BIOLOGY OF REPRODUCTION (2015) **93**(2):43, 1–9
Published online before print 1 July 2015.
DOI 10.1095/biolreprod.115.131359

Embryonic Poly(A)-Binding Protein Is Required During Early Stages of Mouse Oocyte Development for Chromatin Organization, Transcriptional Silencing, and Meiotic Competence[1]

Katie M. Lowther[3] and Lisa M. Mehlmann[2,4]

[3]*Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut*
[4]*Department of Cell Biology, University of Connecticut Health, Farmington, Connecticut*

ABSTRACT

During oocyte maturation, fertilization, and early embryo development until zygotic genome activation (ZGA), transcription is suppressed, and gene expression is dependent upon the timely activation of stored mRNAs. Embryonic poly(A)-binding protein (EPAB) is the predominant poly(A)-binding protein in *Xenopus*, mouse, and human oocytes and early embryos and is important for regulating translational activation of maternally stored mRNAs. EPAB is critical for early development because *Epab*$^{-/-}$ female mice do not produce mature eggs and are infertile. In this study, we further characterize morphological and molecular aspects of *Epab*$^{-/-}$ oocytes. We demonstrated that *Epab*$^{-/-}$ oocytes are smaller in size, contain peripheral germinal vesicles, and are loosely associated with cumulus cells. The chromatin reorganization of the surrounded nucleolus (SN) configuration and transcriptional silencing that normally occurs during oocyte growth does not occur in *Epab*$^{-/-}$ oocytes. Interestingly, microinjection of *Epab* mRNA into *Epab*$^{-/-}$ preantral follicle-enclosed oocytes rescues reorganization of chromatin and oocyte maturation to metaphase II. Overall, these results demonstrate an important role for EPAB during oocyte growth and the acquisition of meiotic competence.

EPAB, meiotic competence, oocyte, translational regulation

INTRODUCTION

In mammals, oocytes enter meiosis during embryonic development and become arrested at prophase of the first meiotic division [1, 2]. Following attainment of reproductive maturity, follicle-enclosed oocytes are selectively recruited to grow in response to the pituitary gonadotropin follicle-stimulating hormone (FSH). Follicular growth is characterized by proliferation of granulosa cells and formation of an antral cavity, as well as oocyte growth and acquisition of meiotic competence. The antrum separates the granulosa cells into 2 functionally distinct compartments: the outer mural granulosa cells that line the basal lamina and the inner cumulus cells that directly surround the oocyte [3]. In response to a preovulatory surge of luteinizing hormone (LH), the oocyte resumes meiosis, and the cumulus and granulosa cells undergo terminal differentiation [4, 5]. These events are coordinated such that a cumulus-surrounded oocyte at metaphase II (MII) is ovulated at the appropriate stage to be fertilized.

Fully grown oocytes become transcriptionally silent prior to meiotic resumption. Transcription remains suppressed during oocyte maturation, fertilization, and early cleavage divisions, until zygotic genome activation (ZGA) [6, 7], which occurs at the 2-cell stage in mice and 4- to 8-cell stage in humans [8–10]. As transcriptional activity is suppressed during this critical stage of development, gene expression is regulated by the timely activation of maternally derived mRNAs synthesized in advance and stored in the oocyte during the first meiotic arrest. These mRNAs are uniquely stable, with a half-life of 8 to 12 days [11, 12], and upon the appropriate stimulus, will drive re-entry into meiosis and control the rate of mitotic cell divisions during cleavage of the early embryo [7, 13].

A main mechanism by which translation is regulated in oocytes is by cytoplasmic polyadenylation [14], whereby maternally derived mRNAs are translationally activated through elongation of their poly(A) tail in the cytoplasm. Although this process has been better delineated in other organisms, conserved sequences and proteins have been identified in mouse oocytes (reviewed in [15]). The best understood pathway involves cytoplasmic polyadenylation element-binding protein 1 (CPEB1), which promotes cytoplasmic lengthening of poly(A) tails on mRNAs that contain cytoplasmic polyadenylation element (CPE) motifs [16, 17]. CPEB1 acts with other regulatory proteins such as symplekin (SYMPK), cytoplasmic polyadenylation specificity factor (CPSF), and GLD2, an atypical poly(A) polymerase [17–19], to regulate poly(A) tail length and thus translation. The process of translational activation during oocyte maturation is complex [20] and additional pathways independent of cytoplasmic polyadenylation, such as those involving deleted in azoospermia-like (DAZL) [21–23], have also been identified. Indeed, many mRNAs contain multiple regulatory elements that facilitate intricate patterns of activation [24].

Another class of highly conserved proteins involved in translational regulation are poly(A)-binding proteins (PABPs), which bind to the poly(A) tail and bring the translational machinery together in a ''closed loop'' configuration [25, 26]. *Xenopus*, mouse, and human oocytes, and early embryonic cells express an embryo-specific poly(A)-binding protein (EPAB) as the predominant PABP that is then replaced by

[1]Supported by a Lalor Foundation fellowship to K.M.L.; U.S. National Institutes of Health grant R01HD059909 to E.S., and by University of Connecticut Health Research Advisory Council award to L.M.M. Presented in part at the 47th Annual Meeting of the Society for the Study of Reproduction, July 19–23, 2014, Grand Rapids, MI; at the Gordon Research Conference: Mammalian Reproduction, August 10–15, 2014, New London, NH; and at the 62nd Annual Meeting of the Society for Reproductive Investigation, March 25–28, 2015, San Francisco, CA.
[2]Correspondence: Lisa M. Mehlmann, Department of Cell Biology, University of Connecticut Health, 263 Farmington Ave., L5074, Farmington, CT 06030. E-mail: lmehlman@uchc.edu

Received: 5 May 2015.
First decision: 5 June 2015.
Accepted: 19 June 2015.

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

somatic poly(A)-binding protein cytoplasmic 1 (PABPC1) after ZGA [27–30]. In *Xenopus*, EPAB is involved in both polyadenylation-dependent and -independent pathways that temporally regulate translational activation of repressed mRNAs upon stimulation of oocyte maturation [31]. Importantly, EPAB prevents deadenylation of mRNAs [29], promotes cytoplasmic polyadenylation [32], enhances translation initiation [30], and is required for maturation of *Xenopus* oocytes [32].

In a previous study, we demonstrated that EPAB is required for female fertility in mice [33]. EPAB-deficient mice are infertile and do not generate mature eggs or embryos in vivo or in vitro. *Epab*$^{-/-}$ oocytes fail to achieve translational activation of maternally stored mRNAs upon stimulation of oocyte maturation, including *CyclinB1* and *Dazl* mRNAs. In addition, late antral follicles in the ovaries of *Epab*$^{-/-}$ mice exhibit impaired cumulus expansion and an 8-fold decrease in ovulation associated with a significant down-regulation of mRNAs encoding EGF-like growth factors and their downstream regulators [33].

Because protein synthesis is not required for germinal vesicle (GV) breakdown in mice [34, 35], it is possible that EPAB is required early during oogenesis for expression of mRNAs that support oocyte growth and acquisition of meiotic competence. In support of this, microinjection of *Epab* mRNA into denuded GV-stage *Epab*$^{-/-}$ oocytes does not rescue oocyte maturation [33]. The goal of the current study was to further delineate the role of EPAB during oogenesis. Our findings collectively suggest that EPAB is a global regulator of gene expression in the oocyte and is required during oocyte development for factors that promote nuclear maturation and meiotic competence.

MATERIALS AND METHODS

Collection of Ovaries, Oocytes, and Follicle-Enclosed Oocytes

Mice were bred and maintained according to Yale University animal research requirements, and all procedures were approved by the Institutional Animal Care and Use Committee (protocol 2011-11207).

