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RESEARCH REPORT

Aurora kinase B inhibits aurora kinase A to control maternal mRNA translation in mouse oocytes

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ABSTRACT

Mammalian oocytes are transcriptionally quiescent, and meiosis and early embryonic divisions rely on translation of stored maternal mRNAs. Activation of these mRNAs is mediated by polyadenylation. Cytoplasmic polyadenylation binding element 1 (CPEB1) regulates mRNA polyadenylation. One message is aurora kinase C (*Aurkc*), encoding a protein that regulates chromosome segregation. We previously demonstrated that AURKC levels are upregulated in oocytes lacking aurora kinase B (AURKB), and this upregulation caused increased aneuploidy rates, a role we investigate here. Using genetic and pharmacologic approaches, we found that AURKB negatively regulates CPEB1-dependent translation of many messages. To determine why translation is increased, we evaluated aurora kinase A (AURKA), a kinase that activates CPEB1 in other organisms. We find that AURKA activity is increased in *Aurkb* knockout mouse oocytes and demonstrate that this increase drives the excess translation. Importantly, removal of one copy of *Aurka* from the *Aurkb* knockout strain background reduces aneuploidy rates. This study demonstrates that AURKA is required for CPEB1-dependent translation, and it describes a new AURKB requirement to maintain translation levels through AURKA, a function crucial to generating euploid eggs.

KEY WORDS: Aurora kinase, CPEB1, Oocyte, Meiosis, Translation, Mouse

INTRODUCTION

Oocyte meiotic maturation involves the integration of nuclear and cytoplasmic changes to prepare an egg for fertilization. Nuclear maturation includes reducing the chromosome content in half and cytoplasmic maturation includes regulating the expression of proteins that support meiosis, fertilization and early embryonic mitoses. Successful completion of these processes is essential to generate healthy offspring.

The aurora kinases (AURK) regulate meiotic maturation. Unlike most somatic cells which express only AURKA and AURKB, mouse oocytes express all three homologs including AURKC (Nguyen and Schindler, 2017; Yao et al., 2004; Nguyen et al., 2018; Balboula and Schindler, 2014; Shuda et al., 2009; Balboula et al., 2016). We have previously demonstrated that these kinases regulate one another during meiotic maturation. We found that AURKB negatively regulates AURKC, because AURKC levels increased when AURKB was deleted (Nguyen et al., 2018). How AURKB negatively regulates AURKC expression is not known, but this regulation is crucial for egg quality because upregulated AURKC correlated with aneuploidy and decreased fertility.

Meiotic maturation occurs in the absence of transcription; protein expression is controlled through post-transcriptional mechanisms (Seli et al., 2005; Guzeloglu-Kayisli et al., 2008; Tadros and Lipshitz, 2009; Ma et al., 2013; Piccioni et al., 2005; Chen et al., 2013). One mechanism involves translation of stored maternal mRNAs (Piqué et al., 2008; Gosden and Lee, 2010) through mRNA polyadenylation and activation (Chen et al., 2011; Ivshina et al., 2014). In *Xenopus* oocytes, cytoplasmic polyadenylation element-binding protein (CPEB) is a master regulator (Mendez and Richter, 2001; Richter, 2007). In mouse, many meiotic messages that regulate chromosome segregation and cell-cycle progression are maternally stored and translated during maturation in a CPEB-dependent manner (Schindler et al., 2012; Han et al., 2017; Chen et al., 2011; Yoshida et al., 2020). In *Xenopus*, AURKA regulates translation through CPEB1 phosphorylation (Keady et al., 2007; Mendez et al., 2000; Setoyama et al., 2007). Because the AURKs can compensate for each other and therefore share substrates, we hypothesized that AURKC increases because AURKB controls CPEB1-mediated translation. Here, we demonstrate that this AURKB role is indirect through regulating AURKA.

RESULTS AND DISCUSSION

AURKB negatively regulates translation

We hypothesized that translation is altered in *Aurkb* conditional knockout (AURKB cKO) oocytes causing an increase in AURKC protein. A floxed allele of *Aurkb* was excised using a Gdf9-driven Cre recombinase (Fernández-Miranda et al., 2011); its depletion has been previously validated (Nguyen et al., 2018). We first assessed protein synthesis levels in wild-type (WT) and AURKB cKO oocytes using Click chemistry to detect L-homopropargylglycine (HPG) incorporation into nascent proteins (Sha et al., 2018) (Fig. 1A). The specificity of HPG was confirmed by halting translation by cycloheximide treatment; we observed significant reduction in HPG intensity (Fig. S1A,B) (Sha et al., 2018). Consistent with our hypothesis, HPG levels at Metaphase I (Met I) were significantly higher in AURKB cKOs compared with WT (Fig. 1B,C), and the elevated translation was rescued upon expression of *Aurkb* in AURKB cKO oocytes (Fig. 1D,E). For further confirmation of this requirement, we conducted additional controls. We found that the change in HPG intensity was specific to meiotic maturation, because HPG labeling in WT and AURKB cKO Prophase I-arrested oocytes, the preceding quiescent stage, did not differ (Fig. S1C,D). A difference in cell-cycle kinetics also did not account for the increase because there was no statistical difference in Met I completion by polar body extrusion timing between WT and AURKB cKO (Fig. S1E). Finally, we conducted HPG incorporation

