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Centromere-specifying nucleosomes persist in aging mouse oocytes in the absence of nascent assembly

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SUMMARY

Centromeres direct genetic inheritance but are not themselves genetically encoded. Instead, centromeres are defined epigenetically by the presence of a histone H3 variant, CENP-A[1]. In cultured somatic cells, an established paradigm of cell cycle-coupled propagation maintains centromere identity: CENP-A is partitioned between sisters during replication and replenished by new assembly, which is restricted to G1. The mammalian female germline challenges this model because of the cell cycle arrest between pre-meiotic S-phase and the subsequent G1, which can last for the entire reproductive lifespan (months to decades). New CENP-A chromatin assembly maintains centromeres during prophase I in worm and starfish oocytes[2,3], suggesting that a similar process may be required for centromere inheritance in mammals. To test this hypothesis, we developed an oocyte specific conditional knockout mouse for *Mis18α*, an essential component of the assembly machinery. We find that embryos derived from *Mis18α* knockout oocytes fail

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AUTHOR CONTRIBUTIONS:

A.D., M.A.L., and B.E.B. designed the project and wrote this manuscript with input from co-authors. M.A.L. and B.E.B. provided supervision and sourced funding. M.A.L. and B.E.B. and K.T. conceived the project. A.D. performed all the experiments and analyzed the data. A.D. and K.G.B. maintained mouse colony, genotyping, and quantified the data. S.B. and K.T. provided the *Mis18α* knockout mouse line and K.T. did initial experimentation.

DECLARATION OF INTERESTS:

The authors declare no competing interests.

INCLUSION AND DIVERSITY

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to assemble CENP-A nucleosomes prior to zygotic genome activation, validating the knockout model. We show that deletion of Mis18α in the female germline at the time of birth has no impact on centromeric CENP-A nucleosome abundance even after 6–8 months of aging. In addition, there is no detectable detriment to fertility. Thus, centromere chromatin is maintained long-term independent of new assembly during the extended prophase I arrest in mouse oocytes.

eTOC Blurb

In brief

To test if centromeres are maintained by new assembly in aging mouse oocytes, Das *et al.* conditionally delete *Mis18α*, a centromere assembly component. They find that centromere-specifying nucleosomes containing the histone H3 variant, CENP-A, are stably maintained even after 6–8 months of aging, with no detriment to fertility.

Keywords

centromeres; meiosis; reproductive aging; oocytes

RESULTS AND DISCUSSION

In contrast to genetic information encoded in our genome, minimal epigenetic information is inherited because most parental epigenetic marks are removed and reprogrammed in germ cells and the early embryo[4,5]. A key exception is the centromeric histone H3 variant, CENP-A[6–8]. Although centromeres direct the process of genetic inheritance by connecting chromosomes to spindle microtubules, they are not encoded in DNA, but rather epigenetically specified by nucleosomes containing CENP-A[9–12]. Thus, CENP-A nucleosomes are inherited to preserve centromere identity.

Studies in cycling somatic cells have established a general paradigm for propagation of epigenetic information between cell cycles. DNA or histone modifications are partitioned between sister chromatids during DNA replication, and then replenished by “reader” proteins that recognize the modification and “writers” that extend it to adjacent nucleosomes[13]. CENP-A, follows this paradigm, with new assembly restricted to G1 by CDK1/2 activity[14–16]. CENP-A and its bound partner histone H4 molecules are remarkably stable in tissue culture cells[17,18], with measured CENP-A turnover rates explained entirely through the dilution when existing CENP-A is partitioned to replicated centromeric DNA during S-phase each cell cycle[19–21]. This paradigm poses a challenge in the mammalian female germline because of the extended prophase I cell cycle arrest, after replication but before an opportunity for new G1 assembly, which can last for the entire reproductive lifespan of the animal[22] (Figure 1A). Centromeres are preserved throughout the arrest (>1 year in mouse) in the absence of new *Cenpa* transcription, as shown by conditional knockout of the *Cenpa* gene[23]. CENP-A nucleosomes are therefore either replenished by new assembly in contrast to the somatic cell paradigm, drawing on a stable pool of CENP-A protein, or stable for the entire duration of the arrest (Figure 1B).

Studies in worm and starfish oocytes show nascent CENP-A chromatin assembly in prophase I (akin to a G2 biochemical cell cycle state in a somatic cell)[2,3]. In mouse, continual deposition of nucleosomes containing another H3 variant, H3.3, during oocyte development is required for oocyte genome integrity and, ultimately, for fertility[24]. These studies suggest that new assembly may maintain CENP-A chromatin through the prophase I arrest. To test this prediction, we created an oocyte specific conditional knockout (cKO) of an essential component of the CENP-A deposition machinery, Mis18α (Mis18a in mouse but referred to as Mis18α for simplicity)[25–29,31], to prevent nascent CENP-A chromatin assembly. Mis18α is part of the Mis18 complex, which recruits the CENP-A chaperone, HJURP, bound to nascent CENP-A/histone H4 dimers[30,32–34], to centromeres. The Mis18 complex is required for both nascent CENP-A chromatin assembly in G1 and replication-coupled CENP-A chromatin assembly in S phase in cycling somatic cells[21,35]. Specifically relevant to our test of ongoing chromatin assembly in the mouse oocyte, prophase I assembly in both worms and starfish oocytes requires the Mis18 complex[2,3]. Thus, if CENP-A chromatin is replenished by new assembly, CENP-A nucleosome levels would decay in *Mis18α* knockout oocytes.

