-D2 localization on male chromosomes despite the absence of AKAP95. We concluded from these observations that mCAP-D2 is targeted to both chromosome complements at first mitosis.

AKAP95 is required for condensation of maternal chromosomes

Targeting of AKAP95 to maternal chromosomes at mitosis

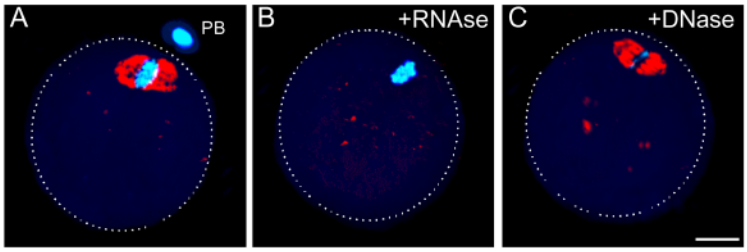

Fig. 2. RNA in situ hybridization analysis of AKAP95 message in MII oocytes. Oocytes were fixed and hybridized with a biotinylated probe against rat AKAP95 cDNA. Hybridization was detected with TRITC-conjugated avidin (red). DNA was counterstained with 0.2 μg/ml Hoechst 33342 (blue). Samples were also pre-treated with DNAse I (+DNAse) or 100 μg/ml RNAse A (+RNAse) prior to fixation. Note the restricted localization of AKAP95 mRNA around the meiotic spindle. PB, polar body. Dotted circles delineate the oocyte. Bar, 20 μm.

suggests that the protein may be involved in the condensation process. To test this hypothesis, we investigated the effect of displacing endogenous AKAP95 in the FPN by microinjecting a competitor GST-AKAP95 fusion peptide encompassing the chromatin-binding domain (residues 387-450), but not the condensin-binding domain (extending beyond residue 450), of human AKAP95 (Eide et al., 2002). This GST-fusion peptide was referred to as AKAP95(387-450). Zygotes were produced by ICSI of Hoechst-labeled sperm in order to distinguish male and female pronuclei. The FPN or the MPN was microinjected with 250 pg of AKAP95(387-450) (Fig. 5A), embryos were allowed to reach mitosis, fixed and DNA conformation and AKAP95 localization were examined. The anti-AKAP95 polyclonal antibody was used to label endogenous AKAP95, while anti-AKAP95 mAb47 was used to detect the AKAP95(387-450) peptide, not recognized by the polyclonal antibody (T. Eide, K. Tasken and P.C., unpublished).

As expected from out previous data, only the FPN contained AKPA95 at the time of peptide injection (Fig. 5A). Injection of AKAP95(387-450) into the MPN promoted binding of the peptide to male chromatin, as detected with mAb47 (Fig. 5B, inset, 'M'), but did not affect AKAP95 localization on female ('F') chromosomes nor condensation of either chromosome complement (Fig. 5B). Similar results were obtained with 500 pg peptide to compensate for the volume difference between FPN and MPN (not shown). Remarkably, however, injection of 250 pg AKAP95(387-450) into the FPN completely inhibited condensation of the female chromatin (Fig. 5C, 'F'). The female chromatin was devoid of endogenous AKAP95, as shown by the lack of polyclonal anti-AKAP95 labeling (Fig. 5C). However, mAb47 immunolabeling indicated that the peptide was bound to the female chromatin and thereby displaced endogenous AKAP95 from maternal chromosomes (Fig. 5C, inset, 'F'). In contrast to female chromatin, male chromosome condensation was not affected by injection of AKAP95(387-450) into the FPN (Fig. 5C, 'M'). As a result, these embryos simultaneously displayed condensed male chromosomes and a decondensed maternal chromatin mass. Labeling of these embryos with an antibody against B-type lamins, a marker of the nuclear envelope, indicated that AKAP95(387-450) did not prevent female pronuclear envelope breakdown (data not shown). Thus, the peptide inhibited maternal chromosome condensation per se. Additionally, injection of the FPN with 250 pg AKAP95(387-692), which binds both chromatin and condensins (Eide et al., 2002) and is detected by the anti-AKAP95 polyclonal antibody (Steen et al., 2000), allowed condensation of female, and male, chromosomes (Fig. 5D), as did a mock-injection with peptide buffer (data not shown). Lastly, FPN injection of the N-terminal domain of human AKAP95 (AKAP95[1-195]), which does not bind chromatin (Eide et al., 2002), or of an AKAP95(387-450) peptide mutated in the zinc finger to abrogate chromatin binding (Eide et al., 2002), did not impair male or female chromosome condensation (data not shown). These controls indicate that inhibition of condensation of female chromatin following FPN injection of AKAP95(387-450) is not due to steric hindrance, but rather to a specific inhibitory effect of the peptide.

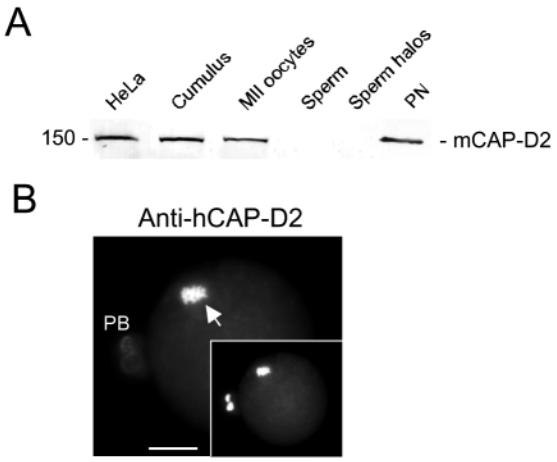

Fig. 3. Distribution of mCAP-D2 in mouse gametes.
(A) Immunoblotting analysis of mCAP-D2 in indicated cell types using an affinity-purified antibody against hCAP-D2.
(B) Immunofluorescence localization of mCAP-D2 in MII oocytes. Arrowhead points to MII chromosomes. DNA was stained with Hoechst 33342. PB, polar body. Bar, 20 μm.