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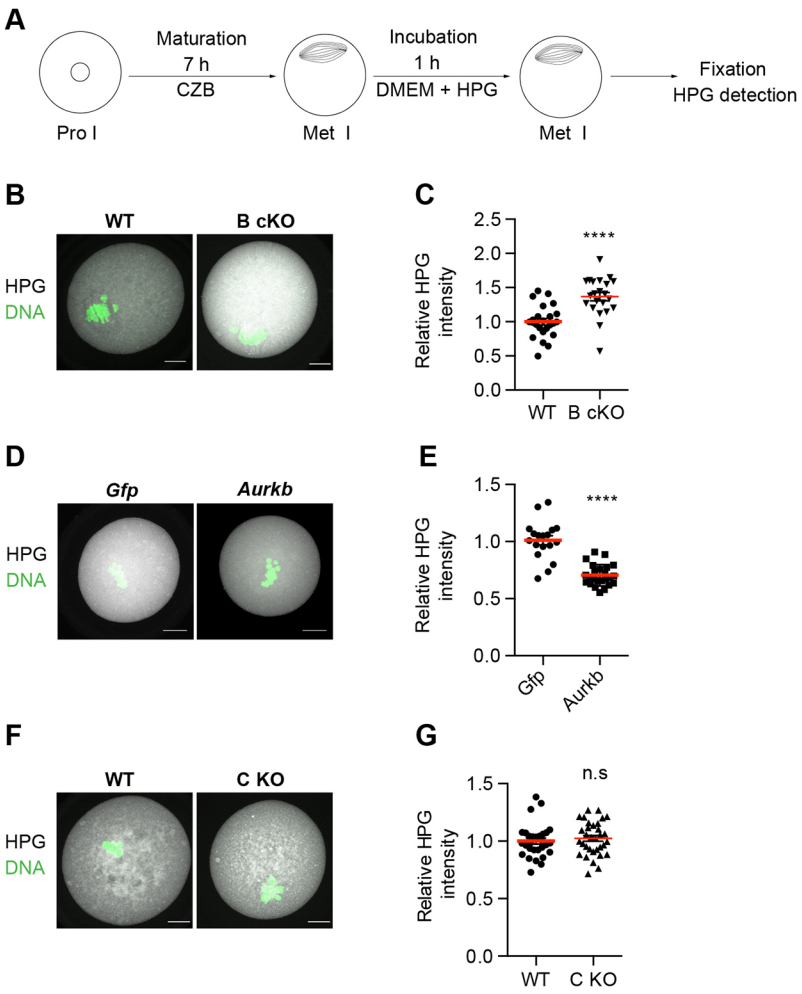

Fig. 1. Translation is upregulated in AURKB cKO oocytes. (A) Schematic of HPG assay. (B-G) Metaphase I (Met I) oocytes were labeled with HPG and stained with anti-HPG (gray) and DAPI (DNA, green). (B) WT and AURKB cKO (B cKO) mice. (C) Relative intensity of HPG from B. Values normalized to WT (number of oocytes: WT 24, B cKO 22). (D) B cKO oocytes microinjected with *Gfp* or *Aurkb* cRNA. (E) Values normalized to *Gfp*-injected (number of oocytes: *Gfp* 18, *Aurkb* 24). (F) Met I oocytes from WT and AURKC KO (C KO). (G) Relative pixel intensity of HPG from F. Values normalized to WT (number of oocytes: WT 29, C KO 33). n.s., not-significant (P=0.4240). Experiments replicated three times; one mouse/genotype/replicate. Data points show individual oocytes. Red horizontal line indicates the mean. ****P<0.0001 (unpaired two-tailed Student's *t*-test). Scale bars: 10 µm.

assays in oocytes lacking *Aurkc*, the homolog that can compensate for AURKB (Kimmins et al., 2007; Schindler et al., 2012), and found that HPG immunoreactivity did not change (Fig. 1F,G). These data suggest that AURKB negatively regulates translation in oocytes. We have previously reported phenotypes such as aneuploidy, premature separation of sister chromatids (PSSC) and subfertility in AURKB cKOs (Nguyen et al., 2018), which could be caused by abnormal expression levels of recruited meiotic proteins, like AURKC, that regulate nuclear maturation.

CPEB1 activity is increased in AURKB cKO oocytes

The resumption of meiosis and cell-cycle progression are coupled with mRNA polyadenylation, translational activation and mRNA degradation (Sha et al., 2017; Yu et al., 2016). The function of CPEB1 in activating translation depends on its hyperphosphorylation, which subsequently triggers its degradation (Sha et al., 2017; Chen et al., 2011; Hodgman et al., 2001; Keady et al., 2007; Ota et al., 2011; Setoyama et al., 2007). Because of the temporal linkage of activation with degradation, CPEB1 turnover reflects CPEB1-driven translation (Han et al., 2017). To confirm these patterns in our laboratory setting, we evaluated CPEB1 phosphorylation and degradation associated with translation during meiotic maturation (Fig. S2). We monitored CPEB1 turnover by western blotting and immunocytochemistry; western blotting also indicates phosphorylation status because hyperphosphorylation induces electrophoretic mobility shifts. Both approaches showed that CPEB1 protein was most abundant in Prophase I (0 h) oocytes and its levels declined during maturation. This decline was coupled with reduced electrophoretic mobility, and CPEB1 protein was lowest at 16 h, when oocytes have arrested at Metaphase II (Met II) (Fig. S2A-D). When we monitored HPG incorporation, the signals peaked at Met I (7 h), when CPEB1 was hyperphosphorylated and declining (Fig. S2E-G).

To determine whether AURKB regulates translation, we evaluated CPEB1 activation by the two methods mentioned. We first evaluated Met II (lowest CPEB1). Compared with WT, we found reduced levels of CPEB1 in AURKB cKOs (Fig. 2A,B). Consistent with the reduction observed by immunocytochemistry, western blotting revealed that total CPEB1 protein levels were significantly reduced in AURKB cKOs. Importantly, before oocytes resumed meiosis (Prophase I), CPEB1 levels were similar between WT and AURKB cKOs, indicating that CPEB1 turnover occurs with faster kinetics and not because there is less CPEB1 to start (Fig. 2C,D). For further validation, we expressed *Aurkb* in *Aurkb* cKO oocytes and detected CPEB1 at Met I; a stage at which translation peaks and CPEB1 degradation is measurable (Fig. S2A,B). The amount of CPEB1 in *Aurkb*-expressing *Aurkb* cKO Met I oocytes increased compared with controls (Fig. 2E,F). These data show that CPEB1 activation and stability are de-regulated in oocytes lacking *Aurkb*, consistent with translation alterations.