For conditional knockout, we used a floxed *Mis18α* allele in which the first two exons, encoding the YIPPEE domain necessary for CENP-A deposition, are flanked by *LoxP* sites[36,37] (Figure 1C, S1). To confirm that Cre-mediated excision generates a null allele, we crossed *Mis18α* heterozygous parents and did not recover any progeny homozygous for the deletion, as expected because *Mis18α* is an essential gene[36] (Figures 1D, S2). Combining the floxed allele with *Cre* recombinase driven by oocyte specific promoters, we generated cKOs of *Mis18α* either early or late in prophase I (Figure S2). The early *Cre* driver (*Gdf9-Cre*) deletes *Mis18α* in arrested oocytes 2 days post birth, preventing assembly of new CENP-A nucleosomes for nearly the entire lifespan of the animal[38]. The late *Cre* driver (*Zp3-Cre*) deletes the gene during oocyte growth, 2–3 weeks prior to ovulation[38] (Figure 2A).

As a functional assay for *Mis18α* deletion, we tested for CENP-A chromatin assembly in embryos from late KO mothers (driven by *Zp3-Cre*) and a wild type father (Figure 2B). Prior to zygotic genome activation (ZGA) at the two-cell stage embryos, CENP-A deposition during the early embryonic mitotic cycles depends solely on maternal Mis18α protein. To assess new chromatin assembly, we injected mRNAs encoding CENP-A-eGFP and H2B-mCherry into one-cell embryos derived from either KO mothers or wild type mothers as controls (Figure 2B). H2B-mCherry serves as a positive control for chromatin assembly because it utilizes a deposition pathway distinct from that of CENP-A and therefore does not require the Mis18 complex. Based on the paradigm established in cycling somatic cells, we expected new CENP-A chromatin assembly in G1 after the first embryonic mitosis. Any Mis18α protein present in the embryo prior to ZGA would be solely contributed maternally from the oocyte. We expect embryos derived from control oocytes would be assembly proficient whereas embryos from KO oocytes lacking Mis18α would fail to assemble. Indeed, CENP-A-eGFP localized to centromeres in all control embryos (100%, N = 19) from wild type mothers. In contrast, none of the embryos from late *Mis18α* KO mothers (0%, N = 20) contained detectable centromeric CENP-A-eGFP (Figure 2C). H2B-mCherry was present in chromatin in 100% of both control and KO injected embryos as expected.

The all-or-none effect of *Mis18α* KO is due to the high efficiency of Cre excision and the relatively fast turnover of *Mis18α* mRNA in oocytes following excision (Figure S3). This finding establishes that even after a relatively short duration KO, *Mis18α* deletion in oocytes abrogates nascent CENP-A chromatin assembly in early embryos.

Next, we measured fertility of *Mis18α* KO mothers as the ultimate test of centromere function, because centromeres are required for the meiotic divisions and for maternal centromere inheritance, and even partial reduction of maternal CENP-A chromatin lowers fertility[8]. All *Mis18α* early and late KO mothers were fertile when crossed to wild type males (Figure 3A). We also confirmed that both *Cre* recombinases have a 100% deletion efficiency as we did not recover a floxed allele of *Mis18α* inherited from a cKO mother (Figure 3B, C). Combined with our embryo experiments, this result confirms that every oocyte with a combination of floxed alleles and *Cre* lacks Mis18α protein and that fertility is not a consequence of inefficient *Cre* activity in some oocytes. Thus, centromere identity is maintained in aging oocytes without Mis18α protein or nascent CENP-A assembly.

Even though the cKO mice are fertile, there could still be a reduction in CENP-A nucleosomes over time. Therefore, we tested if CENP-A levels decay in the late KO oocytes. We predict that if CENP-A nucleosomes are continually replenished by new assembly from a stable pool of CENP-A protein, centromeric CENP-A levels would decline in the KO oocytes compared to control oocytes. We did not see any reduction in centromeric CENP-A levels in late KO oocytes compared to controls (Figures 4A, B). However, *Mis18α* is deleted for only 2–3 weeks before ovulation in the late KO, leaving open the possibility of a more dramatic reduction in CENP-A nucleosomes on longer timescales.

Thus, we leveraged the early KO (*Gdf9-Cre*) oocytes that delete *Mis18α* shortly after birth, to test if centromeric CENP-A levels decline after months without new assembly (Figures 4C, D). We aged control and early KO females for 6–8 months, representing most of the fertile lifespan, and found that CENP-A levels were not significantly reduced compared to the controls. Although not statistically significant, we measured a ~3% decrease in CENP-A levels in the KO oocytes relative to control (Figure 4D) that equates to loss at a rate of ~0.02% per day over 180 days. Such loss in signal would be in the range of one nucleosome per month, assuming ~200 CENP-A nucleosomes per centromere as estimated in human cells[19]. In comparison, new assembly in starfish oocytes is estimated at a rate of 2% per day, based on centromere localization of GFP-tagged exogenous CENP-A in cells cultured *in vitro* for 10 days[3]. Our result is consistent with our previous study, where we conditionally deleted the *Cenpa* gene using *Gdf9-Cre* and followed CENP-A nucleosome stability further and measured no substantial decrease[23].