Collectively, these results indicate that AKAP95(387-450) displaces endogenous AKAP95 from maternal chromatin. This correlates with inhibition of mitotic maternal chromosome condensation whereas paternal chromosome condensation remains unaffected. The chromatin- and condensin-binding peptide AKAP95(387-692) has no inhibitory effect, suggesting that the AKAP95(387-450) peptide acts as a dominant negative. These observations suggest an essential role of AKAP95 in the condensation of maternal chromosome in mitotic zygotes.

AKAP95(387-450) abolishes recruitment of mCAP-D2 to maternal chromosomes

Human AKAP95 was recently proposed to act as a targeting molecule for the condensin complex, a five-subunit structure

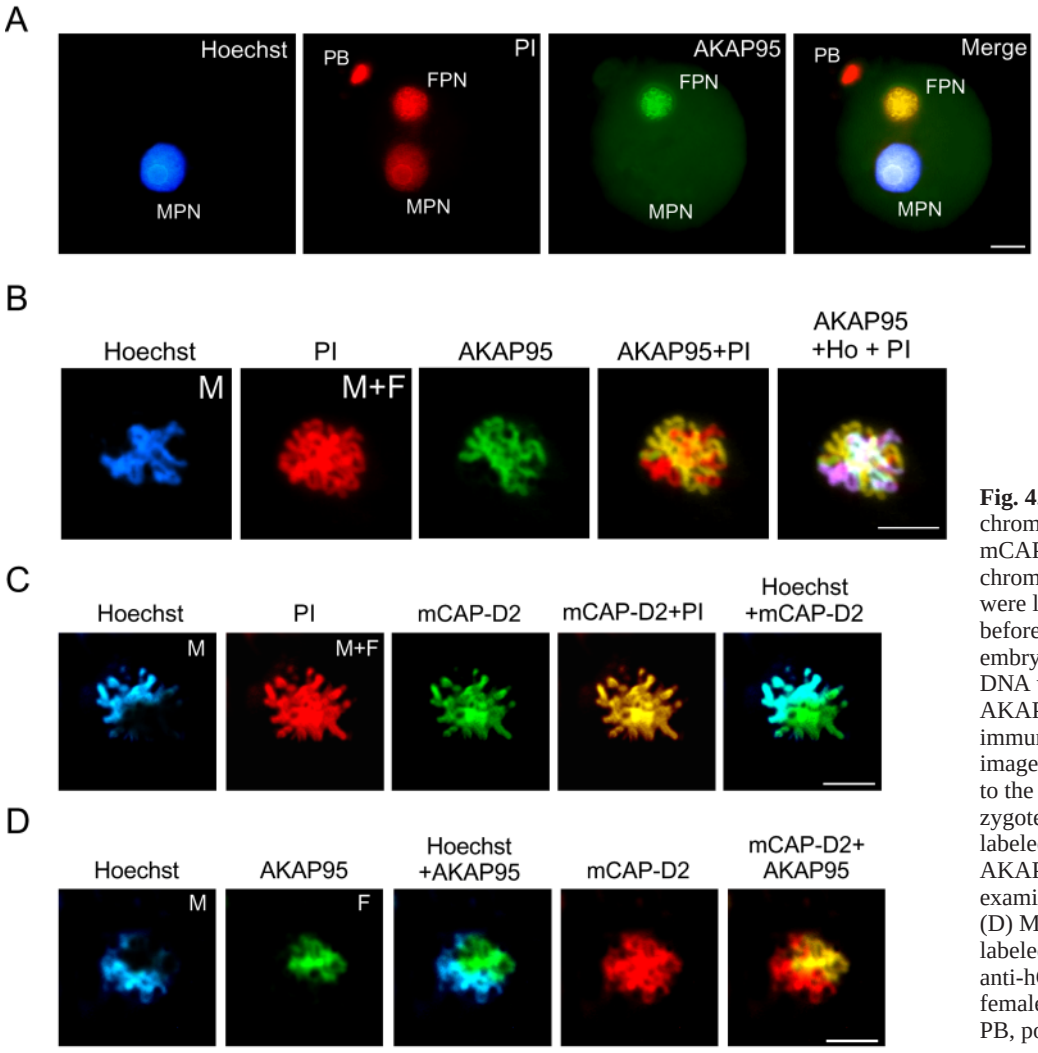

Fig. 4. AKAP95 associates with maternal chromosomes at first mitosis, whereas mCAP-D2 is recruited to both chromosome complements. (A) Sperm were labeled with Hoechst 33342 (blue) before ICSI into oocytes. Resulting ICSI embryos were fixed at the PN stage, total DNA was labeled with PI (red) and AKAP95 localized by immunofluorescence (green). Merge image shows AKAP95 labeling restricted to the FPN (yellow). (B,C) Mitotic zygotes produced by ICSI of Hoechst-labeled sperm as in A were fixed and AKAP95 (B) and mCAP-D2 (C) were examined by immunofluorescence. (D) Mitotic embryos were also double-labeled with anti-AKAP95 mAb47 and anti-hCAP-D2 antibodies. 'F' and 'M', female and male chromatin, respectively; PB, polar body. Bars, 20 μm.