Aurkc is a maternal message that is translated in a CPEB1-dependent manner (Schindler et al., 2012). Because AURKC levels are increased in AURKB cKO oocytes (Nguyen et al., 2018), we asked whether other proteins that undergo cytoplasmic

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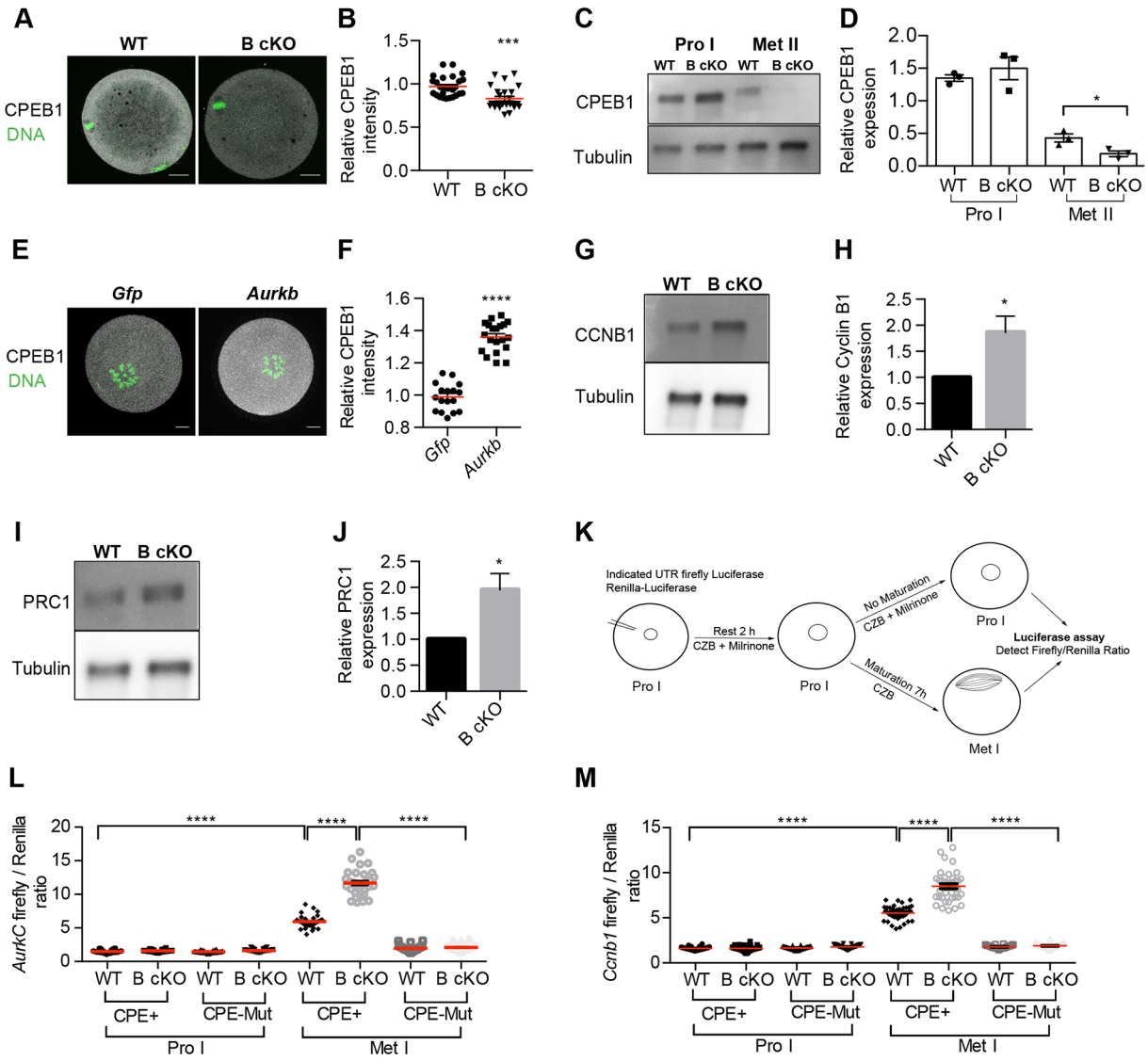

Fig. 2. CPEB1 activity is increased in AURKB cKO oocytes. (A) Met II eggs from WT and AURKB cKO (B cKO) mice stained with anti-CPEB1 (gray) and DAPI (DNA, green). (B) Relative intensity of CPEB1 from A. Values normalized to WT (number of oocytes: WT 27, B cKO 25). (C) Representative western blot detecting CPEB1 from WT and B cKO (30/lane). Loading control: α-tubulin. (D) Quantification of CPEB1 after normalizing C to α-Tubulin in three experiments. (E) B cKO oocytes were microinjected with *Gfp* or *Aurkb* cRNA, matured to Met I and stained with anti-CPEB1 (gray) and DAPI (DNA, green). (F) Relative CPEB1 intensity from E. Values normalized *Gfp*-injected (Number of oocytes: *Gfp*-17; *Aurkb*-20). (G,I) Western blot of Met I oocytes detecting CCNB1 (25/lane; G) or PRC1 (25/lane; I). (H,J) Relative CCNB1 or PRC1expression from G and I, respectively. Values normalized to α-tubulin. (K) Schematic of luciferase assay. (L,M) Prophase I (Pro I) oocytes were co-injected with luciferase RNAs: *Aurkc* (L) and *Ccnb1* (M) fused with CPE+ or CPE-mutated (Mut) UTR. Luminescence was measured and quantified as firefly/*Renilla* (number of oocytes: L – WT-CPE+ 29, WT-CPE-Mut 30, B cKO-CPE+ 29, B cKO-CPE-Mut 27; M – WT-CPE+ 40, WT-CPE-Mut 34, B cKO-CPE+ 39, B cKO-CPE-Mut 33). Experiments repeated three times; total 3-5 mice/genotype. Red horizontal line indicates the mean. **P*<0.05; ****P*<0.001; *****P*<0.0001 (unpaired two tailed Student's *t*-test). Scale bars: 10 µm.