To collect GV-stage oocytes, ovaries were obtained from 12-week-old wild-type *Epab*$^{+/+}$ C57BL/6 and *Epab*$^{-/-}$ mice [33] 44 to 48 h after intraperitoneal injection of 5 IU of equine chorionic gonadotropin (eCG; Sigma, St. Louis, MO). Antral follicles were selectively punctured, and GV-stage oocytes were collected in α-minimum essential medium (MEMα; Life Technologies, Grand Island, NY) supplemented with 20 mM Hepes, 75 μg/ml penicillin G (Sigma), 50 μg/ml streptomycin sulfate (Sigma), 0.1% polyvinyl alcohol (PVA; Sigma), and 10 μM milrinone (Sigma) to prevent meiotic resumption. Prior to removal of the surrounding cumulus cells, oocytes were scored for the presence of cumulus cells as fully enclosed, partially enclosed, or denuded. For overnight culture, oocytes were transferred to MEMα supplemented with 25 mM $NaHCO_3$, 75 μg/ml penicillin G, 50 μg/ml streptomycin sulfate, 5% fetal bovine serum (FBS; product no. 12000-022; Life Technologies), and 10 μM milrinone and incubated in a humidified atmosphere at 37°C with 5% CO_2 and 95% air. To examine in vitro maturation, 10 μM of milrinone was excluded from the medium, and oocytes were assessed after 18 hours of culture for GV breakdown (GVBD) and progression to MII.

Preantral follicles (~120–140 μm in diameter) were dissected from ovaries of unprimed 23- to 29-day-old wild-type and *Epab*$^{-/-}$ mice. Ovaries were removed from the bursa, and follicles were gently teased away from the ovary, using a forceps and a needle [36]. The culture medium for follicle collection and culture was MEMα supplemented with 25 mM $NaHCO_3$, 75 μg/ml penicillin, 50 μg/ml streptomycin sulfate, 20% FBS, 50 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium (ITS; Sigma), and 10 ng/ml ovine FSH (National Hormone and Peptide Program, Torrance, CA).

Measurement of Oocyte Size and GV Position

Oocyte diameter was measured using transmitted images of freshly isolated, denuded oocytes obtained by confocal microscopy. Excluding the zona pellucida, the vertical, horizontal, and diagonal diameters were measured for each oocyte by using Image J software (National Institutes of Health, Bethesda, MD) after calibration with a scale bar. The diameter of the oocyte was recorded as the average of the 3 measurements. The same images were used to determine the location of the GV. Oocytes were scored as having either central or peripheral GVs by drawing a horizontal and vertical line through the oocyte to designate the center. GVs that crossed the center were scored as central, and GVs located outside the center were scored as peripheral.

Assessment of Chromatin Configuration

GV-stage oocytes were collected from eCG-primed 12-week-old *Epab*$^{+/+}$ and *Epab*$^{-/-}$ mice by selectively puncturing antral follicles. Directly after isolation, oocytes were washed with phosphate-buffered saline (PBS) with 0.1% PVA and fixed with 2% formaldehyde (Sigma) in 100 mM Hepes, 50 mM EGTA, 10 mM $MgSO_4$, and 0.2% Triton X-100 for 1 h at 37°C. Following fixation, oocytes were incubated in 5 μM SYTOX Orange (Life Technologies) for 10 min at room temperature, and washed 3 times in PBS/PVA before being imaged on a 510 model confocal microscope (Carl Zeiss Microscopy, Thornwood, NY). Oocytes were observed with a 63× 1.2-numerical aperture lens, using excitation at 543 nm and emission at 570 nm. Chromatin was classified as nonsurrounded nucleolus (NSN), surrounded nucleolus (SN), or partially surrounded nucleolus (PSN).

Assessment of Transcriptional Activity

GV-stage oocytes were isolated from 12-week-old eCG-primed *Epab*$^{+/+}$ and *Epab*$^{-/-}$ mice by selectively puncturing antral follicles, and transcriptional activity was determined by incorporating 5-bromouridine (BrUrd) into nascent RNA transcripts [37]. Oocytes were microinjected with 10 pl of 5-bromouridine 5′ triphosphate sodium salt (Br-UTP) (Sigma; 100 mM in 2 mM PIPES buffer [pH 7.4] containing 140 mM KCl), cultured for 45 min at 37°C and fixed in 4% formaldehyde (Sigma) in PBS/PVA for 30 min at 37°C. Following fixation, oocytes were treated with 0.5% Triton X-100 in PBS/PVA, blocked in 2% bovine serum albumin (BSA), and incubated in primary antibody (anti-BrdU; Sigma) diluted 1:300 in blocking buffer overnight at 4°C. Oocytes were then washed in blocking buffer, incubated in secondary antibody (anti-mouse-488; Life Technologies) diluted 1:200 in blocking buffer for 2 h at room temperature, washed again, and imaged on a 510 model confocal microscope using a 63×, 1.2 NA lens with excitation at 488 nm and emission at 530 nm. Oocytes were scored as transcriptionally active based on positive Br-UTP staining in the GV.

Preparation and Microinjection of Epab mRNA into Preantral Follicle-Enclosed Oocytes

pCR2.1-mEpab-HA vector was prepared as previously described [33]. To generate an empty pCR2.1-GFP vector, green fluorescent protein (GFP) was amplified from the pCMV6-GFP vector (Origene, Rockville, MD) using the primers: 5′ GGGAAATTCGAAATGGA-GAGCGACGAGAGC 3′ and 5′ GGGAAAAAGCTTCTCGAGTTAAACTCTTTCTTCACCG-GC 3′. The PCR product was then cloned into the pCR2.1 vector by using a TOPO-TA cloning kit (Invitrogen). Insert direction and sequences were confirmed by sequencing with M13 forward and reverse primers. The pCR2.1-mEpab-HA and pCR2.1-GFP vectors were linearized with *Hind*III and used as a template for in vitro transcription using mMESSAGE mMACHINE T7 kit (Ambion, Austin, TX). Following in vitro transcription, mRNAs were polyadenylated using a poly(A) tailing kit (Ambion) and stored at −80°C in nuclease-free water until microinjection.

Microinjection of oocytes and follicle-enclosed oocytes was carried out as described previously [36, 38]. Follicles were loaded into an injection chamber between 2 coverslips spaced ~100 μm apart. Quantitative microinjection was performed using pipettes backfilled with mercury, and concentrations of injected substances were calculated based on an oocyte volume of 200 pl [38]. mRNA was injected in a total volume of 10 pl. Injected follicle-enclosed oocytes were plated on collagen type I culture inserts (Becton Dickinson Biosciences, San Jose, CA) and cultured in a humidified atmosphere at 37°C with 5% CO_2 and 95% air. Following 9 to 11 days of culture, oocytes were removed from the follicle and assessed either immediately or following 17 to 18 h of in vitro maturation.

Determination of Metaphase II Arrest by Spindle Immunofluorescence

Following in vitro maturation, oocytes were fixed in 2% formaldehyde (in buffer containing 100 mM Hepes, 50 mM EGTA, 10 mM $MgSO_4$, and 0.2%

Triton X-100) for 1 h at 37°C, washed 3 times in PBS/PVA with 0.01% Triton X-100, incubated in blocking buffer (PBS containing 0.01% Triton X-100, 0.1% PVA, and 3% BSA) for 30 min at room temperature and then in anti-tubulin primary antibody (AbD Serotec, Raleigh, NC) diluted 1:100 in blocking buffer overnight at 4°C. Oocytes were then washed 3 times in blocking buffer and incubated in secondary antibody (anti-RAT 488; Invitrogen) diluted 1:200 in blocking buffer for 1 h at room temperature. Finally, oocytes were washed 4 times with PBS/PVA, with 5 μM SYTOX Orange in the first wash. Oocytes were examined usind a 510 confocal microscope, with 40× 1.2 NA lens using excitation at 488 nm and emission at 530 nm (tubulin) and excitation at 543 nm and emission at 570 nm (Sytox).

Statistical Analysis

Data are representative of at least 3 independent experiments, unless otherwise specified. Values were analyzed either by Student *t*-test, 1-way ANOVA, or 2-way ANOVA, as described in each figure legend. Percentages of data were transformed via arcsin square root prior to statistical analysis [39]. All statistical analyses were performed using Prism software (Graph Pad, San Diego, CA) and significance was assessed at a P value of ≤ 0.05.