required for chromosome condensation (Steen et al., 2000). To account for the failure of maternal chromosomes condensation after FPN injection of AKAP95(387-450), decondensed female chromatin and condensed male chromosomes obtained at mitosis after FPN injection with AKAP95(387-450) (Fig. 5C) were labeled using anti-hCAP-D2 antibodies. mCAP-D2 was not detected on decondensed female chromatin (Fig. 6A, 'F'), whereas male chromosomes ('M') were labeled (Fig. 6A, 'M'). mCAP-D2 targeting to paternal or maternal chromosomes was not abolished by injection of AKAP95(387-450) into the MPN (not shown), nor by FPN injection of AKAP95(387-692) or AKAP95(1-195) (Fig. 6B). Therefore, displacement of endogenous AKPA95 by the competitor AKAP95(387-450) peptide correlated with the lack of mCAP-D2 association with maternal chromosomes.

AKAP95(387-692) restores condensation of maternal chromatin

To further demonstrate a requirement of AKAP95 for female chromatin condensation, we determined whether the chromatin- and condensin-binding AKAP95(387-692) peptide would restore condensation of decondensed maternal chromosomes. Cytoplasmic injection of a 20× mass excess (5 ng) of AKAP95(387-692) in embryos containing a decondensed female chromatin mass (as in Fig. 5C) restored recruitment of mCAP-D2 to female chromosomes and condensation (Fig. 6C). These chromosomes were seen either distant from the already condensed paternal chromatin or closely apposed to male chromosomes as in a normal mitosis. Cytoplasmic injection of AKAP95(1-195) (not shown) or AKAP95(387-450) was ineffective in rescuing female chromatin condensation (Fig. 6D). Immunolabeling with the anti-AKAP95 polyclonal antibody suggested association of AKAP95(387-692) with female chromosomes (Fig. 6C). We concluded that restoration of binding of a functional AKAP95 peptide to decondensed maternal chromosomes rescued mCAP-D2 targeting to chromatin and condensation. This suggests that chromatin association of AKAP95 is necessary for condensin recruitment to maternal chromosomes and condensation. In contrast, condensins appear to associate with paternal chromatin independently of AKAP95.