polyadenylation element (CPE)-mediated translation are also upregulated (Fig. S3A). Despite similar starting levels at Prophase I (Fig. S3B-G,J,K), oocytes lacking *Aurkb* expressed more CCNB1, PRC1, MOS and HEC1 (NDC80) after meiotic maturation compared with WT, consistent with excess translation of CPE-containing messages (Fig. 2G-L, Fig. S3H,I,M,N). We then selected two candidates, *Aurkc* and *Ccnb1*, to validate recruitment using a luciferase-based assay (Fig. 2K). Here, *Aurkc* and *Ccnb1* 3′ untranslated regions (3′UTRs), which contain CPEB1-binding sites, drive expression of a firefly luciferase reporter (Schindler et al., 2012; Han et al., 2017; Yang et al., 2017; Murai et al., 2010). We also mutated the CPE-binding sites for controls (supplementary Materials and Methods). *Renilla* luciferase mRNA was injected for normalization. In WT, *Aurkc* and *Ccnb1* firefly luciferase accumulated ∼4-fold; recruitment was abolished when expressing CPE-mutant UTRs. Luciferase levels doubled in AURKB cKOs (∼8- to 12-fold) and was dependent upon the CPE (Fig. 2L,M). These data support a model that increased translation of CPE-containing mRNAs arises because CPEB1 activity is upregulated in AURKB cKO oocytes.

AURKA regulates translation in mouse oocytes

AURKA activation is increased in AURKB cKOs (Nguyen et al., 2018). Because AURKA regulates translation in female germlines of other organisms, we hypothesized that AURKB regulates translation through AURKA (Mendez et al., 2000; Piqué et al., 2008; Barnard

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et al., 2004; Groisman et al., 2001). We first validated that AURKA has increased activity by evaluating phosphorylated CDC25B, an AURKA substrate (Zhao et al., 2015), in AURKB cKO oocytes. Compared with WT, pCDC25B intensity was significantly increased in AURKB cKOs (Fig. S4A,B). We hypothesized that this increase drives more CPEB1 activation and excess translation. To test this hypothesis, we first inhibited AURKA in AURKB cKO oocytes using a specific small-molecule inhibitor, MLN8237, and detected HPG. Compared to AURKB cKO oocytes in DMSO, inhibition of AURKA reduced translation (Fig. 3A,B). Furthermore, AURKB/C inhibition in WT oocytes via ZM447439 did not affect HPG incorporation, whereas AURKA inhibition reduced HPG (Fig. S4C,D). Finally, we evaluated HPG incorporation in AURKA cKO oocytes; *Aurka* is removed by Gdf9-Cre and has been validated elsewhere (Blengini et al., 2021). Compared with WT, HPG intensity decreased significantly in AURKA cKO oocytes (Fig. 3C,D), and this reduction was rescued when *Aurka* was expressed in AURKA cKO oocytes (Fig. 3E,F). These results, combined with the precedence of AURKA regulation of translation in other systems, strongly suggest that AURKA positively regulates translation in mouse oocytes. Furthermore, these results suggest that AURKB negatively regulates translation indirectly through AURKA.

We next evaluated whether the increased AURKA activity alters CPEB1 activity. In *Xenopus* oocytes, AURKA phosphorylates CPEB1 and induces its ability to control translation (Mendez et al., 2000; Groisman et al., 2001; Barnard et al., 2004; Piqué et al., 2008). AURKA is not involved in CPEB1 phosphorylation in bovine and porcine oocytes (Komrskova et al., 2014; Uzbekova et al., 2008), and in mouse oocytes AURKA control of CPEB1 is not clear. Hodgman et al. (2001) indicated that AURKA phosphorylates CPEB1 whereas Han et al. (2017) reported that AURKA inhibition did not alter CPEB1 electrophoretic mobility. Consistent with Hodgman, we found that CPEB1 phosphorylation is perturbed in WT oocytes treated with an AURKA inhibitor because the electrophoretic mobility of CPEB1 at 7 h post MLN8237-treatment was more rapid compared with DMSO controls (Fig. S4E,F). Finally, we investigated a requirement for AURKA in CPEB1 regulation using a genetic approach. First, we evaluated CPEB1 levels in WT and AURKA cKO Met I oocytes; AURKA cKO oocytes arrest at Met I (Blengini et al., 2021) precluding Met II evaluation. Compared with WT, CPEB1 intensity