RESULTS

Epab$^{-/-}$ *Oocytes Exhibit Morphological Abnormalities*

Because *Epab*$^{-/-}$ oocytes are incapable of progressing to MII, we examined morphological characteristics associated with meiotic competence such as the association with cumulus cells, oocyte size, and GV location [40–44]. Due to the increase in secondary follicle formation in *Epab*$^{-/-}$ ovaries [33], antral follicle-enclosed oocytes were selectively punctured to include only this population of oocytes. Approximately 50% of *Epab*$^{-/-}$ oocytes released from antral follicles were completely denuded, and only ~25% were fully enclosed by cumulus cells (Fig. 1). This is in contrast to *Epab*$^{+/+}$, where 14% were denuded and 62% were fully enclosed. Percentages of partially enclosed oocytes for *Epab*$^{+/+}$ were similar to that for *Epab*$^{-/-}$ oocytes (Fig. 1B). Oocytes were then stripped of cumulus cells and evaluated for size and GV location (Fig. 2A). *Epab*$^{-/-}$ oocytes were slightly but significantly smaller in size than *Epab*$^{+/+}$ oocytes (69.7 μm vs. 71.5 μm, respectively, $P < 0.0001$) (Fig. 2, B and C). In addition, 89% of *Epab*$^{-/-}$ oocytes contained peripheral GVs (Fig. 2D) compared to only 11% of *Epab*$^{+/+}$ oocytes. Overall, *Epab*$^{-/-}$ oocytes isolated from antral follicles exhibit morphological abnormalities such as peripheral GV location and fewer connections with cumulus cells. The small decrease in size of *Epab*$^{-/-}$ oocytes may not be meaningful in terms of oocyte development because the oocytes grew beyond 60 μm [44].

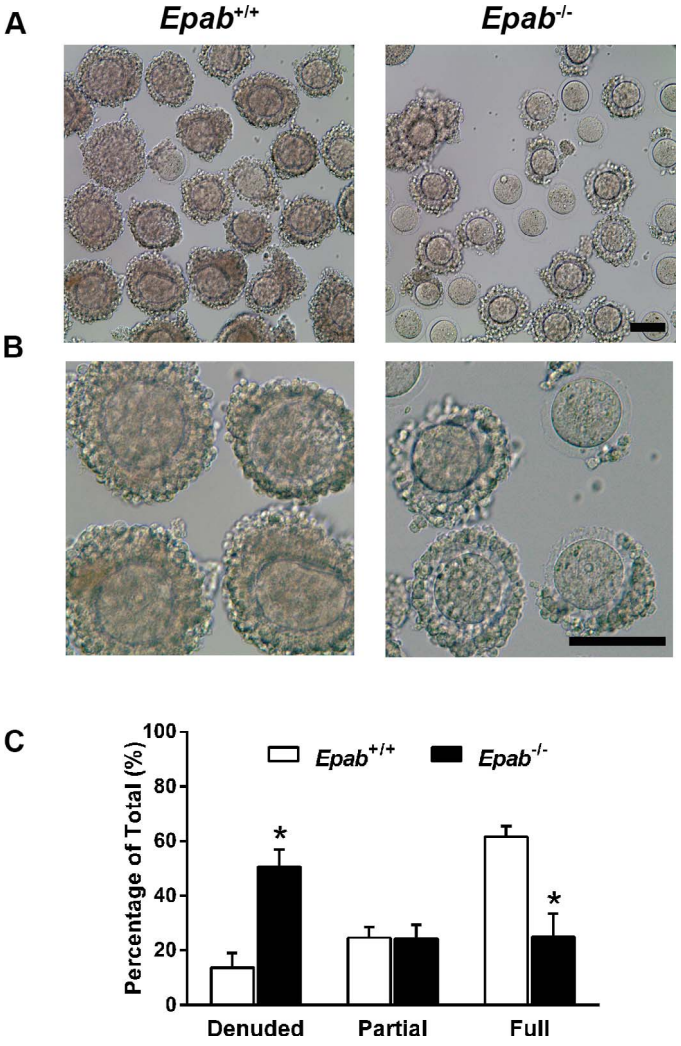

FIG. 1. *Epab*$^{-/-}$ oocytes isolated from antral follicles have fewer connections with cumulus cells. **A**) Representative images of *Epab*$^{+/+}$ and *Epab*$^{-/-}$ cumulus-oocyte complexes directly after antral follicles were punctured. Bar = 100 μm. **B**) Higher 3× magnification of images shown in **A**. Bar = 100 μm. **C**) Oocytes were scored as either denuded (lacking cumulus cells), partial (partially enclosed), or full (fully enclosed). Results are means ± SEM. *Significant difference compared to *Epab*$^{+/+}$, as determined by Student *t*-test, $P \leq 0.05$. Four *Epab*$^{+/+}$ and 3 *Epab*$^{-/-}$ mice from 2 litters were evaluated, and a total of at least 75 oocytes were scored for each morphological characteristic.

Epab$^{-/-}$ *Oocytes Fail to Progress to the SN-Type Chromatin Configuration and Become Transcriptionally Silent*

Chromatin configuration and transcriptional activity are often used as markers of meiotic and developmental competence as well as ovulation rates [45–47], all of which are abnormal in *Epab*$^{-/-}$ mice [33]. Chromatin configuration and transcriptional activity also correlate with oocyte size and GV location [37, 48, 49]. Oocytes initially contain uncondensed chromatin (NSN) that is permissive to transcriptional activity. However, as a follicle-enclosed oocyte grows, the DNA condenses and forms a tight ring around the nucleolus (SN). This transition from NSN to SN is thought to occur around the time of antrum formation and precedes oocyte maturation [46, 49, 50]. Generally, smaller oocytes with peripheral GVs are isolated at a more immature follicular stage and thus have NSN-type chromatin. Larger oocytes with central GVs are isolated from large antral follicles and have SN-type chromatin. Additionally, the somatic cells surrounding the oocyte are important for modulating chromatin reorganization and transcriptional silencing [51].

Chromatin configurations were examined in oocytes isolated from antral follicles of *Epab*$^{+/+}$ and *Epab*$^{-/-}$ ovaries by fixing and staining DNA with SYTOX Orange. Approximately 55% of *Epab*$^{-/-}$ oocytes contained uncondensed NSN chromatin, and only 25% of *Epab*$^{-/-}$ oocytes progressed to the SN configuration. In contrast, 25% of *Epab*$^{+/+}$ oocytes contained NSN chromatin, and 75% contained SN chromatin. The number and morphology of the heterochromatin spots in *Epab*$^{+/+}$ and *Epab*$^{-/-}$ NSN oocytes were similar (data not shown). Interestingly, 19% of *Epab*$^{-/-}$ oocytes exhibited chromatin that did not completely surround the nucleolus, which we termed PSN (Fig. 3, A and B). The PSN chromatin configuration was not observed in *Epab*$^{+/+}$ oocytes.

It has previously been shown that the SN configuration begins to form when oocytes reach ~50 μm and the percentage of SN oocytes increases significantly when antral follicles