PKA anchoring to AKAP95 is dispensable for maternal chromosome condensation

We previously reported that AKAP95 function in chromatin

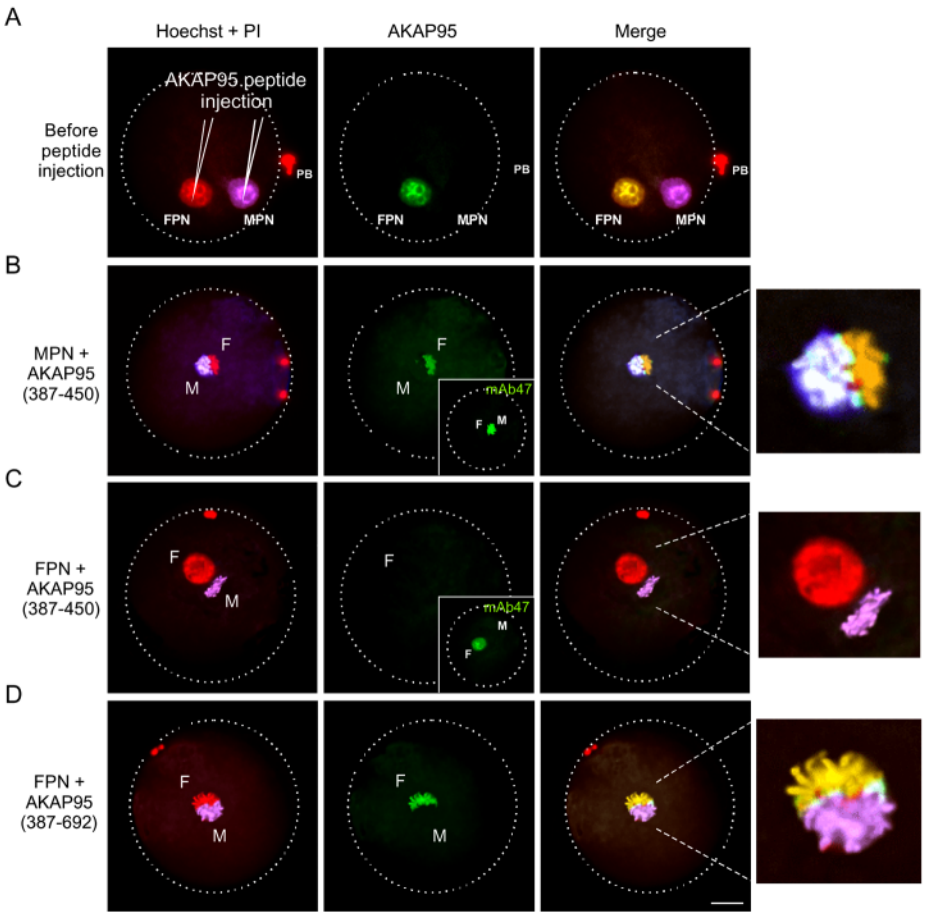

Fig. 5. AKAP95 is required for condensation of maternal chromosomes. (A) Zygotes produced by ICSI of Hoechst-labeled sperm were injected into the FPN or the MPN with 250 pg AKAP95(387-450). Embryos were fixed, counterstained with PI (red) and labeled using anti-AKAP95 polyclonal antibodies (green). (B-D) Injected embryos were cultured to mitosis, fixed and analyzed by DNA staining and AKAP95 immunofluorescence using the polyclonal antibody or mAb47 (insets in B and C only). (B) Mitosis after MPN injection. (C) Mitosis after FPN injection. (D) Mitosis after FPN injection with 250 pg AKAP95(387-692). 'F' and 'M', female and male chromatin, respectively; PB, polar body. Dotted circles delineate embryos. Bar, 20 μm.

condensation in mitotic extract and in vivo was independent of PKA anchoring to AKAP95, as shown by microinjection of Ht31, a well-characterized AKAP-binding competitor peptide (Carr et al., 1991; Collas et al., 1999). To determine whether AKAP95 function in zygotic maternal chromatin condensation required PKA anchoring, the FPN was injected with 750 nM Ht31, or 750 nM of a control Ht31-P mutant which does not bind PKA (Carr et al., 1991). This Ht31 concentration, adjusted to account for the ~15-fold increase in volume between a somatic nucleus and a FPN, was shown to disrupt PKA-AKAP95 anchoring (Collas et al., 1999). Neither peptide affected maternal chromosome condensation at mitosis (data not shown). Thus, as in mitotic cells, PKA anchoring to AKAP95 is probably not required for AKAP95 function in zygotic chromatin condensation.

Paternal chromosome condensation is AKAP95-independent

The lack of AKAP95 labeling of male chromosomes led us to determine whether AKAP95 was required for condensation of male chromosomes at first mitosis. Endogenous pronuclear AKAP95 was removed by non-invasive enucleation of the FPN from normally fertilized pronuclear zygotes. The resulting androgenetic embryos harbored only the MPN devoid of detectable AKAP95 (Fig. 7A). These androgenetic embryos progressed through mitosis (Fig. 7B) and cleaved to at least two cells (Fig. 7C). Mitotic androgenotes displayed condensed chromosomes devoid of detectable AKAP95 (Fig. 7B). At the two-cell stage, however, AKAP95 staining was detected in the nucleus of each blastomere (Fig. 7C; yellow labeling). Thus, AKAP95 is clearly dispensable for condensation and segregation of paternal chromosomes. Nevertheless, paternal genes are capable of transcribing AKAP95 at the two-cell stage in androgenotes, suggesting that the protein might be necessary from this stage of development onwards.

Association of AKAP95 with chromatin is required for cleavage to the four-cell stage

Requirements for AKAP95 anchoring to chromatin for condensation of two-cell stage chromosomes and cleavage was determined. The nucleus of a blastomere of a normal fertilized two-cell stage embryo (Fig. 8, left panel) was microinjected with 250 pg of the AKAP95(387-450) peptide together with a 150-kDa FITC-conjugated dextran to trace the peptide-injected blastomere (Collas et al., 1999). The second blastomere was mock-injected with peptide buffer. AKAP95(387-450) inhibited chromosome condensation and cleavage of the injected blastomere in 14/15 embryos (Fig. 8, Mitosis, arrow). Chromosomes of mock-injected blastomeres, however, condensed normally (Fig. 8, Mitosis, arrowhead) and blastomeres cleaved at least twice in these embryos (Fig. 8, '8-16-cell'). Additional mock injections of 250 pg of the functional AKAP95(387-692) peptide or of the non-chromatin binding AKAP95(1-195) peptide in a similar number of embryos were not inhibitory (Fig. 8, Mock-injected). These results indicate that AKAP95 is implicated in chromatin condensation and cleavage in early mouse embryos.

Discussion

Results from this study postulate a differential requirement for AKAP95 for the condensation of paternal and maternal chromosomes at first embryonic mitosis in mouse embryos. Condensin targeting to chromosomes also seems to be differentially regulated: AKAP95 allows binding of mCAP-