CONCLUSION

In conclusion, these results provide clear evidence supporting long term retention of CENP-A nucleosomes assembled prior to birth as the dominant pathway for maintaining centromere identity in mammalian oocytes. This is in stark contrast to H3.3, whose ongoing deposition into bulk chromatin during prophase I is essential for normal oocyte chromatin structure and fertility[24]. In addition, our findings have implications for human female

meiosis, which is inherently error prone and especially vulnerable to aging. With advancing maternal age at childbirth, mechanisms that preserve centromeres in aging oocytes gain increasing significance. Although maternal knockout of *Mis18α* in mice preserves fertility due to early zygotic genome activation, maternal depletion of *Mis18α* is expected to have more severe consequences in human embryos, where activation occurs later. Our findings can now direct future research into the mechanisms that underlie CENP-A retention in mammalian oocytes. Previous studies of centromere chromatin suggest multiple potential molecular mechanisms that could contribute to its stability: the relatively low level of transcription of centromeric DNA[39–42] relative to genic regions harboring histone H3.3 nucleosomes[43]; structural features that differ from canonical nucleosomes, including internal structural rigidity at the CENP-A/histone H4 interface[44,45]; and non-histone CCAN components that bind and stabilize CENP-A nucleosomes in tissue culture cells[17,18,46]. In sum, it remains to be seen if the mechanisms that function to retain CENP-A chromatin over short periods of time in cycling cells also contribute to extreme stability during oogenesis.

STAR METHODS:

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ben E. Black (blackbe@pennmedicine.upenn.edu).

Materials availability—Mouse lines generated in this study are available upon request.

Data and code availability—All imaging data reported in this paper will be shared by the lead contact upon request. This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All animal experiments and protocols were approved by the Institutional Animal Use and Care Committee of the University of Pennsylvania and were consistent with National Institutes of Health guidelines (protocol #803994). Experimental animals were compared to age, gender and genetic background matched controls. The *Mis18a*$^{fl/fl}$ strain is a previously published and validated knockout strain[36]. This strain is in a mixed genetic background of C57BL6/J/129Sv/CBA/J (generated by Sung Hee Baek, Seoul National University and obtained from Kikue Tachibana, Max Planck Institute). Oocyte specific conditional knockout strains *Mis18a*$^{fl/fl}$*;Zp3-Cre* (*Mis18α* late KO) and *Mis18a*$^{fl/fl}$*;Gdf9-iCre* (*Mis18α* early KO) were generated by crossing the original *Mis18a*$^{fl/fl}$ strain to either *C57BL/6-Tg(Zp3-cre)93Knw/J* (RRID:IMSR_JAX:003651, Jackson Laboratory) or *Tg(Gdf9-icre)5092Coo/J* (RRID:IMSR_JAX:011062, Jackson Laboratory). The following primers were used to genotype the animals: 1) *Mis18a*$^{fl/fl}$: SDL-Forward: TGC CTA TTG GTG TAC CTT CCA GTG, LOXP-Reverse: CCT AAG TCG TTG ACC TGA CCG AGG, 2) *Mis18a*$^{fl/Δ}$: Mis18-DeltaAT2 Reverse: GGA CAG GAA TAG GAC ACT TTC

AAC combined with SDL-Forward (Figure S1). For testing fertility, age matched single conditional knockout (*Mis18α* early or late KO) or control mothers (*Mis18a*$^{fl/fl}$) were mated in cages to single males (either *Mis18a*$^{fl/fl}$ or *Mis18a*$^{fl/fl}$). Litter sizes were determined for multiple mating pairs per cross. Oocytes and embryos were collected from multiple mothers for all experiments.

METHOD DETAILS

Oocyte collection, meiotic maturation, and culture—Female mice were hormonally primed with 5 U of pregnant mare serum gonadotropin (PMSG, Peptides International) 44–48 h before oocyte collection. Germinal vesicle intact oocytes were collected in bicarbonate-free minimal essential medium (M2, Sigma), denuded from cumulus cells, and cultured in Chatot–Ziomek–Bavister[47] (CZB, Fisher Scientific) medium covered with mineral oil (Sigma, BioXTRA) in a humidified atmosphere of 5% CO_2 in air at 37 °C. During collection, meiotic resumption was inhibited by addition of 2.5 mM milrinone (Sigma). Milrinone was subsequently washed out to allow meiotic resumption and oocytes were fixed 6–7 h later at metaphase I.

Oocyte immunocytochemistry—Oocytes were fixed in 2% paraformaldehyde (Sigma) in phosphate buffered saline (PBS) with 0.1% Triton X-100 (Sigma), pH 7.4, for 20 min at room temperature (r.t.), permeabilized in PBS with 0.2% Triton X-100 for 15 min at r.t., placed in blocking solution (PBS containing 0.3% bovine serum albumin (BSA) and 0.01% Tween-20) for 20 minutes at r.t., treated with λ-phosphatase (1,600 U, NEB) for 1 h at 30 °C for CENP-A staining, incubated for 1 h with primary antibody in blocking solution, washed three times for 10 min each, incubated for 1 h with secondary antibody, washed three times for 10 min each, and mounted in Vectashield with 4′,6-diamidino-2-phenylindole (DAPI; Vector) to visualize the chromosomes. The primary antibody was rabbit anti-mouse CENP-A (1:200, Cell Signaling, C51A7). The secondary antibody was donkey anti-rabbit Alexa Fluor 488 (1:500, Invitrogen).

Microscopy—Confocal images were collected as *z*-stacks with 0.5-μm intervals, using a microscope (DMI4000 B; Leica) equipped with a 63× 1.3-NA glycerol-immersion objective lens, an *x*–*y* piezo Z stage (Applied Scientific Instrumentation), a spinning disk confocal scanner (Yokogawa Corporation of America), an electron-multiplying charge-coupled device camera (ImageEM C9100–13; Hamamatsu Photonics) and either an LMM5 (Spectral Applied Research) or Versalase (Vortran Laser Technology) laser merge module, controlled by MetaMorph software (Molecular Devices, v7.10.3.294). Images were acquired using the same laser settings and all images in a panel were scaled the same. Single channels are shown wherever quantifications were performed.