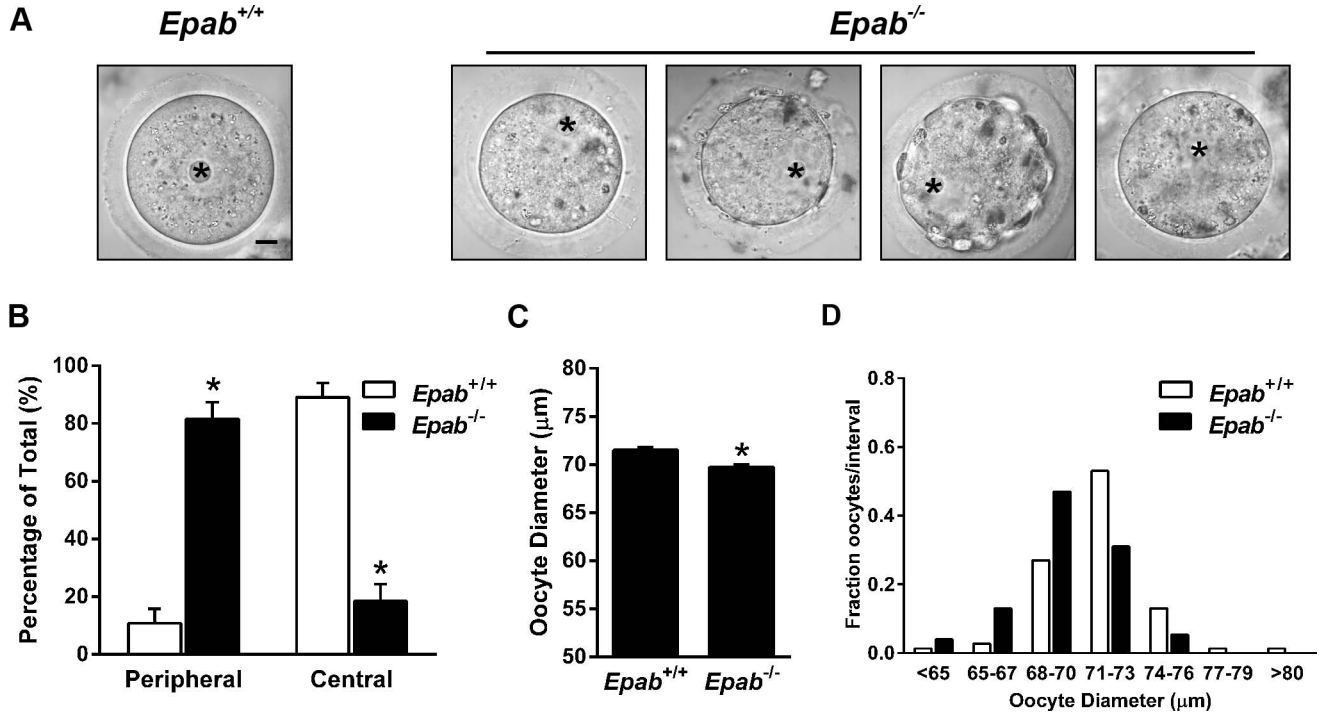

FIG. 2. Morphological characteristics of $Epab^{-/-}$ oocytes. Oocytes collected from eCG-primed $Epab^{+/+}$ and $Epab^{-/-}$ mice were characterized based on location of the GV and oocyte size. **A**) Representative transmitted light confocal images of $Epab^{+/+}$ and $Epab^{-/-}$ oocytes. Bar = 10 μm. *GVs. **B**) Location of the GV was scored by confocal microscopy as either peripheral or central. Results are means ± SEM. *Significant differences compared to $Epab^{+/+}$ as determined by Student *t*-test, $P \leq 0.01$. **C**) Oocyte size was determined using Image J analysis after calibration with a scale bar. Results are means ± SEM. *Significant differences compared to $Epab^{+/+}$ as determined by Student *t*-test, $P \leq 0.0001$. **D**) Distribution of $Epab^{+/+}$ and $Epab^{-/-}$ oocyte sizes in 3-μm intervals. Four $Epab^{+/+}$ and 3 $Epab^{-/-}$ mice from 2 litters were evaluated, and a total of at least 75 oocytes were scored for each morphological characteristic.

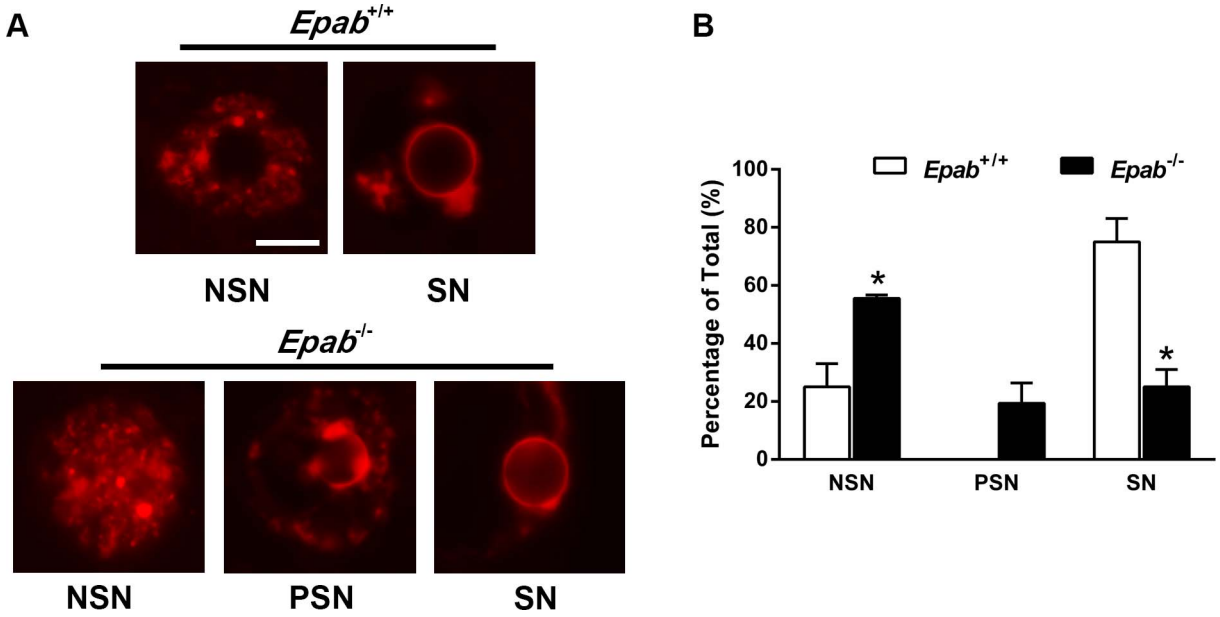

FIG. 3. $Epab^{-/-}$ oocytes did not progress to the SN-type chromatin configuration. Oocytes collected from eCG-primed mice were fixed and stained with SYTOX Orange. Chromatin configuration was evaluated by confocal microscopy and classified as nonsurrounded (NSN), surrounded (SN), or partially surrounded (PSN). **A**) Representative images of the chromatin configuration in oocytes isolated from $Epab^{+/+}$ and $Epab^{-/-}$ mice. Bar = 10 μm. **B**) Percentages of $Epab^{+/+}$ oocytes (n = 98) and $Epab^{-/-}$ oocytes (n = 84) exhibiting the different chromatin configurations are shown. Results are means ± SEM. *Significant differences compared to $Epab^{+/+}$ as determined by Student *t*-test, $P \leq 0.05$. Four $Epab^{+/+}$ and 3 $Epab^{-/-}$ mice from 2 litters were evaluated.

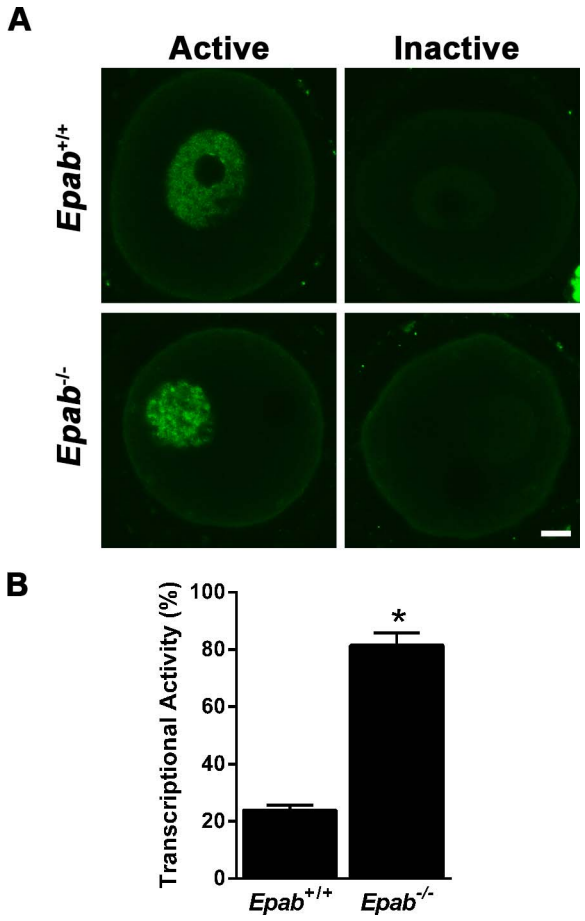

FIG. 4. *Epab*$^{-/-}$ oocytes remain transcriptionally active. Oocytes were collected from eCG-primed *Epab*$^{+/+}$ and *Epab*$^{-/-}$ mice, injected with Br-UTP, and examined for incorporation into nascent mRNA transcripts by immunofluorescence and confocal microscopy. **A**) Representative images show transcriptional activity. Bar = 10 μm. **B**) Percentages of *Epab*$^{+/+}$ oocytes (n = 46) and *Epab*$^{-/-}$ oocytes (n = 51) were scored as transcriptionally active. Results are means ± SEM. *Significant differences in transcriptional status between *Epab*$^{+/+}$ and *Epab*$^{-/-}$ oocytes, as determined by Student *t*-test, $P \leq 0.05$. Four *Epab*$^{+/+}$ and 4 *Epab*$^{-/-}$ mice from 4 litters were evaluated, and a total of at least 75 oocytes were scored.

develop and oocytes reach ~70 μm [49]. Although *Epab*$^{-/-}$ oocytes were smaller in size, the average size of *Epab*$^{-/-}$ oocytes from antral follicles was 69.7 μm, and most oocytes were larger than 68 μm (Fig. 2C). This finding suggests that size alone is most likely not responsible for the fewer SN oocytes in *Epab*$^{-/-}$ ovaries. Rather, the finding that *Epab*$^{-/-}$ oocytes maintain fewer connections with cumulus cells may be a contributing factor [51]. Overall, even in the presence of antrum formation, most *Epab*$^{-/-}$ oocytes fail to reach the SN configuration.