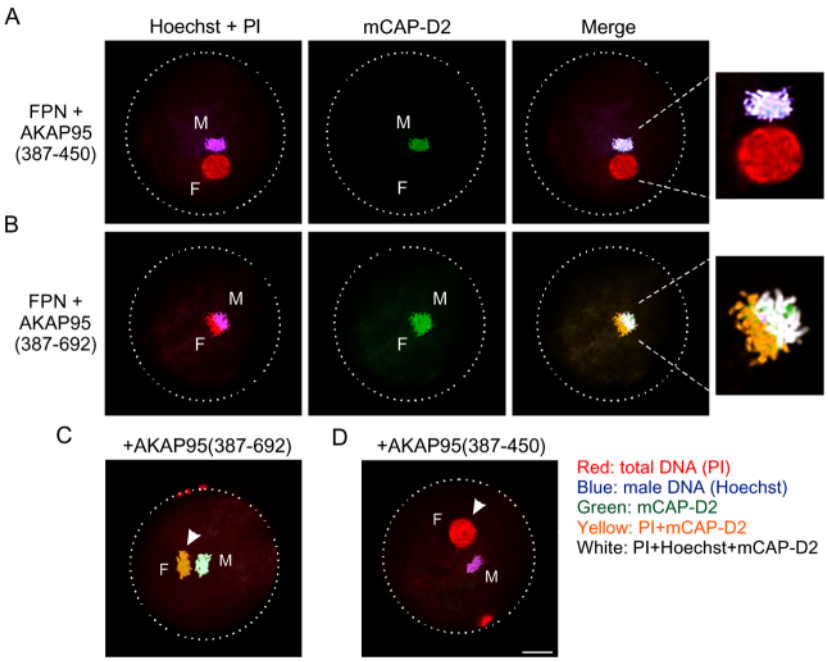

Fig. 6. AKAP95 is required for mCAP-D2 targeting to maternal, but not paternal, chromatin. (A,B) Injection of AKAP95(387-450) in the FPN abolishes mCAP-D2 targeting to female chromatin. The FPN of zygotes produced as in Fig. 5A was injected with (A) 250 pg AKAP95(387-450) or (B) 250 pg AKAP95(387-692). Embryos progressed to mitosis and were examined by total DNA staining with PI (red) and mCAP-D2 immunofluorescence (green). (C,D) AKAP95(387-692) rescues condensation of maternal chromosomes. Mitotic embryos with decondensed female chromatin and condensed male chromosomes were cytoplasmically injected with (C) 5 ng AKAP95(387-692) or (D) 5 ng AKAP95(387-450) as a negative control. Embryos were fixed 2 hours later and analyzed by DNA labeling with PI and by immunofluorescence using the anti-AKAP95 polyclonal antibody. Arrowheads point to female chromatin. Color coding for C and D is shown. 'F' and 'M', female and male chromatin, respectively. Dotted circles delineate the embryos. Bar, 20 μm.

D2, the putative mouse homologue of hCAP-D2, to maternal chromatin, whereas mCAP-D2 is recruited to male chromosomes independently of AKAP95. However, in either situation mCAP-D2 targeting to both parental genomes correlates with chromosome condensation activity.

At fertilization, AKAP95 is translated from a maternal pool of mRNA upon oocyte activation, assembles in the FPN and associates with maternal chromatin at mitosis. What restricts AKAP95 to the FPN remains speculative. Recombinant AKAP95 binds sperm chromatin in vitro (our unpublished results) and in vivo, thus differential AKAP95 targeting is unlikely to result from differences in chromatin composition between the parental genomes. Pronuclear accumulation of AKAP95 may be regulated at the level of the nuclear import machinery or at the level of the female pronuclear envelope; however, these possibilities remain to be examined. Alternatively, a putative AKAP95 docking protein might be absent or non-functional in the MPN. Our unpublished immunoprecipitation studies suggest that AKAP95 interacts in interphase HeLa nuclei with the nuclear matrix protein, NuMA (Compton et al., 1992). Whereas mouse MPNs are devoid of NuMA immunoreactivity, FPNs display variable amounts of NuMA (our unpublished results). Thus, NuMA may constitute a possible AKAP95 anchor specific for the FPN.

A third attractive possibility is that the specific localization of the AKAP95 protein may be a consequence of the site of AKAP95 translation. Polarized mRNA localization and site of translation is a well known mechanism elaborated in (usually large) eggs of several non-mammalian species and in some differentiated somatic cells to ensure efficient protein function at a specific locus. Localized RNAs establish axial pattern formation and act as cell fate determinants during development in, for example, *Caenorhabditis elegans* (Kemphues, 2000), *Drosophila* (Lasko et al., 1999), zebrafish (Suzuki et al., 2000) and *Xenopus* (King et al., 1999; Alarcon and Elinson, 2001). Localized RNAs also contribute to maintaining the specialized characteristics of differentiated somatic cells such as neurons, oligodentrocytes and fibroblasts (Bashirullah et al., 1998). Our RNA in situ hybridization results show a highly restricted concentration of AKAP95 mRNA near the meiotic spindle in mature mouse oocytes. Whether the AKAP95 message is associated with spindle microtubules, and the fate of the mRNA during resumption of meiosis remain undetermined at present. It would be interesting to determine whether the RNA remains in the oocyte cytoplasm upon second polar body emission; in particular since no AKAP95 protein was detected in polar bodies (see, for example, Fig. 1D,E and Fig. 2A). Upon formation of the FPN, AKAP95 translation may take place concomitant with, or shortly after, disassembly of the meiotic spindle. The protein may either be engulfed in the nucleus by

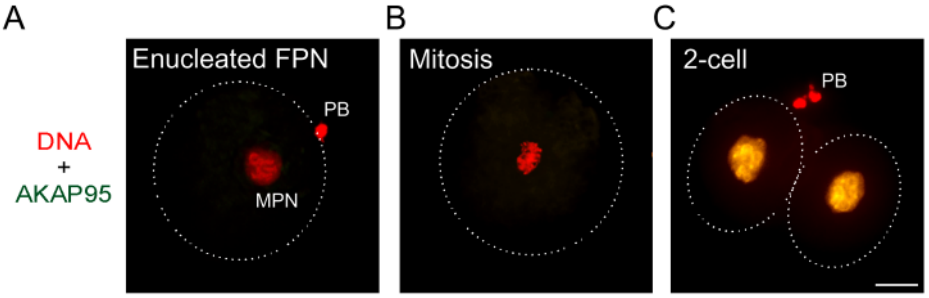

Fig. 7. Male chromosomes condense and segregate at mitosis in the absence of AKAP95. (A) The FPN of normal fertilized PN stage embryos was mechanically removed and (B) resulting embryos were allowed to enter mitosis and (C) cleave to the two-cell stage. Indicated stages were examined by DNA staining with PI (red) and immunofluorescence analysis of AKAP95 (green). Merged fluorescence images are shown. Dotted circles delineate embryos or blastomeres. PB, polar body. Bar, 20 μm.