Embryo collection, microinjection and culture—*Mis18a*$^{fl/fl}$ or *Mis18a*$^{fl/fl}$*;Zp3-Cre* (*Mis18α* late KO) females were hormonally primed with 5 U of PMSG (Peptides International) and the oocytes were matured *in vivo* with 5 U of human chorionic gonadotropin (hCG; Sigma) before mating with B6D2F1/J males (F1 hybrid of a cross between C57BL6/J and DBA2/J; RRID:IMSR_JAX:100006, Jackson Laboratory). Males were fed a special low soymeal diet (5LG4 irradiated diet, Labdiet) and housed singly.

Embryos were collected 14–16 h post hCG in M2 containing hyaluronidase (0.3 mg ml^{-1}) to remove cumulus cells and subsequently washed in M2 (Sigma). and cultured in EmbryoMax Advanced KSOM (AKSOM, Millipore Sigma) with humidified air and 5% CO_2.

Embryos were then subjected to inter-cytoplasmic microinjection in M2 medium covered with mineral oil (Sigma, BioXtra) at r.t. with a micromanipulator (Narishige) and a picoinjector (Medical Systems Corp). Each embryo was injected with 2 pl of cRNA, then cultured in EmbryoMax Advanced KSOM (AKSOM, Millipore Sigma) with humidified air and 5% CO_2 until 2 cell stage and fixed in 2% paraformaldehyde. The following cRNAs were used for microinjection: H2B-mCherry (human histone H2B with mCherry at the C-terminus) at 25 ng/ul, CENP-A-EGFP (mouse CENP-A with EGFP at the C-terminus) at 20 ng/ul. The cRNAs were synthesized using the T7 mScript Standard mRNA kit (Thermo Fisher Scientific) and purified by phenol-chloroform extraction.

mRNA quantification in oocytes—Total RNA was extracted from at least 20 full-grown oocytes from two females each for control and late conditional knockout (*Zp3-Cre* mediated) using Arcturus Picopure RNA isolation kit (Thermofisher Scientific), and cDNA was prepared by reverse transcription of total RNA with Superscript III First Strand Synthesis system (Thermofisher) using oligo dT primers. *Mis18α* was amplified for standard PCR using Kapa polymerase (Roche) from 1 μg of cDNA. Real time PCR was performed using *Mis18α* Taqman probes and H2A serving as the endogenous control. Each sample was run twice in triplicate. Quantification was performed using the comparative Ct method (Livak method) on an Applied Biosystems ViiA 7 machine.

Quantification and statistical analysis—To quantify centromere signal ratios, a sum intensity Z-projection was made using Fiji/ImageJ software. Circles of constant diameter were drawn around individual centromeres and the average intensity was calculated for each centromere after subtracting background, obtained from nearby regions. Raw centromere intensities were obtained from several controlled independent experiments and multiple cells were analyzed from each animal. Normalization of centromere intensities was performed using age- and gender-matched controls for each independent experiment. Statistical tests (Mann-Whitney U test) were performed using the Graphpad Prism software. Details of the p-values and error bars are provided in figure legends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Genetic removal of *Mis18α* tests current models for centromere inheritance in oocytes
- Mis18a removal has no impact on CENP-A nucleosome levels in aging oocytes
- Mis18a removal disrupts CENP-A nucleosome assembly in the early embryo
- CENP-A assembled prior to birth is sufficient for fertility in aging mice