Global transcriptional silencing occurs concomitantly with changes in chromatin structure. Oocytes with SN-type chromatin configuration are generally transcriptionally silent, whereas oocytes with the NSN-type chromatin configuration are generally transcriptionally active [45]. Transcriptional activity was detected by injecting oocytes with Br-UTP and examining the incorporation into nascent mRNAs by immunofluorescence and confocal microscopy (Fig. 4A). Consistent with previous reports, ~24% of *Epab*$^{+/+}$ oocytes remained transcriptionally active, whereas most underwent transcriptional silencing. In contrast, ~82% of *Epab*$^{-/-}$ oocytes remained transcriptionally active and failed to undergo transcriptional silencing (Fig. 4B). Overall, the number of oocytes that exhibited NSN chromatin and transcriptional activity was significantly higher in *Epab*$^{-/-}$ mice than in *Epab*$^{+/+}$ mice.

Microinjection of Epab *mRNA into* Epab$^{-/-}$ *Follicle-Enclosed Oocytes at Preantral Stage Rescues Follicle Growth, Chromatin Reorganization, and Oocyte Maturation*

Previously, we showed that microinjection of *Epab* mRNA into denuded GV-stage oocytes from *Epab*$^{-/-}$ mice failed to rescue in vitro maturation [33], suggesting that EPAB is required at earlier steps during oogenesis. To determine the stage of oocyte development at which EPAB becomes necessary, we injected *Epab* mRNA into preantral follicle-enclosed oocytes (FEOs) isolated from *Epab*$^{-/-}$ mice. Oocytes within follicles at the preantral stage have not completed growth and are meiotically incompetent. Therefore, after microinjection, the follicle-enclosed oocytes were cultured on a collagen membrane for 9 to 11 days in order to promote oocyte and follicle growth. Uninjected *Epab*$^{-/-}$ follicles did not grow beyond 230 μm during the culture period, and only 38% resumed meiosis when isolated from the follicle (Fig. 5, A and C). Maturation did not proceed normally in *Epab*$^{-/-}$ oocytes because none of the oocytes that resumed meiosis formed morphologically normal spindles (Fig. 5, B and C). In contrast, uninjected *Epab*$^{+/+}$ follicles and *Epab*$^{-/-}$ follicles that were injected with *Epab* mRNA grew larger than 320 μm (Fig. 5A). A majority of these oocytes underwent GVBD following isolation from the follicle (97% and 77%, respectively) and formed morphologically normal spindles (84% and 75%, respectively) (Fig. 5, B and C). Injection of *Gfp* mRNA into follicle-enclosed *Epab*$^{-/-}$ oocytes served as a negative control; 29% of these oocytes underwent GVBD but did not progress normally to MII (Fig. 5, A–C).

In addition to oocyte maturation, we also examined whether microinjection of *Epab* mRNA into preantral follicle-enclosed *Epab*$^{-/-}$ oocytes could restore the progression from NSN- to SN-type chromatin configuration. Following the culture period, oocytes were isolated from the follicles, fixed, and stained with SYTOX Orange. The chromatin configurations for *Epab*$^{+/+}$ and *Epab*$^{-/-}$ follicle-enclosed oocytes grown in vitro were similar to that of our previous results where the oocytes were freshly isolated from antral follicles. A majority of *Epab*$^{+/+}$ oocytes (91%) contained SN-type chromatin, compared to only 33% of *Epab*$^{-/-}$ oocytes. Injection of *Epab* mRNA into *Epab*$^{-/-}$ follicle-enclosed oocytes significantly increased the percentage of oocytes that transitioned to the SN configuration (84%) (Fig. 6). We also measured the sizes of uninjected *Epab*$^{+/+}$ and *Epab*$^{-/-}$ oocytes and those injected with *Epab* mRNA after the culture period. The average size for uninjected *Epab*$^{-/-}$ oocytes was ~67 μm, whereas *Epab*$^{-/-}$ oocytes injected with *Epab* mRNA grew to ~75 μm, which was similar to the average size of 76 μm for *Epab*$^{+/+}$ oocytes (data not shown). Thus, injection of *Epab* mRNA into *Epab*$^{-/-}$ preantral follicle-enclosed oocytes restores follicle and oocyte growth, as well as the progression from NSN to SN-type chromatin configuration.

DISCUSSION

In the present study, we demonstrated that EPAB is essential during early stages of oogenesis for acquisition of meiotic competence. *Epab*$^{-/-}$ oocytes are smaller, with peripheral GVs, and have fewer connections with cumulus cells. *Epab*$^{-/-}$