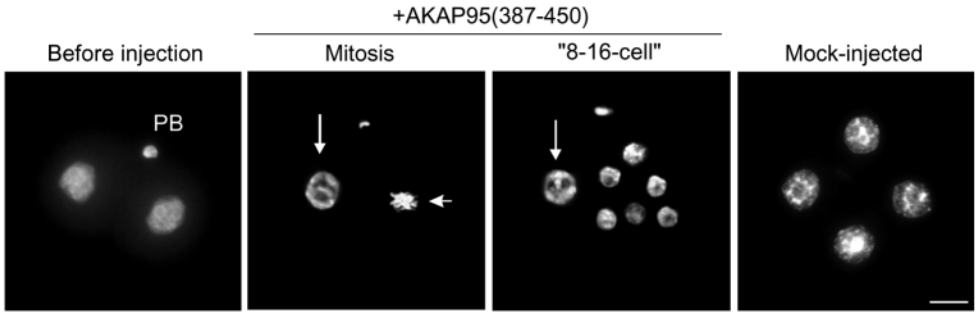

Fig. 8. AKAP95 is required for cleavage beyond the two-cell stage. The nucleus of one blastomere of a two-cell stage embryo was injected with 250 pg AKAP95(387-450) in a buffer containing a 150 kDa FITC-dextran as a tracer (arrow), while the second blastomere was injected with unlabeled peptide buffer. Each nucleus of control two-cell stage embryos was also mock-injected with buffer (Mock-injected, right panel). Embryos were allowed to cleave to the 8-16-cell stage. Nuclei were labeled with Hoechst 33342. Arrowheads point to a metaphase plate in the cleaving buffer-injected blastomere (Mitosis). PB, polar body. Bar, 20 μm.

the targeted nuclear membrane precursors or selectively imported in the FPN. This remains to be investigated. In any event, restriction of AKAP95 mRNA to the meiotic spindle may provide a regulatory mechanism for efficient AKAP95 targeting to the FPN upon activation.

Perhaps as a consequence of association with the FPN, AKAP95 specifically translocates to maternal chromosomes at first mitosis. This observation is reminiscent of the prompt redistribution of AKAP95 from the nuclear matrix to chromatin prior to, or during, somatic nuclear envelope breakdown in mitotic extract (Steen et al., 2000). Thus, AKAP95 may simply not be available to paternal chromatin. This view is supported by the binding of AKAP95 peptides to male chromatin when made available by co-incubation with sperm chromatin (our unpublished results) or by injection into the oocyte cytoplasm or into the MPN (this paper). High affinity association of AKAP95 with maternal chromosomes may prevent its release and binding to the closely apposed paternal chromosomes; we are currently exploring this possibility. Selective targeting of AKAP95 to maternal chromatin at first mitosis may have implications on the extent or kinetics of female chromosome condensation. For example, maternal chromosomes appear more compact than paternal chromosomes in mitotic zygotes (Donahue, 1972; Kaufman, 1973; Dyban and Sorokin, 1983). A correlation between the extent of compaction and resolution of HeLa chromosomes in mitotic extract and the amount of AKAP95(387-692) peptide bound to chromatin was shown previously, and this paralleled increasing amounts of hCAP-D2 recruited to the chromosomes (Steen et al., 2000). Thus, the different extent of compaction of male and female chromosomes at first mitosis may relate to the absence or presence of AKAP95.

A second issue is how mCAP-D2 and, potentially, the entire condensin complex, are recruited to paternal chromatin independently of AKAP95. Condensins may be targeted to male chromosomes via a putative loading factor distinct from AKAP95 (Kimura et al., 2001) or by binding DNA directly. Condensin association with chromatin has been proposed to involve a binding of SMCs to DNA stabilized by the non-SMC subunits (Hirano and Hirano, 1998; Akhmedov et al., 1998; Kimura et al., 2001). Condensins may also have a higher affinity for DNA modifications (Howlett and Reik, 1991) or histone alterations (Adenot et al., 1997) that characterize male chromatin. Both these modifications are expected to alter chromatin conformation. The higher affinity of SMCs for DNA sequences (such as AT-rich regions) with a propensity to form secondary structures (Kimura and Hirano, 1997; Akhmedov et al., 1998) supports this hypothesis.

A question that remains to be addressed is how condensation of foreign chromatin, such as that of a somatic nucleus, is regulated in the mature oocyte cytoplasm following somatic nuclear transplantation (‘cloning’). A consistent phenomenon in nuclear transfer embryos is the premature condensation of the transplanted chromatin into a metaphase-like conformation in the MII oocyte cytoplasm (e.g. Collas and Robl, 1991). As AKAP95 is present in somatic cell nuclei (Fig. 1), it is anticipated that it remains associated with the condensing chromosomes. Whether it is required for condensation is not yet known; however, studies of somatic chromosome condensation in mitotic HeLa cell extracts suggest that AKAP95 function is necessary for condensation to take place (Collas et al., 1999). It would be interesting to examine somatic chromosome condensation requirements in a mammalian meiotic egg extract.