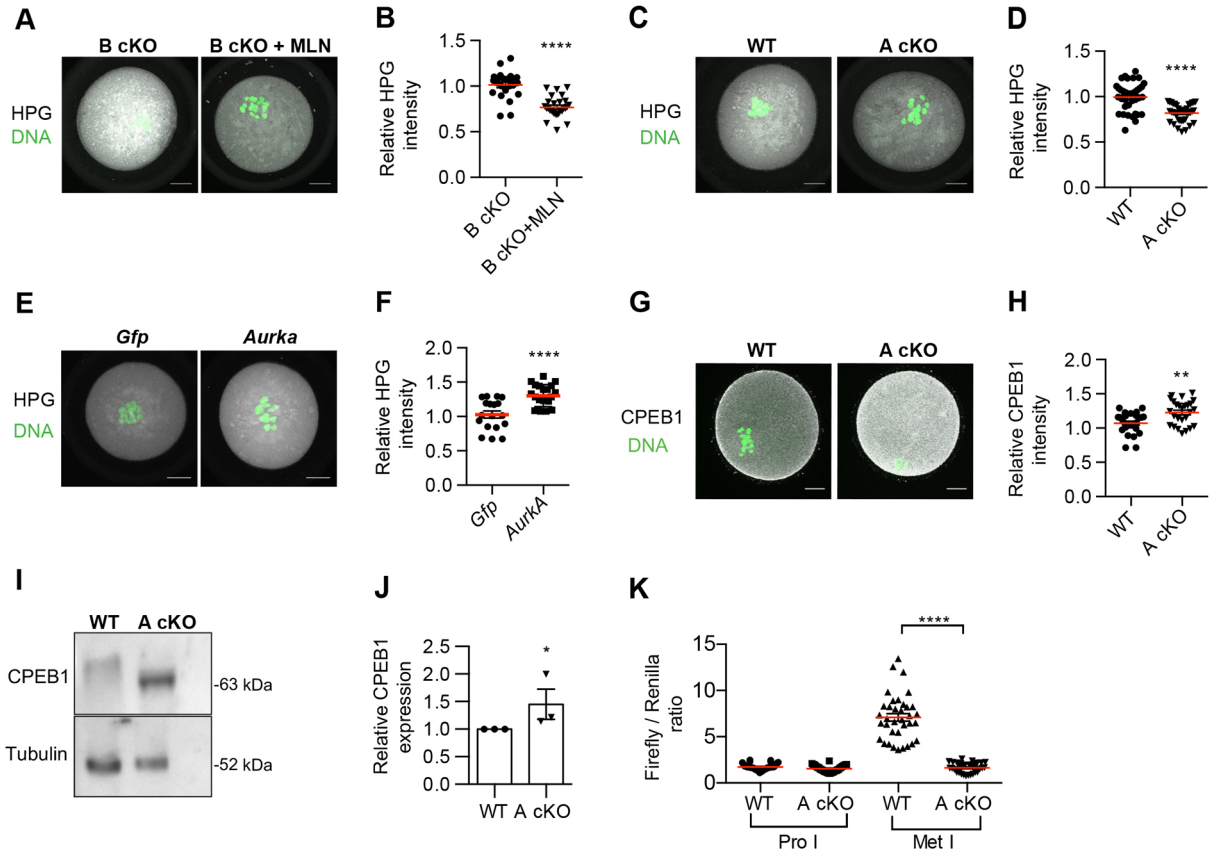

Fig. 3. AURKA is required for translation and CPEB1 regulation. (A) Metaphase I (Met I) oocytes from AURKB cKO (B cKO) treated with DMSO or 1 µM MLN8237 (MLN), labeled with HPG and stained with anti-HPG (gray) and DAPI (DAPI, green). (B) Relative intensity of HPG from A (number of oocytes: B cKO 23; B cKO+MLN 27). (C) Met I oocytes from WT and AURKA cKO (A cKO) mice were labeled with HPG and stained with anti-HPG (gray) and DAPI (DNA, green). Shown are representative confocal *z*-projections. (D) Relative intensity of HPG from C. Values normalized to WT (number of oocytes: WT 39, A cKO 39). (E) A cKO oocytes were microinjected with *Gfp* or *Aurka* cRNA, matured to Met I, labeled with HPG and stained with anti-HPG (gray) and DNA (DAPI, green). (F) Values normalized to *Gfp*-injected group (number of oocytes: Gfp 18, *Aurka* 21). (G) Met I oocytes from WT and A cKO mice were stained with anti-CPEB1 (gray) and DAPI (DNA, green). (H) Relative intensity of CPEB1 from G. Values normalized to WT (number of oocytes: WT 27, A cKO 26). (I) Met I oocytes from WT and A cKO mice were collected for western blotting to detect CPEB1 (30/lane). Loading control: α-tubulin. (J) Quantification of CPEB1 after normalizing I to α-tubulin. (K) Prophase I (Pro I) oocytes were co-injected with luciferase RNAs as in Fig. 2M. Luminescence was measured and quantified (number of oocytes: WT 36, A cKO 32). Experiments A-F replicated three times; one mouse/genotype/replicate; experiments G-I repeated three times; total 3-5 mice/genotype. Red horizontal line indicates the mean. **P*<0.05; ***P*<0.01; *****P*<0.0001 (unpaired two tailed Student's *t*-test). Scale bars: 10 µm.

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was significantly higher in AURKA cKOs (Fig. 3G,H). Furthermore, by western blot, CPEB1 protein was more abundant and migrated with a faster electrophoretic mobility in AURKA cKOs (Fig. 3I,J). We did not find any change in CPEB1 intensity or abundance in AURKC KOs (Fig. S5), suggesting an AURKA-specific function.

To test whether AURKA is required for CPEB1-mediated translation, we performed the luciferase assay (Fig. 2K) in AURKA cKO oocytes. Compared with the 4-fold enrichment of firefly luciferase in WT, there was no enrichment of luciferase in AURKA cKOs (Fig. 3K). These data indicate that AURKA controls CEPB1 activity and is required for maternal message translation. Moreover, these data support the model that, in the absence of AURKB, excess AURKA activity drives upregulation of CPE-dependent translation.

Excess AURKA activity upregulates CPEB1 activity

To confirm antagonistic function of AURKB on AURKA and translation, we evaluated CPEB1 levels in AURKB cKO oocytes with reduced *Aurka* copy number [i.e. conditionally heterozygous for *Aurka* (cHet)]. Reduction of AURKA activity in these oocytes compared with AURKB cKO oocytes was validated by observing reduced pCDC25B levels (Fig. S4A,B). Compared with AURKB cKO oocytes, in which CPEB1 is turned over, reduction of *Aurka* copy number (AURKB cKO/AURKA cHet) increased CPEB1 abundance when assessed by immunocytochemistry and western blotting (Fig. 4A-D). Therefore, reducing *Aurka* copy number resulted in stabilizing and dampening CPEB1 activity in AURKB cKOs. These results strongly suggest AURKB-dependent CPEB1 regulation occurs indirectly through controlling AURKA activation. To confirm whether this effect on CPEB1 affects translation, we evaluated the HPG incorporation in AURKB cKO/AURKA cHet oocytes compared with AURKB cKO oocytes. We found no significant difference in HPG levels in prophase I oocytes (Fig. S1C,D), although there was a significant reduction in HPG signal at Met I (Fig. 4E,F). Furthermore, the translation difference was not related to meiotic stage differences because there was no statistical difference in PBE timing between oocytes from the different strains (Fig. S1E).