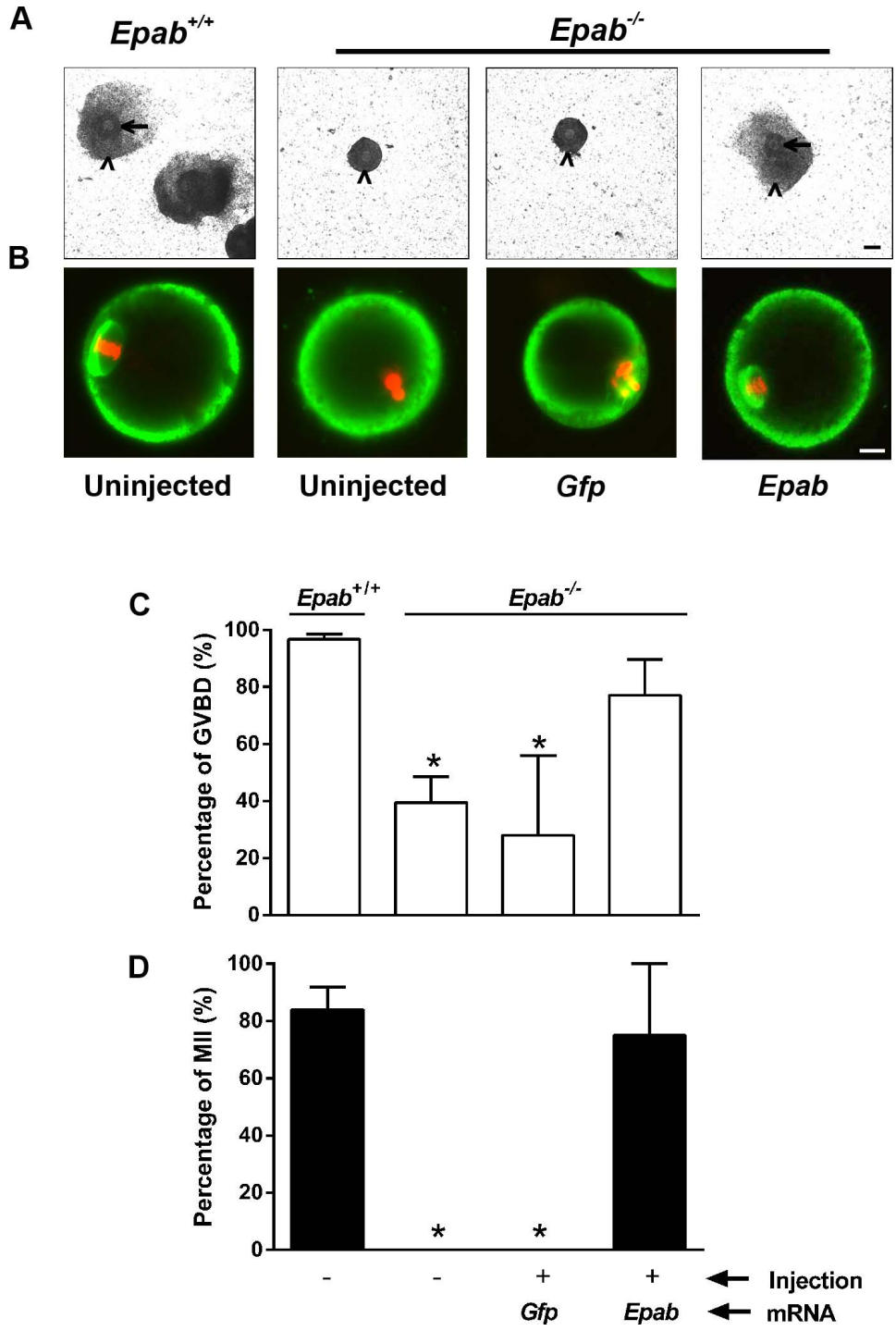

FIG. 5. Microinjection of *Epab* mRNA into *Epab*$^{-/-}$ preantral follicle-enclosed oocytes rescued oocyte maturation. Preantral follicle-enclosed oocytes were injected with *Epab* or *Gfp* mRNA and cultured on a collagen membrane for 9 to 11 days. After the culture period, oocytes were evaluated for GVBD and MII spindle formation. **A**) Representative images of follicles are shown after the culture period. Bar = 100 μm. Arrowhead indicates granulosa cells, and arrow indicates cumulus-enclosed oocyte complexes within the follicle. **B**) Representative images of spindle immunofluorescence shown after overnight maturation. Tubulin is shown in green, and DNA is shown in red. Bar = 10 μm. **C** and **D**) Percentage of oocytes that underwent GVBD (**C**) and matured normally to metaphase II (**D**) is shown. Results are means ± SEM. *Significantly lower number of *Epab*$^{-/-}$ oocytes underwent GVBD or formed normal MII spindles than *Epab*$^{+/+}$ oocytes ($P \leq 0.01$). Significance was determined by one-way ANOVA, Bonferroni's multiple comparisons test (n = 70 for *Epab*$^{+/+}$; n = 27 for uninjected *Epab*$^{-/-}$; n = 10 for *Gfp* injected *Epab*$^{-/-}$; and n = 18 for *Epab*-injected *Epab*$^{-/-}$ FEOs).

oocytes also fail to progress to SN-type chromatin configuration and become transcriptionally silent. The requirement of EPAB during oocyte growth is further supported by the finding that chromatin configuration and oocyte maturation can be rescued when *Epab* mRNA is introduced into follicle-enclosed *Epab*$^{-/-}$ oocytes at the preantral stage. Overall, these results demonstrate that EPAB is required not only during oocyte maturation for translational activation of maternal mRNAs, but also during oocyte development for establishing factors that regulate nuclear maturation and meiotic competence.

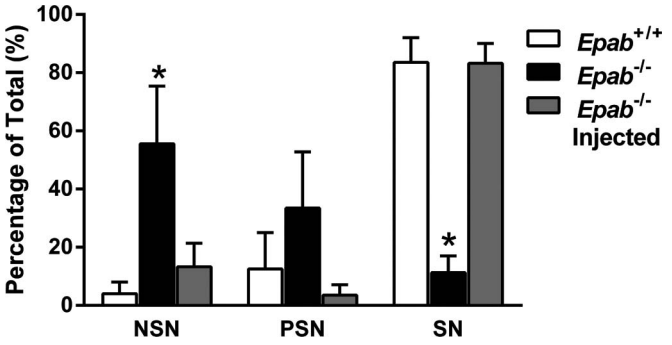

FIG. 6. Microinjection of *Epab* mRNA into $Epab^{-/-}$ preantral follicle-enclosed oocytes rescued progression to SN-type chromatin. Preantral follicles were dissected from $Epab^{+/+}$ and $Epab^{-/-}$ ovaries, and $Epab^{-/-}$ FEOs were injected with *Epab* mRNA. After 9 to 11 days of culture on a collagen membrane, oocytes were isolated from the follicles, fixed, and stained with SYTOX Orange for assessment of chromatin configuration. Graph represents the percentage of oocytes that contained NSN, PSN, or SN-type chromatin for uninjected $Epab^{+/+}$ (n = 23), uninjected $Epab^{-/-}$ (n = 27), and injected $Epab^{-/-}$ FEOs (n = 19). Data are means ± SEM. *Statistically significant differences compared to $Epab^{+/+}$ as determined by two-way ANOVA, multiple comparisons test, $P \leq 0.05$.

The oocyte nucleus, or GV, contains a unique chromatin configuration that is subject to dynamic modifications during oogenesis. Initially, oocytes contain uncondensed NSN-type chromatin that is dispersed throughout the GV. Around the time of antrum formation, the chromatin condenses and reorganizes into the SN-type configuration that surrounds the nucleolus [46, 49, 50, 52]. Chromatin configuration is associated with transcriptional activity such that oocytes with NSN chromatin have increased levels of transcriptional activity, whereas oocytes with SN chromatin exhibit repressed transcription (reviewed in [45]). Although the NSN-to-SN transition occurs concomitantly with transcriptional silencing, these 2 processes are likely to be regulated by different mechanisms [53]. Importantly, chromatin condensation and the associated decrease in transcriptional activity are often used as markers of meiotic and developmental competence. Although both NSN and SN oocytes can mature, they do so with different rates and characteristics [50]. Studies have shown that a higher percentage of oocytes with the SN configuration undergo GVBD and progress to MII compared to oocytes with the NSN configuration. More striking is the association between chromatin configuration and developmental competence because NSN oocytes are incapable of developing past the 2-cell stage in vitro [54, 55]. The timing of transcriptional silencing also appears to be important. If the period between transcriptional repression and maturation is experimentally extended, cleavage rates and blastocyst formation are significantly reduced [51]. Despite the association of chromatin configuration and transcriptional activity with early development, these processes alone do not determine competence [54]. Rather, changes in nuclear and cytoplasmic factors that occur simultaneously are more likely to be important [56]. It remains to be determined what these factors are and whether EPAB is directly responsible for regulating their expression.

Interestingly, the surrounding cumulus granulosa cells play an active role in modulating chromatin structure and transcriptional activity in the oocyte and ultimately coordinate nuclear and cytoplasmic meiotic competence [51, 57–59]. Somatic cells also have been shown to regulate translational activation of maternal mRNAs during oocyte maturation [60]. However, the signal(s) originating from the cumulus cells that are important for these processes remain to be identified. Most oocytes isolated from $Epab^{-/-}$ ovaries are either denuded or have fewer connections with cumulus cells. It is possible that the lack of oocyte–granulosa interactions could be a contributing factor associated with meiotic incompetence and failed chromatin reorganization and transcriptional silencing. In addition, this finding suggests that the somatic cells are also affected by EPAB deficiency, and this is consistent with impaired cumulus expansion and ovulation in $Epab^{-/-}$ mice. Ultimately, the somatic cell phenotype in $Epab^{-/-}$ mice must derive from a problem with the oocyte because EPAB is oocyte-specific. Because bidirectional communication between oocytes and somatic cells is critical for the function of both compartments [43, 61], the lack of EPAB in the oocyte could impair the differentiation or function of somatic cells, which could then lead to further problems in the oocyte. Therefore, miscommunication between the oocyte and somatic compartment could contribute to meiotic incompetence of $Epab^{-/-}$ oocytes.

The significance of EPAB in the function of the somatic compartment is currently being investigated. Perhaps the expression of oocyte-derived factors is affected in $Epab^{-/-}$ mice or an independent pathway that regulates folliculogenesis is involved. Another possibility is that miscommunication between the oocyte and somatic compartment is physical, such that gap junctions, transzonal processes, or other channels do not function properly. This would disrupt not only the transfer of oocyte-derived factors, but it would also disrupt signals initiated by the somatic cells. Whether the abnormalities we observed in $Epab^{-/-}$ oocytes are directly the result of EPAB deficiency in the oocyte or are an indirect secondary effect of an abnormal follicular environment is not known.

Two RNA binding proteins that are also essential for translational regulation during oocyte maturation are CPEB and DAZL. Similar to EPAB, both CPEB and DAZL are not only required for regulation of gene expression during oocyte maturation, but they are also important in the oocyte at earlier stages of gametogenesis. Deletion of CPEB results in germ cells that are arrested at the pachytene stage of prophase I. This arrest is due to loss of synaptonemal complex protein and failure of homologous chromosome pairing [62]. When CPEB is specifically reduced in prophase I oocytes, the poly(A) tail elongation of a number of key mRNAs, including *Gdf9, Smad5*, and *Hifoo*, is lost [63]. There is also a decreased number of oocytes and follicles, increased apoptosis, abnormal polar body formation, parthenogenetic cell division, and oocyte detachment from cumulus cells [63]. DAZL has been identified as an intrinsic factor required for germ cell entry into meiosis [64], and deletion of DAZL results in the complete loss of female germ cells before birth [65, 66]. Furthermore, specific knockdown of *Dazl* in prophase I-arrested oocytes followed by in vitro maturation results in lower percentage of oocytes that undergo GVBD and expel a polar body as well as formation of abnormal spindles. DAZL knockdown also completely prevents fertilization, as demonstrated by the absence of 2-cell embryos [21]. Overall, these studies emphasize the importance of RNA binding proteins at several steps during gametogenesis.