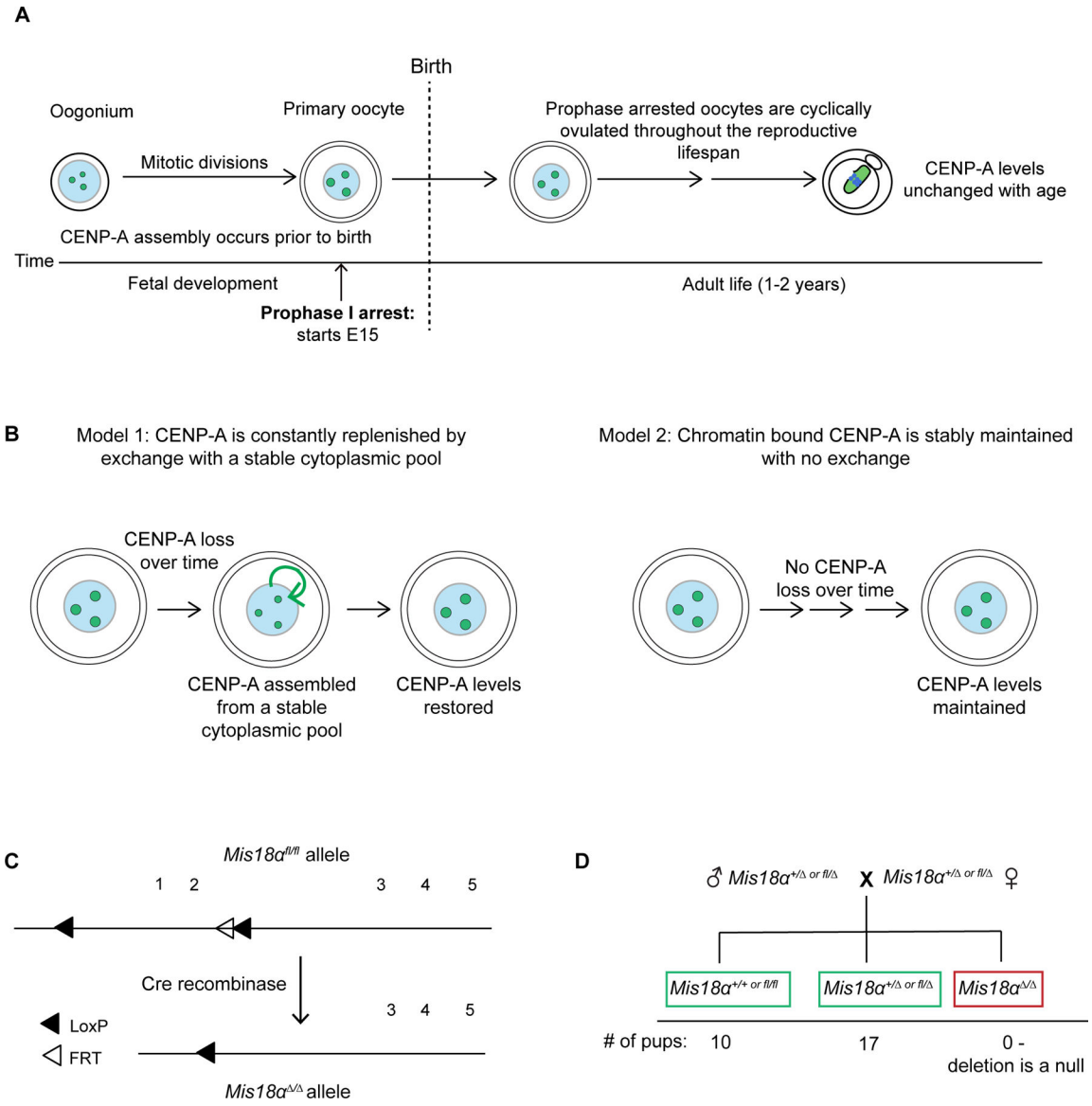


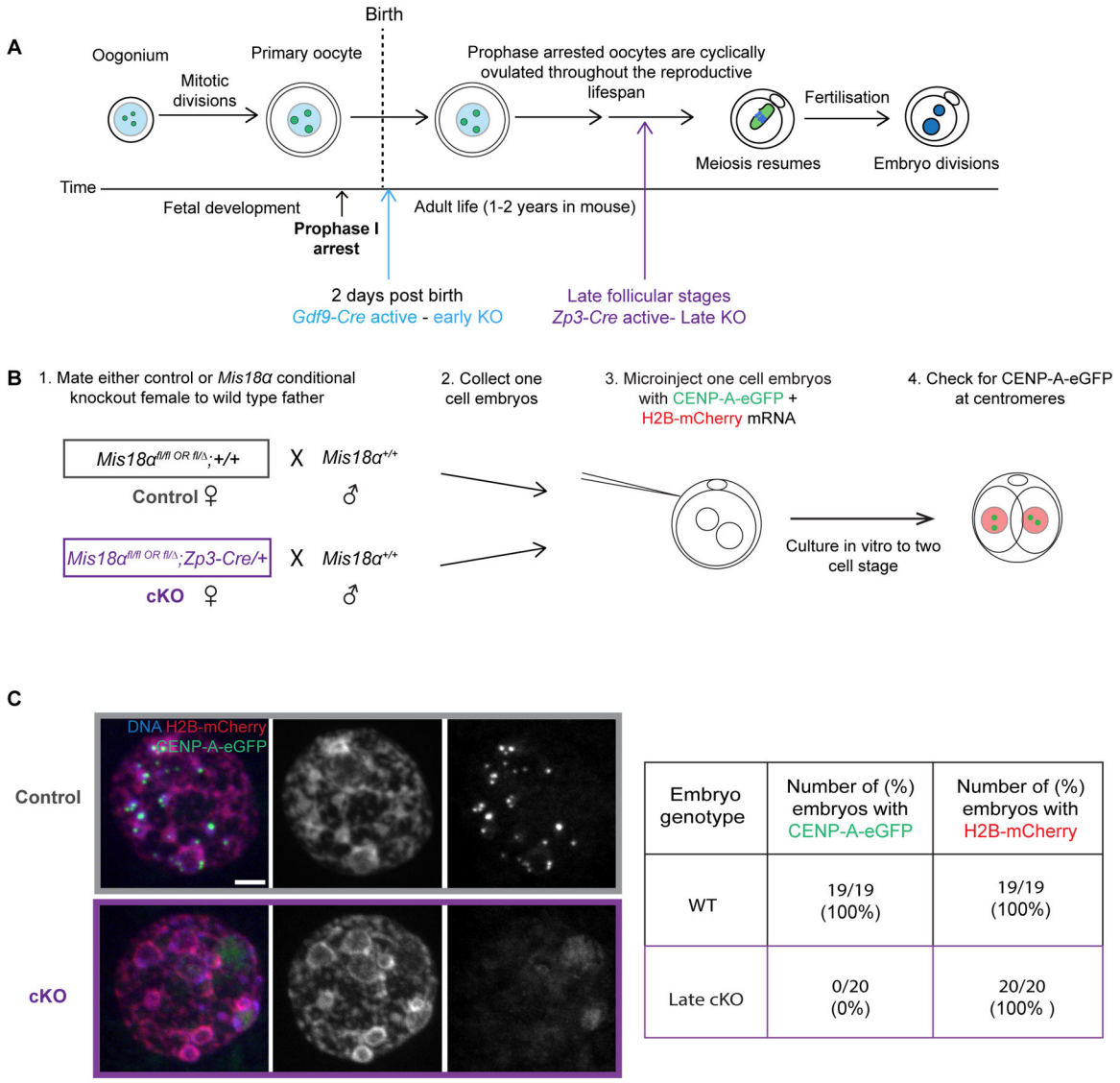
Figure 1: Testing models for stable retention of CENP-A chromatin in oocytes