Genetic, nuclear transplantation and pathological evidence suggests that normal mammalian development requires the contribution of maternal and paternal genomes (McGrath and Solter, 1984; Cattanach and Kirk, 1985; Surani et al., 1986; Lalande, 1996). These may function in distinct entities during preimplantation development when extensive epigenetic remodeling and nuclear reprogramming take place. This view is supported by the topological separation of the two genomes at first mitosis (this paper) and up to the four-cell stage, as shown by BrdU labeling of sperm and differential heterochromatin staining in mouse interspecific hybrid embryos (Mayer et al., 2000). Complementarity of genome function is reflected by distinct patterns of expression of maternally and paternally derived alleles of imprinted genes (Tilghman, 1999). In addition, relative to the FPN, the MPN displays earlier initiation of replication and transcription (Bouniol-Baly et al., 1997), greater transcriptional activity (Adenot et al., 1997; Aoki et al., 1997) and an enrichment in hyperacetylated histone H4 (Adenot et al., 1997). The FPN harbors AKAP95, a major component of the nuclear matrix and chromatin interface. Interestingly, AKAP95 is found in hypoacetylated chromatin in mouse cumulus cells and fibroblasts, as shown in chromatin immunoprecipitation studies using an anti-acetylated histone H4 antibody (our unpublished results). This suggests that at least a fraction of AKAP95 is enriched in transcriptionally silent chromatin. Collectively, these observations raise the possibility that AKAP95 is involved in imposing a repressive chromatin structure, or acts as an anchoring protein for chromatin

remodeling complexes or transcriptional regulators. It is tempting to speculate that implications of AKAP95 association with the maternal genome after fertilization may extend beyond a role in the regulation of mitotic chromosome condensation.

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References

Adenot, P., Mercier, Y., Reinsch, S. and Thompson, E. M. (1997). Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos. *Development* **124**, 4615-4625.

Akhmedov, A. T., Frei, C., Tsai-Pflugfelder, M., Kemper, B., Gasser, S. M. and Jessberger, R. (1998). Structural maintenance of chromosomes protein C-terminal domains bind preferentially to DNA with secondary structure. *J. Biol. Chem.* **273**, 24088-24094.

Alarcon, V. B. and Elinson, R. P. (2001). RNA anchoring in the vegetal cortex of the *Xenopus* oocyte. *J. Cell Sci.* **114**, 1731-1741.

Aoki, F., Worrad, D. M. and Schultz, R. M. (1997). Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev. Biol.* **181**, 296-307.

Bashirullah, A., Cooperstock, R. L. and Lipshitz, H. D. (1998). RNA localization in development. *Annu. Rev. Biochem.* **67**, 335-394.

Bouniol-Baly, C., Nguyen, E., Besombes, D. and Debey, P. (1997). Dynamic organization of DNA replication in one-cell mouse embryos: relationship to transcriptional activation. *Exp. Cell Res.* **236**, 201-211.

Carr, D. W., Stofko-Hahn, R. E., Fraser, I. D., Bishop, S. M., Acott, T. S., Brennan, R. G. and Scott, J. D. (1991). Interaction of the regulatory subunit (RII) of cAMP-dependent protein kinase with RII-anchoring proteins occurs through an amphipathic helix binding motif. *J. Biol. Chem.* **266**, 14188-14192.

Cattanach, B. M. and Kirk, M. (1985). Differential activity of maternally and paternally derived chromosome regions in mice. *Nature* **315**, 496-498.

Ciemerych, M. A. and Czolowska, R. (1993). Differential chromatin condensation of female and male pronuclei in mouse zygotes. *Mol. Reprod. Dev.* **34**, 73-80.

Coghlan, V. M., Langeberg, L. K., Fernandez, A., Lamb, N. J. and Scott, J. D. (1994). Cloning and characterization of AKAP95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. *J. Biol. Chem.* **269**, 7658-7665.

Collas, P. and Aleström, P. (1998). Nuclear localization signals enhance germline transmission of a transgene in zebrafish. *Transgenic Res.* **7**, 303-309.

Collas, P. and Robl, J. M. (1991). Relationship between nuclear remodeling and development in nuclear transplant rabbit embryos. *Biol. Reprod.* **45**, 455-465.

Collas, P., le Guellec, K. and Tasken, K. (1999). The A-kinase anchoring protein, AKAP95, is a multivalent protein with a key role in chromatin condensation at mitosis. *J. Cell Biol.* **147**, 1167-1180.

Compton, D. A., Szilak, I. and Cleveland, D. W. (1992). Primary structure of NuMA, an intranuclear protein that defines a novel pathway for segregation of proteins at mitosis. *J. Cell Biol.* **116**, 1395-1408.

Donahue, R. P. (1972). Fertilization of the mouse oocyte: sequence and timing of nuclear progression to the two-cell stage. *J. Exp. Zool.* **180**, 305-318.

Dyban, A. P. and Sorokin, A. V. (1983). Comparison of the size of paternal and maternal homologous chromosomes during the first 2 cleavage divisions in mouse embryos. *Ontogenez* **14**, 238-246.