Deletion of other RNA binding proteins in the oocyte also produces similar phenotypes associated with chromatin organization and transcriptional activity. For example, MSY2 is an oocyte-specific RNA binding protein that regulates mRNA stability [67–71]. The absence of MSY2 perturbs oocyte growth and maturation, RNA stability, and the transcriptome. MSY2-deficient oocytes also do not undergo transcriptional silencing or progression to SN and are loosely associated with cumulus cells [72]. Another example is

ELAVL2, an AU-rich element binding protein specific to the oocyte that acts as a translational repressor. Interestingly, ELAVL2 is abundant in NSN oocytes but is ablated during progression from NSN to SN. ELAVL2 appears to be important for oocyte growth and meiotic competence because prematurely reduced *Elavl2* expression results in lower yields of fully grown and meiotically matured oocytes. Furthermore, *Elavl2* knockdown promotes translational activity in fully grown oocytes [73]. Thus, several RNA binding proteins in addition to EPAB are important for producing fully grown oocytes that have complete developmental competence, underscoring the importance of additional pathways that regulate gene expression during oocyte development.

In summary, EPAB is important in the oocyte not only for translational activation of maternal mRNAs during maturation but also for acquisition of meiotic competence during oocyte development. It is not known whether the observed phenotypes are a secondary effect of a disrupted follicular environment or a more direct role of EPAB in regulating specific mRNAs in the oocyte. Overall, based on these findings, it is reasonable to propose that EPAB interacts with different complexes to regulate translation at different times during oocyte growth and oocyte maturation. Future studies are aimed toward identifying which mRNAs EPAB binds to and is directly involved in regulating their translation, as well as other regulatory proteins that EPAB associates with in mouse oocytes. In *Xenopus* oocytes, EPAB associates with CPEB and components of the cytoplasmic poly(A) machinery [31], PUM-2 [23] and DAZL [22]. It will be interesting to determine if these interactions are conserved in mouse oocytes and whether EPAB-bound mRNAs can be identified based on CPE motifs and DAZL-binding sites. Such studies will not only provide insight into additional pathways that regulate post-transcriptional gene expression but may also uncover novel mRNAs that are essential during oocyte formation and early embryonic development.

ACKNOWLEDGMENT

We thank Emre Seli and laboratory members for helpful discussions and Marina Freudzon and Jan Ellenberg (European Molecular Biology Laboratory, Heidelberg, Germany) for providing their method of culturing preantral follicle-enclosed oocytes, which was modified for this work. We also thank Laurinda Jaffe (Department of Cell Biology, University of Connecticut Health, Farmington, CT) and Center for Cellular and Molecular Imaging (Yale School of Medicine, New Haven, CT) for use of confocal microscopes.

REFERENCES

1. Eppig JJ. Oocyte-somatic cell communication in the ovarian follicles of mammals. Semin Dev Biol 1994; 5:51–59.
2. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 1996; 17:121–155.
3. Mehlmann LM. Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. Reproduction 2005; 130:791–799.
4. Eppig JJ. Intercommunication between mammalian oocytes and companion somatic cells. Bioessays 1991; 13:569–574.
5. Richards JS, Russell DL, Ochsner S, Espey LL. Ovulation: new dimensions and new regulators of the inflammatory-like response. Annu Rev Physiol 2002; 64:69–92.
6. Matova N, Cooley L. Comparative aspects of animal oogenesis. Dev Biol 2001; 231:291–320.
7. Schultz RM. Regulation of zygotic gene activation in the mouse. Bioessays 1993; 15:531–538.
8. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 1988; 332:459–461.
9. Clegg KB, Piko L. RNA synthesis and cytoplasmic polyadenylation in the one-cell mouse embryo. Nature 1982; 295:343–344.
10. Flach G, Johnson MH, Braude PR, Taylor RA, Bolton VN. The transition from maternal to embryonic control in the 2-cell mouse embryo. EMBO J 1982; 1:681–686.
11. Brower PT, Gizang E, Boreen SM, Schultz RM. Biochemical studies of mammalian oogenesis: synthesis and stability of various classes of RNA during growth of the mouse oocyte in vitro. Dev Biol 1981; 86:373–383.
12. Bachvarova R, De Leon V. Polyadenylated RNA of mouse ova and loss of maternal RNA in early development. Dev Biol 1980; 74:1–8.
13. Tadros W, Lipshitz HD. The maternal-to-zygotic transition: a play in two acts. Development 2009; 136:3033–3042.
14. Radford HE, Meijer HA, de Moor CH. Translational control by cytoplasmic polyadenylation in *Xenopus* oocytes. Biochim Biophys Acta 2008; 1779:217–229.
15. Clarke HJ. Post-transcriptional control of gene expression during mouse oogenesis. Results Probl Cell Differ 2012; 55:1–21.
16. Gebauer F, Richter JD. Mouse cytoplasmic polyadenylylation element binding protein: an evolutionarily conserved protein that interacts with the cytoplasmic polyadenylylation elements of c-mos mRNA. Proc Natl Acad Sci U S A 1996; 93:14602–14607.
17. Hake LE, Richter JD. CPEB is a specificity factor that mediates cytoplasmic polyadenylation during *Xenopus* oocyte maturation. Cell 1994; 79:617–627.
18. Barnard DC, Ryan K, Manley JL, Richter JD. Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. Cell 2004; 119: 641–651.
19. Stebbins-Boaz B, Hake LE, Richter JD. CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte maturation in *Xenopus*. EMBO J 1996; 15:2582–2592.
20. Vasudevan S, Seli E, Steitz JA. Metazoan oocyte and early embryo development program: a progression through translation regulatory cascades. Genes Dev 2006; 20:138–146.
21. Chen J, Melton C, Suh N, Oh JS, Horner K, Xie F, Sette C, Blelloch R, Conti M. Genome-wide analysis of translation reveals a critical role for deleted in azoospermia-like (Dazl) at the oocyte-to-zygote transition. Genes Dev 2011; 25:755–766.
22. Collier B, Gorgoni B, Loveridge C, Cooke HJ, Gray NK. The DAZL family proteins are PABP-binding proteins that regulate translation in germ cells. EMBO J 2005; 24:2656–2666.
23. Padmanabhan K, Richter JD. Regulated Pumilio-2 binding controls RINGO/Spy mRNA translation and CPEB activation. Genes Dev 2006; 20:199–209.
24. Pique M, Lopez JM, Foissac S, Guigo R, Mendez R. A combinatorial code for CPE-mediated translational control. Cell 2008; 132:434–448.
25. Brook M, Smith JW, Gray NK. The DAZL and PABP families: RNA-binding proteins with interrelated roles in translational control in oocytes. Reproduction 2009; 137:595–617.
26. Kahvejian A, Roy G, Sonenberg N. The mRNA closed-loop model: the function of PABP and PABP-interacting proteins in mRNA translation. Cold Spring Harb Symp Quant Biol 2001; 66:293–300.
27. Guzeloglu-Kayisli O, Pauli S, Demir H, Lalioti MD, Sakkas D, Seli E. Identification and characterization of human embryonic poly(A) binding protein (EPAB). Mol Hum Reprod 2008; 14:581–588.
28. Seli E, Lalioti MD, Flaherty SM, Sakkas D, Terzi N, Steitz JA. An embryonic poly(A)-binding protein (ePAB) is expressed in mouse oocytes and early preimplantation embryos. Proc Natl Acad Sci U S A 2005; 102: 367–372.
29. Voeltz GK, Ongkasuwan J, Standart N, Steitz JA. A novel embryonic poly(A) binding protein, ePAB, regulates mRNA deadenylation in Xenopus egg extracts. Genes Dev 2001; 15:774–788.
30. Wilkie GS, Gautier P, Lawson D, Gray NK. Embryonic poly(A)-binding protein stimulates translation in germ cells. Mol Cell Biol 2005; 25: 2060–2071.
31. Kim JH, Richter JD. RINGO/cdk1 and CPEB mediate poly(A) tail stabilization and translational regulation by ePAB. Genes Dev 2007; 21: 2571–2579.
32. Friend K, Brook M, Bezirci FB, Sheets MD, Gray NK, Seli E. Embryonic poly(A)-binding protein (ePAB) phosphorylation is required for Xenopus oocyte maturation. Biochem J 2012; 445:93–100.
33. Guzeloglu-Kayisli O, Lalioti MD, Aydiner F, Sasson I, Ilbay O, Sakkas D, Lowther KM, Mehlmann LM, Seli E. Embryonic poly(A)-binding protein (EPAB) is required for oocyte maturation and female fertility in mice. Biochem J 2012; 446:47–58.
34. de Vant'ery C, Gavin AC, Vassalli JD, Schorderet-Slatkine S. An accumulation of p34cdc2 at the end of mouse oocyte growth correlates with the acquisition of meiotic competence. Dev Biol 1996; 174:335–344.
35. Schultz RM, Wassarman PM. Biochemical studies of mammalian