(A) Schematic showing maintenance of CENP-A chromatin across the oogenesis timeline. Oocytes arrested in prophase I are cyclically recruited to begin growth before ovulation and maturation. Prophase arrest prior to recruitment can last up to 2 years in mice. Centromeric CENP-A levels are stable over time even if the *Cenpa* gene is eliminated shortly after birth[23].

(B) Models for retention of CENP-A in meiotic prophase I arrested eggs.

(C) Schematic of the *Mis18α* conditional knockout gene locus[36]. The 1st and 2nd *Mis18α* protein coding exons are flanked by LoxP sites (floxed, fl). The FRT site is a remnant from FLP mediated excision of the Neomycin cassette in the original construct used to generate the KO animals (see Figure S1 for genotyping).

(D) Genotype frequencies of the progeny from a cross of *Mis18α* $^{+/\Delta \text{ or } fl/\Delta}$ heterozygotes (See Figure S2 for generation of heterozygotes). Number of litters = 6, number of pups = 27.



Embryo genotype	Number of (%) embryos with CENP-A-eGFP	Number of (%) embryos with H2B-mCherry
WT	19/19 (100%)	19/19 (100%)
Late cKO	0/20 (0%)	20/20 (100%)

Figure 2: Maternally deposited Mis18α protein is eliminated in late KO oocytes

(A) Schematic showing early and late *Mis18α* knockout depending on the timing of *Cre* recombinase expression driven by either *Gdf9* or *Zp3* promoters (colored arrows).

(B) Experimental design to test for a stable pool of Mis18α protein in the maternal cytoplasm. CENP-A-eGFP and H2B-mCherry mRNA are injected into one cell embryos, and CENP-A foci are assessed after the first embryonic mitosis when assembly is expected to occur but before zygotic genome activation.

(C) Images show H2B-mCherry, CENP-A-eGFP, and DNA (DAPI) in interphase two cell embryos from control or cKO mothers. Table shows frequencies of detectable assembly for CENP-A-eGFP and H2B-mCherry, obtained from 2 independent matings. Scale bars = 5 μm. Also see Figure S2 and S3.