Finally, to determine the biological significance of excess AURKA driving translation, we assessed levels of aneuploidy comparing AURKB cKO with AURKB cKO/AURKA cHet.

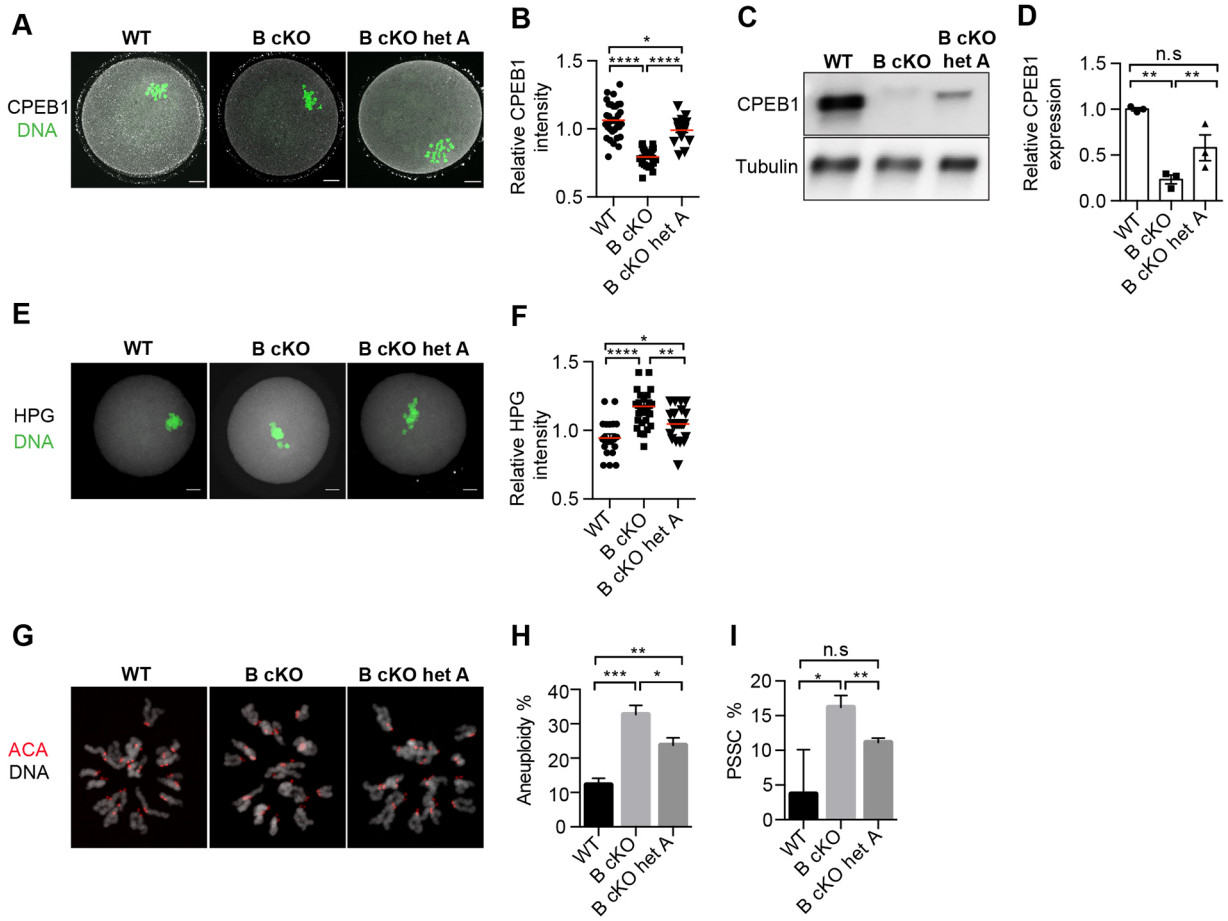

Fig. 4. Reduction of AURKA activity in AURKB cKO oocytes partially rescues CPEB1, translation and meiosis defects. (A) Metaphase I (Met I) oocytes from WT, AURKB cKO (B cKO) and AURKB cKOs heterozygous for *Aurka* (B cKO het A) mice were stained to detect CPEB1 (gray) and DAPI (DNA, green). (B) Relative intensity of CPEB1 from A. Values normalized to WT (number of oocytes: WT 34, B cKO 25, B cKO het A 24). (C) Met I oocytes from WT, B cKO and B cKO het A mice were collected for western blotting to detect CPEB1 (30/lane). Loading control: α-tubulin. (D) Quantification of CPEB1 after normalizing C to α-tubulin. (E) Met I oocytes were stained to detect HPG (gray) and DAPI (DNA, green). (F) Relative intensity of CPEB1 from E. Values normalized to WT (number of oocytes: WT 22, B cKO 30, B cKO het A 21). (G) Representative images of chromosome spreads of Metaphase II eggs stained with Anti-Centromeric Antibodies (ACA, red) and DAPI (gray). (H) Quantification of the aneuploidy. (I) Quantification of the premature separation of sister chromatids (PSSC). Number of oocytes in H,I: WT 29, B cKO 31, B cKO het A 36. Experiments repeated three times; total 3-4 mice/genotype. Red horizontal line indicates the mean. Error bars indicate s.e.m. **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001 (one-way ANOVA). n.s., not significant. Scale bars: 10 µm.

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Importantly, aneuploidy and PSSC levels significantly decreased upon removal of one *Aurka* allele (Fig. 4G-I). These data suggest that a consequence of abnormal translation is aneuploidy and subfertility, observed in AURKB cKO eggs. Further studies are still required to investigate how AURKB inhibits AURKA activity and to identify other differentially expressed proteins that can be driving the aneuploidy phenotype.