Eide, T., Coghlan, V., Orstavik, S., Holsve, C., Solberg, R., Skalhegg, B. S., Lamb, N. J., Langeberg, L., Fernandez, A., Scott, J. D. et al. (1998). Molecular cloning, chromosomal localization, and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95. *Exp. Cell Res.* **238**, 305-316.

Eide, T., Carlson, C. R., Tasken, K. A., Hirano, T., Tasken, K. and Collas, P. (2002). Distinct but overlapping domains of AKAP95 are implicated in chromosome condensation and condensin targeting. *EMBO Rep.* **3**, 433-437.

Giet, R. and Glover, D. M. (2001). *Drosophila* aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J. Cell Biol.* **152**, 669-682.

Hirano, M. and Hirano, T. (1998). ATP-dependent aggregation of single-stranded DNA by a bacterial SMC homodimer. *EMBO J.* **17**, 7139-7148.

Hirano, T. (2000). Chromosome cohesion, condensation, and separation. *Annu. Rev. Biochem.* **69**, 115-144.

Hirano, T., Kobayashi, R. and Hirano, M. (1997). Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a *Xenopus* homolog of the *Drosophila* Barren protein. *Cell* **89**, 511-521.

Howlett, S. K. and Reik, W. (1991). Methylation levels of maternal and paternal genomes during preimplantation development. *Development* **113**, 119-127.

Kaufman, M. H. (1973). Timing of the first cleavage division of haploid mouse eggs, and the duration of its component stages. *J. Cell Sci.* **13**, 553-566.

Kemphues, K. (2000). PARsing embryonic polarity. *Cell* **101**, 345-348.

Kimura, K. and Hirano, T. (1997). ATP-dependent positive supercoiling of DNA by 13S condensin: a biochemical implication for chromosome condensation. *Cell* **90**, 625-634.

Kimura, K., Hirano, M., Kobayashi, R. and Hirano, T. (1998). Phosphorylation and activation of 13S condensin by Cdc2 in vitro. *Science* **282**, 487-490.

Kimura, K., Rybenkov, V. V., Crisona, N. J., Hirano, T. and Cozzarelli, N. R. (1999). 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. *Cell* **98**, 239-248.

Kimura, K., Cuvier, O. and Hirano, T. (2001). Chromosome condensation by a human condensin complex in *Xenopus* egg extracts. *J. Biol. Chem.* **276**, 5417-5420.

King, M. L., Zhou, Y. and Bubunenko, M. (1999). Polarizing genetic information in the egg: RNA localization in the frog oocyte. *Bioessays* **21**, 546-557.

Lalande, M. (1996). Parental imprinting and human disease. *Annu. Rev. Genet.* **30**, 173-195.

Lasko, P. (1999). RNA sorting in *Drosophila* oocytes and embryos. *FASEB J.* **13**, 421-433.

Martins, S. B., Eide, T., Steen, R. L., Jahnsen, T., Skålhegg, B. S. and Collas, P. (2000). HA95 is a protein of the chromatin and nuclear matrix regulating nuclear envelope dynamics. *J. Cell Sci.* **113**, 3703-3713.

Mayer, W., Smith, A., Fundele, R. and Haaf, T. (2000). Spatial separation of parental genomes in preimplantation mouse embryos. *J. Cell Biol.* **148**, 629-634.

McGrath, J. and Solter, D. (1984). Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development in vitro. *Science* **226**, 1317-1319.

Schmiesing, J. A., Ball, A. R. J., Gregson, H. C., Alderton, J. M., Zhou, S. and Yokomori, K. (1998). Identification of two distinct human SMC protein complexes involved in mitotic chromosome dynamics. *Proc. Natl. Acad. Sci. USA* **95**, 12906-12911.

Schmiesing, J. A., Gregson, H. C., Zhou, S. and Yokomori, K. (2000). A human condensin complex containing hCAP-C-hCAP-E and CNAP1, a homolog of *Xenopus* XCAP-D2, colocalizes with phosphorylated histone H3 during the early stage of mitotic chromosome condensation. *Mol. Cell. Biol.* **20**, 6996-7006.

Stanker, L. H., Wyrobek, A., McKeown, C. and Balhorn, R. (1993). Identification of the binding site of two monoclonal antibodies to human protamine. *Mol. Immunol.* **30**, 1633-1638.

Steen, R. L. and Collas, P. (2001). Mistargeting of B-type lamins at the end of mitosis: implications on cell survival and regulation of lamins A/C expression. *J. Cell Biol.* **153**, 621-626.

Steen, R. L., Cubizolles, F., le Guellec, K. and Collas, P. (2000). A-kinase anchoring protein (AKAP)95 recruits human chromosome-associated protein (hCAP)-D2/Eg7 for chromosome condensation in mitotic extract. *J. Cell Biol.* **149**, 531-536.

Surani, M. A., Barton, S. C. and Norris, M. L. (1986). Nuclear transplantation in the mouse: heritable differences between parental genomes after activation of the embryonic genome. *Cell* **45**, 127-136.

Suzuki, H., Maegawa, S., Nishibu, T., Sugiyama, T., Yasuda, K. and Inoue, K. (2000). Vegetal localization of the maternal RNA encoding and EDEN-BP/Bruno-like protein in zebrafish. *Mech. Dev.* **93**, 205-209.

Tilghman, S. M. (1999). The sins of the fathers and mothers: genomic imprinting in mammalian development. *Cell* **96**, 185-193.