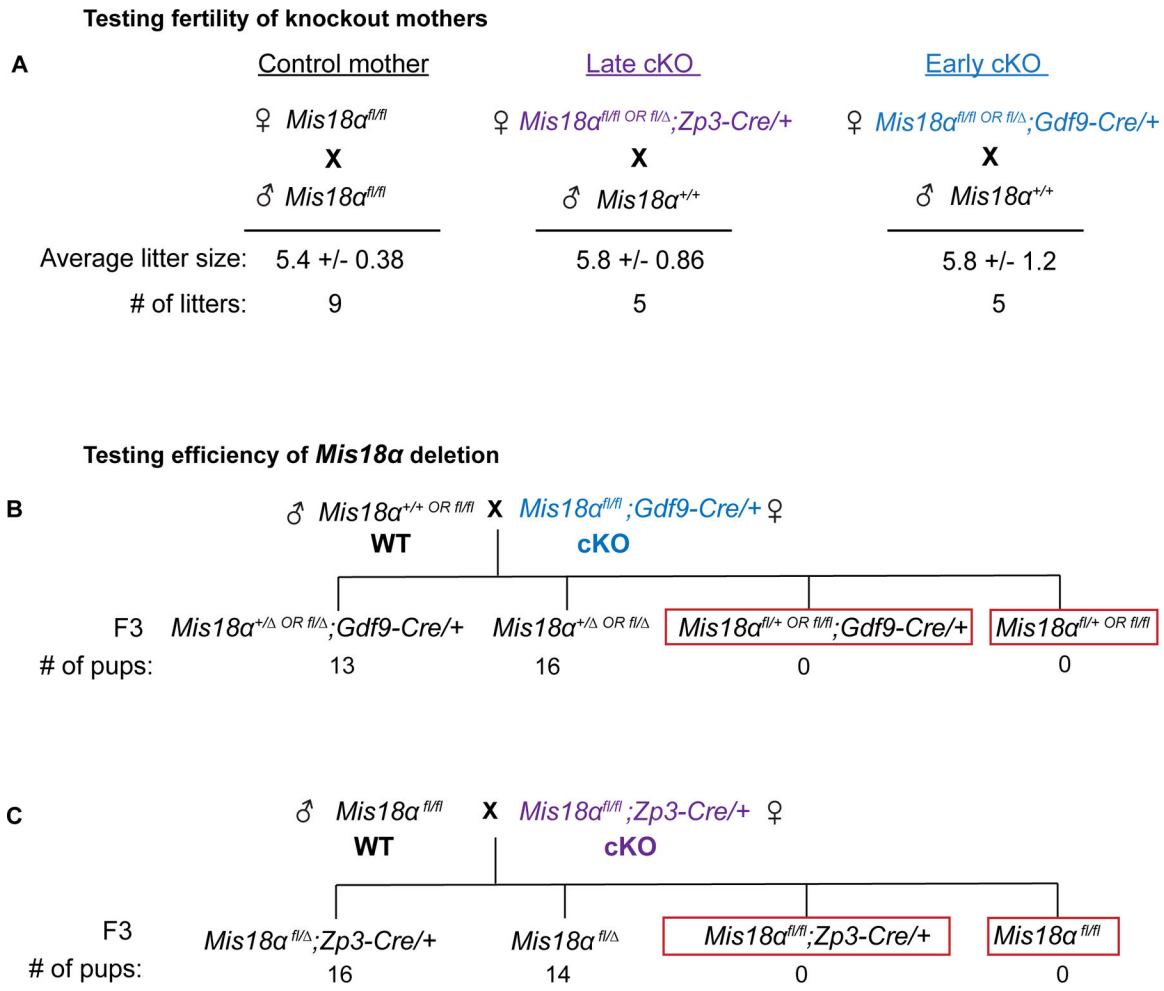


Figure 3: *Mis18α* knockout mothers can support fertility

(A) Litter sizes for control, early cKO, and late cKO mothers (age 2–4 months) crossed to wild type males. See Figure S2 for generating cKO mothers.

(B, C) Genotype frequencies of the progeny from a cross between a WT or *Mis18α*$^{fl/fl}$ father and an early or late cKO mother respectively (N= 29 pups, 5 litters; N = 30 pups, 5 litters).

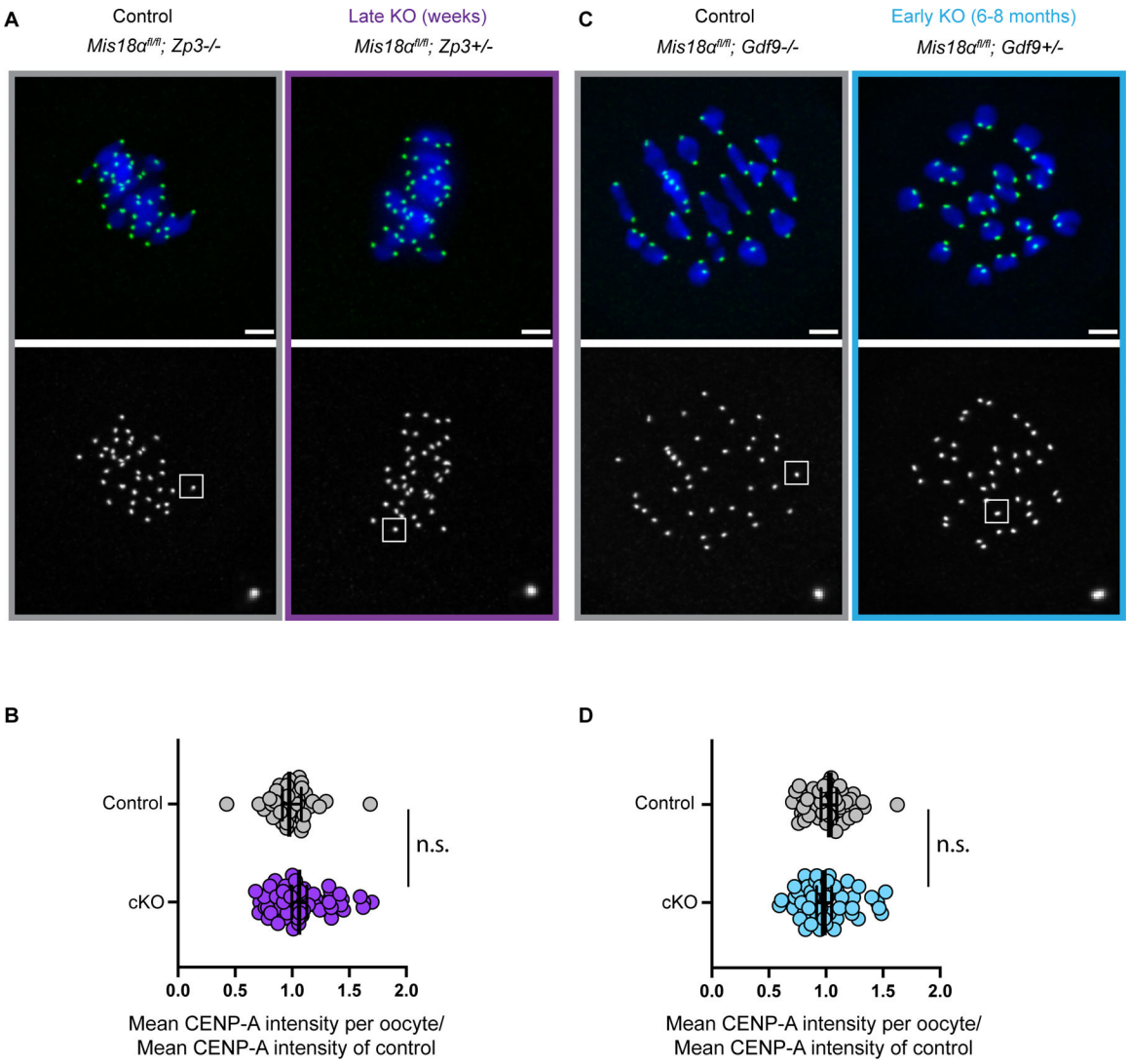


Figure 4: Pre-meiotically assembled CENP-A chromatin is maintained in prophase arrested oocytes without new assembly

(A, C) Images show metaphase I oocytes stained for CENP-A (green) and DNA (DAPI, blue) from late cKO (A) or early cKO (C) mothers. Scale bars = 5 μm.