In summary, we describe a new meiotic requirement of AURKB to inhibit AURKA activity. This regulation is important to control CPEB1-mediated translation. Abnormal translation could affect the expression levels of important meiotic proteins resulting in aneuploid eggs. Moreover, we demonstrate that CPEB1 activity is controlled by AURKA in mouse oocytes, thereby clarifying conflicting findings in the literature. CPEB1 upregulation resulted in elevated translation of CPE-containing genes such as *Aurkc*, *Ccnb1*, *Prc1* and *Hec1* in AURKB cKO oocytes and this translation was abolished in AURKA cKO oocytes. Although we do not understand how AURKB inhibits AURKA, we know that competition among the three aurora kinases for binding activating partners occurs and is a prevalent control mechanism in mouse oocytes (Nguyen et al., 2018). Here, we show that an antagonistic relationship between AURKB and AURKA is important for tight control of translation. AURKA is the most abundantly expressed and most activated of the three aurora kinases (Nguyen et al., 2018; Shuda et al., 2009). We speculate that this imbalance of expression levels therefore requires an oocyte-specific, inter-kinase competition to carefully regulate AURKA activity and ensure gamete quality.

MATERIALS AND METHODS

Mice

Generation of *Aurkb*$^{fl/fl}$ *Gdf9-Cre*, *Aurka*$^{fl/fl}$ *Gdf9-Cre* and *Aurkc*$^{-/-}$ mice has been described previously (Kimmins et al., 2007; Schindler et al., 2012; Fernández-Miranda et al., 2011; Wellard et al., 2021; Blengini et al., 2021). Control (WT) mice are from the same genetic background as experimental transgenic animals but lack the Cre recombinase transgene. All animals were bred and housed in the animal facility at Rutgers University (NJ, USA), with a 12 h light/12 h dark cycle, constant temperature and food and water provided *ad libitum*. All animal experiments performed in this study were approved by the Rutgers Institutional Animal Care and Use Committee and follow guidelines set by the National Institutes of Health.

Oocyte, egg collection and microinjection

Collection of prophase I-arrested oocytes was performed as previously described (Nguyen et al., 2018) in minimal essential medium (MEM) containing 2.5 µM milrinone (Sigma-Aldrich, M4659) to prevent oocyte maturation. Mice (~8 weeks old) were primed by injection of 5 I.U. of pregnant mare serum gonadotropin (PMSG, Lee Biosolutions, 493-10) 48 h before collection. After harvest, oocytes were matured in Chatot, Ziomek and Bavister (CZB) medium without milrinone in 5% CO_2 and 37°C for the desired time of maturation (7 h for Met I and 16 h for Met II). MLN8237 (Alisertib; Selleckchem, S1133) and ZM447439 (Tocris, 2458) were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and added to the CZB culture media at a final concentration of 1 µµ and 5 µµ respectively. Cycloheximide (Sigma-Aldrich, C7698) was added to the CZB culture media at a final concentration of 20 µµ. For Met II egg isolation, mice were primed with PMSG followed by 5 I.U. of human chronic gonadotrophin (hCG; Sigma-Aldrich, CG5) 48 h later. Eggs were collected 14 h after hCG injection from oviducts in MEM media containing 3 mg/ml hyaluronidase (Sigma-Aldrich, H3506). Generation of *Aurkb* and *Aurka* construct has been previously described (Nguyen et al., 2018). To generate cRNA, the plasmids were linearized with NdeI, purified (Qiagen, QIAquick PCR Purification) and *in vitro* transcribed using an mMessage mMachine T7 kit (Ambion), according to the manufacturer's protocol. The synthesized cRNAs were then purified using an RNAeasy kit (Qiagen) and stored at −80°C. cRNAs were injected at a concentration of at least 500 ng/µl.

Protein synthesis detection assay

To detect protein synthesis levels in oocytes, an L-HPG-Translation kit was used (Rong et al., 2019; Sha et al., 2018). Oocytes were incubated with HPG in DMEM medium lacking methionine (Life Technologies, 21013-024; 1:100) for 1 h. HPG was detected using Click-iT HPG Alexa Fluor 488 and 594 Protein Synthesis Assay kits (Life Technologies, C10428 and C10429), used according to the manufacturer's instructions.

Western blotting

With slight modifications, western blotting was performed as previously described (Nguyen et al., 2018). A total of 30-50 oocytes were lysed in sodium dodecyl sulphate sample buffer (2× Laemmli Sample Buffer; Bio-Rad, 1610737) and denatured at 95°C for 5 min. Membranes were incubated with primary antibodies (anti-CPEB1, anti-CCNB1, anti-PRC1 and anti-α-tubulin for 1 h at room temperature) and then were incubated with horseradish peroxidase-conjugated secondary antibodies (Kindle Biosciences, digital anti-Rabbit-HRP, R1006 or anti-mouse, R1005) for 1 h at room temperature. Protein bands were detected using ECL Substrate Solution (KwikQuant Ultra Digital-ECL Substrate Solution, R1002) following the manufacturer's protocol using KwikQuant Imager (KwikQuant Imager, Kindle Biosciences, D1001) and Imager manager software (KwikQuant Image Manager Software, Kindle Biosciences, D1016). See 'Antibodies and drugs' below for full details of antibodies used.

Live cell imaging

To evaluate the timing of polar body extrusion, we matured oocytes *in vitro* for 24 h while imaging using an EVOS FL Auto Imaging System (Life Technologies) with a 10× objective. The microscope stage was heated to 37°C and 5% CO_2 was maintained using the EVOS Onstage Incubator. Bright-field images of individual cells were acquired every 20 min and processed using National Institutes of Health (NIH) ImageJ Software.