(B, D) Quantifications of CENP-A intensities in metaphase I oocytes from late cKO (B) or early cKO (D) mothers. Each data point represents average CENP-A levels at centromeres in each oocyte normalized to the control mean. Late cKO (*Zp3-Cre,* purple): mean ± S.E.M. = 1.1 ± 0.034 compared to control, number of oocytes = 58, number of centromeres = 1512, N = 7 females) and *Cre* negative control oocytes (number of oocytes = 40, number of centromeres = 962, N = 5 females). Early cKO (*Gdf9-Cre,* blue): mean ± S.E.M. = 0.97 ± 0.068 (number of oocytes = 47, number of centromeres = 1746, N = 7 females) relative to control (number of oocytes = 50, number of centromeres = 2019, N = 8 females); n.s.: Mann-Whitney U Test. Error bars: geometric mean ± 95% confidence interval. The early cKO mothers are aged 6–8 months, and their oocytes lack Mis18α for the entire lifespan. Also see Figure S2 for generation of cKO animals.

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Anti-CENP-A (C51A7)	Cell Signaling	2048; RRID: AB_1147629
Alexa Fluor 488 conjugate donkey anti-rabbit IgG (H+L)	Molecular Probes (Invitrogen)	A-21206 RRID:AB_2535792
Chemicals, peptides, and recombinant proteins		
Lambda phosphatase	New England Biolabs	P0753L
Milrinone	Sigma	M4659
Mineral Oil (BioXtra)	Sigma	M8410
Mineral Oil (Fuji)	Fuji Film Irvine	9305-500 ml
Pregnant Mare Serum Gonadotropin (PMSG)	Peptides International	HOR_272-5000 IU
Human Chorionic Gonadotropin	Sigma	CG10-1VL
Vectashield Antifade (DAPI)	Vector Laboratories	H-1200
CZB	Fisher Scientific	MR-019-D
EmbryoMax M2 medium, with Phenol Red	Millipore Sigma	MR-015
Embryomax Advanced KSOM	Millipore Sigma	MR-101-D
Phenol (UltraPure buffer saturated)	Thermofisher Scientific	15513039
Dulbecco's PBS (without Ca and Mg)	Corning	MT21-031-CV
Triton X 100	Sigma	X100-500 ml
RedExtract N-Amp PCR ready Mix	Millipore Sigma	R4775
Kapa Polymerase Hifi Hotstart Ready Mix	Roche	KK2602
Taqman Fast Advanced Master Mix	Thermofisher Scientific	4444557
Extraction Solution	Millipore Sigma	E7526
Tissue Preparation Solution	Millipore Sigma	T3073
Neutralization Solution B	Millipore Sigma	N3910
1 Kb Plus ladder	Thermofisher	SM1333
Stellar Competent cells	Takara Bio	636766
Nucleospin Plasmid kit	Takara Bio	740588.250
Critical commercial assays		
T7 mScript Standard mRNA	Cell Script	C-MSC100625
Superscript III First Strand Synthesis	Thermofisher Scientific	18080051
Arcturus Picopure RNA Isolation Kit	Thermofisher Scientific	KIT0204
Taqman Assay (*Mis18α)*	Thermofisher Scientific	Mm01209645_m1
Taqman Assay (*Hist2h2)*	Thermofisher Scientific	Mm0051974_s1
Experimental models: Organisms/strains		
Mouse: *Mis18α*$^{fl/fl}$; B6-129-CBA-*Mis18a*$^{fl/fl}$	Kim et al, 2012[36]	N/A
Mouse: *Mis18α* early KO; B6-129-CBA- *Mis18a*$^{fl/fl}$*;Gdf9-Cre*	This paper	N/A
Mouse: *Mis18α* late KO; B6–129-CBA- *Mis18a*$^{fl/fl}$*;Zp3-Cre*	This paper	N/A
Mouse: *Zp3-Cre*; B6-*Tg(Zp3-cre)93Knw/J*	Jackson laboratory	RRID:IMSR_JAX:003651

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Mouse: *Gdf9-Cre*; B6-*Tg(Gdf9-icre)5092Coo/J*	Jackson laboratory	RRID:IMSR_JAX:011062
Oligonucleotides		
SDL-Forward: 5'-TGC CTA TTG GTG TAC CTT CCA GTG-3'	Kim et al, 2012[36]	N/A
LOX-Reverse: 5'-CCT AAG TCG TTG ACC TGA CCG AGG-3'	Kim et al, 2012[36]	N/A
Mis18-DeltaAT2: 5'-GGA CAG GAA TAG GAC ACT TTC AAC-3'	Kim et al, 2012[36]	N/A
Mis18F (exons 1–4): 5'-AGA AGT GGG CAA ACA TGT CG-3'	This paper	N/A
Mis18R (exons 1–4): 5'-GAG GAC CCT AAG GTG TAA CTT TCA A-3'	This paper	N/A
Mis18F (exons 2–4): 5'- AGC GTC TCC TGT AAC GTC TC-3'	This paper	N/A
Mis18R (exons 2–4): 5'-GTC AGG ACT TCT TCC ATC TGC TT-3'	This paper	N/A
Recombinant DNA		
CENPA-eGFP	Smoak et al, 2016[23]	N/A
H2B-mCherry	Akera et al, 2017[48]	N/A
Software and algorithms		
GraphPad Prism 9.3.1 (350)	GraphPad	http://www.graphpad.com/
FIJI/ImageJ	Schindelin et al, 2012[49]	https://fiji.sc/