Immunocytochemistry

Following meiotic maturation, oocytes were fixed in PBS-containing paraformaldehyde (PFA; Sigma-Aldrich, P6148) at room temperature (CPEB1, HEC1 and MOS: 2% PFA for 20 min; pCDC25B: 3.7% PFA+0.01% Triton X-100 for 1 h at room temperature) followed by two consecutive washes through blocking buffer [PBS+0.3% (w/v) bovine serum albumin (BSA)+0.1% (v/v) Tween-20]. Before immunostaining, oocytes were permeabilized for 20 min in PBS containing 0.1% (v/v) Triton X-100 and 0.3% (w/v) BSA followed by 10 min in blocking buffer. Immunostaining was performed by incubating cells in primary antibody (CPEB1, HEC1 and MOS: 1 h at room temperature; pCDC25B for 3 h at room temperature) followed by three consecutive 10 min incubations in blocking buffer. After washing, secondary antibodies were diluted 1:200 in blocking solution and the sample was incubated for 1 h at room temperature followed by three consecutive 10 min incubations in blocking buffer. The cells were next mounted in Vectashield (Vector Laboratories, H-1000) with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Life Technologies; 1:170). See 'Antibodies and drugs' below for full details of antibodies used.

Luciferase assay

The cRNAs of firefly luciferase fused to the 3′ UTR of *Aurkc* (250 ng/µl) or *Ccnb1* (250 ng/µl) and *Renilla* luciferase (25 ng/µl) were prepared as previously described (Murai et al., 2010; Schindler et al., 2012). Mutation of the CPE-binding sites for *Aurkc* and *Ccnb1* 3′ UTRs was performed using the multi-site directed mutagenesis kit (Agilent Technologies, 210515) as previously described (Schindler et al., 2012; Yang et al., 2017). See supplementary Materials and Methods for sequences. The luciferase cRNAs were co-injected into Prophase I-arrested oocytes from indicated genotypes and incubated *in vitro* for 2 h in CZB medium containing milrinone. Some groups of the injected oocytes were matured to Met I (7 h) while others were kept arrested at Prophase I. After washing in PBS+polyvinylpyrroilidone (PVP), oocytes were collected and lysed in Passive Lysis Buffer (Promega, E1910; 20 µl/oocyte) using a 96-well microplate for 15 min at room temperature with mixing, followed by processing with the Dual Luciferase reporter assay system (Promega, E1910) according to the manufacturer's

instructions. Signal intensities were obtained using a Microplate Luminometer (Veritas Microplate Luminometer, Turned Biosystems, version 1.5). Background fluorescence was subtracted by measuring signals in uninjected oocytes and firefly luciferase activities were normalized to that of *Renilla* luciferase.

In situ chromosome counting

Prophase I oocytes were *in vitro* matured to Met II. Then, eggs were incubated with 100 µM Monastrol (Sigma-Aldrich, M8515) in CZB medium for 2 h. Finally, the eggs were fixed in 2% PFA in PBS for 20 min and permeabilized in PBS containing 0.2% Triton X-100 for 20 min. Eggs were stained with Anti-Centromeric Antibodies (ACA) to detect centromeres and DAPI to detect DNA. Normal chromosome counting for a mouse egg is 20 pairs of sister chromatids; any deviation of this number was considered an aneuploid egg. Eggs were imaged using the Leica SP8 confocal with 0.5 µm *z*-intervals. Chromosome counting was performed with NIH ImageJ software, using cell counter plugins. See 'Antibodies and drugs' below for full details of antibodies used.

Microscopy and image analysis

Images were captured using a Leica TCS SP8 confocal microscope equipped with a 63× objective, 1.40 N.A. oil immersion objective. For each image, optical *z*-slices were obtained using a 0.5-1 µm interval with a zoom setting of 1.5-4. For comparison of pixel intensities, the laser power was kept constant for each oocyte and group in an experiment. Images were analyzed using NIH ImageJ software. *Z*-slices from each image were merged into a projection. For HPG intensity measurement, the cytoplasmic area was selected. The average intensity was recorded using the measurement tool for pixel intensity analysis.

Antibodies and drugs

The following primary antibodies were used for western blotting (WB) and immunocytochemistry (IF): rabbit anti-α-tubulin (WB: 1:1000; Cell Signaling Technology, 11H10; Giurisato et al., 2018), rabbit anti-CPEB1 (WB: 1:1000, IF: 1:100; Abcam, ab73287; Daldello et al., 2019), rabbit anti-Mos (IF: 1:100; Lifespan Biosciences, LS-C164489), mouse anti-CCNB1 (WB: 1:500; Cell Signaling Technology, 4135; Han et al., 2017), rabbit HEC1 (IF: 1:100; gift from Dr Robert Benezra, Memorial Sloan-Kettering Cancer Center, New York, USA), rabbit anti-PRC1 (WB: 1:1000; Proteintech, 15617-1-AP), rabbit pCDC25B (Ser353) (IF: 1:100; American Research Products, SAB-11949) and human anti-ACA (IF: 1:30; Antibodies Incorporated, 15-234). Mouse anti-α-tubulin Alexa Fluor 488-conjugated (IF: 1:200; Life Technologies, 322588), goat anti-human-Alexa-633 (IF: 1:200; Life Technologies, A21091) and donkey anti-rabbit-Alexa568 (IF: 1:200; Life Technologies, A10042), anti-HRB (WB: 1:1000; rabbit R1006 or mouse R1005, Kindle Biosciences) were used as secondary antibodies.

Statistical analysis

One-way ANOVA and unpaired two-tailed Student's *t*-test were used to evaluate the differences between groups using GraphPad Prism. The differences of $P<0.05$ were considered significant. The error bars indicate s.e.m.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: K.S.; Methodology: M.A.; Formal analysis: M.A.; Investigation: M.A.; Writing - original draft: M.A.; Writing - review & editing: M.A., K.S.; Supervision: K.S.; Project administration: K.S.; Funding acquisition: K.S.

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