oogenesis: Protein synthesis during oocyte growth and meiotic maturation in the mouse. J Cell Sci 1977; 24:167–194.
36. Jaffe LA, Norris RP, Freudzon M, Ratzan WJ, Mehlmann LM. Microinjection of follicle-enclosed mouse oocytes. Methods Mol Biol 2009; 518:157–173.
37. Bouniol-Baly C, Hamraoui L, Guibert J, Beaujean N, Szollosi MS, Debey P. Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes. Biol Reprod 1999; 60:580–587.
38. Kline D. Quantitative microinjection of mouse oocytes and eggs. Methods Mol Biol 2009; 518:135–156.
39. Steel R, Torrie J. Principles and Procedures of Statistics: a Biometrical Approach. New York: McGraw-Hill Book Co; 1980.
40. Brower PT, Schultz RM. Intercellular communication between granulosa cells and mouse oocytes: existence and possible nutritional role during oocyte growth. Dev Biol 1982; 90:144–153.
41. Brunet S, Maro B. Germinal vesicle position and meiotic maturation in mouse oocyte. Reproduction 2007; 133:1069–1072.
42. Chesnel F, Wigglesworth K, Eppig JJ. Acquisition of meiotic competence by denuded mouse oocytes: participation of somatic-cell product(s) and cAMP. Dev Biol 1994; 161:285–295.
43. Kidder GM, Vanderhyden BC. Bidirectional communication between oocytes and follicle cells: ensuring oocyte developmental competence. Can J Physiol Pharmacol 2010; 88:399–413.
44. Sorensen R, Wassarman P. Relationship between growth and meiotic maturation of the mouse oocyte. Dev Biol 1976; 50:531–536.
45. De La Fuente R. Chromatin modifications in the germinal vesicle (GV) of mammalian oocytes. Dev Biol 2006; 292:1–12.
46. Mattson BA, Albertini DF. Oogenesis: chromatin and microtubule dynamics during meiotic prophase. Mol Reprod Dev 1990; 25:374–383.
47. Tan JH, Wang HL, Sun XS, Liu Y, Sui HS, Zhang J. Chromatin configurations in the germinal vesicle of mammalian oocytes. Mol Hum Reprod 2009; 15:1–9.
48. Bellone M, Zuccotti M, Redi CA, Garagna S. The position of the germinal vesicle and the chromatin organization together provide a marker of the developmental competence of mouse antral oocytes. Reproduction 2009; 138:639–643.
49. Zuccotti M, Piccinelli A, Giorgi Rossi P, Garagna S, Redi CA. Chromatin organization during mouse oocyte growth. Mol Reprod Dev 1995; 41: 479–485.
50. Debey P, Szollosi MS, Szollosi D, Vautier D, Girousse A, Besombes D. Competent mouse oocytes isolated from antral follicles exhibit different chromatin organization and follow different maturation dynamics. Mol Reprod Dev 1993; 36:59–74.
51. De La Fuente R, Eppig JJ. Transcriptional activity of the mouse oocyte genome: companion granulosa cells modulate transcription and chromatin remodeling. Dev Biol 2001; 229:224–236.
52. Wickramasinghe D, Ebert KM, Albertini DF. Meiotic competence acquisition is associated with the appearance of M-phase characteristics in growing mouse oocytes. Dev Biol 1991; 143:162–172.
53. De La Fuente R, Viveiros MM, Burns KH, Adashi EY, Matzuk MM, Eppig JJ. Major chromatin remodeling in the germinal vesicle (GV) of mammalian oocytes is dispensable for global transcriptional silencing but required for centromeric heterochromatin function. Dev Biol 2004; 275: 447–458.
54. Inoue A, Nakajima R, Nagata M, Aoki F. Contribution of the oocyte nucleus and cytoplasm to the determination of meiotic and developmental competence in mice. Hum Reprod 2008; 23:1377–1384.
55. Zuccotti M, Ponce RH, Boiani M, Guizzardi S, Govoni P, Scandroglio R, Garagna S, Redi CA. The analysis of chromatin organisation allows selection of mouse antral oocytes competent for development to blastocyst. Zygote 2002; 10:73–78.
56. Ma JY, Li M, Luo YB, Song S, Tian D, Yang J, Zhang B, Hou Y, Schatten H, Liu Z, Sun QY. Maternal factors required for oocyte developmental competence in mice: transcriptome analysis of non-surrounded nucleolus (NSN) and surrounded nucleolus (SN) oocytes. Cell Cycle 2013; 12: 1928–1938.
57. Carabatsos MJ, Sellitto C, Goodenough DA, Albertini DF. Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. Dev Biol 2000; 226: 167–179.
58. Eppig JJ. Oocyte control of ovarian follicular development and function in mammals. Reproduction 2001; 122:829–838.
59. Liu H, Aoki F. Transcriptional activity associated with meiotic competence in fully grown mouse GV oocytes. Zygote 2002; 10:327–332.
60. Chen J, Torcia S, Xie F, Lin CJ, Cakmak H, Franciosi F, Horner K, Onodera C, Song JS, Cedars MI, Ramalho-Santos M, Conti M. Somatic cells regulate maternal mRNA translation and developmental competence of mouse oocytes. Nat Cell Biol 2013; 15:1415–1423.
61. Su YQ, Sugiura K, Eppig JJ. Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. Semin Reprod Med 2009; 1:32–42.
62. Tay J, Richter JD. Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. Dev Cell 2001; 1: 201–213.
63. Racki WJ, Richter JD. CPEB controls oocyte growth and follicle development in the mouse. Development 2006; 133:4527–4537.
64. Lin Y, Gill ME, Koubova J, Page DC. Germ cell-intrinsic and -extrinsic factors govern meiotic initiation in mouse embryos. Science 2008; 322: 1685–1687.
65. Lin Y, Page DC. Dazl deficiency leads to embryonic arrest of germ cell development in XY C57BL/6 mice. Dev Biol 2005; 288:309–316.
66. Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P, Dorin J, Cooke HJ. The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. Nature 1997; 389:73–77.
67. Medvedev S, Yang J, Hecht NB, Schultz RM. CDC2A (CDK1)-mediated phosphorylation of MSY2 triggers maternal mRNA degradation during mouse oocyte maturation. Dev Biol 2008; 321:205–215.
68. Yang J, Medvedev S, Yu J, Tang LC, Agno JE, Matzuk MM, Schultz RM, Hecht NB. Absence of the DNA-/RNA-binding protein MSY2 results in male and female infertility. Proc Natl Acad Sci U S A 2005; 102: 5755–5760.
69. Yu J, Deng M, Medvedev S, Yang J, Hecht NB, Schultz RM. Transgenic RNAi-mediated reduction of MSY2 in mouse oocytes results in reduced fertility. Dev Biol 2004; 268:195–206.
70. Yu J, Hecht NB, Schultz RM. Expression of MSY2 in mouse oocytes and preimplantation embryos. Biol Reprod 2001; 65:1260–1270.
71. Yu J, Hecht NB, Schultz RM. RNA-binding properties and translation repression in vitro by germ cell-specific MSY2 protein. Biol Reprod 2002; 67:1093–1098.
72. Medvedev S, Pan H, Schultz RM. Absence of MSY2 in mouse oocytes perturbs oocyte growth and maturation, RNA stability, and the transcriptome. Biol Reprod 2011; 85:575–583.
73. Chalupnikova K, Solc P, Sulimenko V, Sedlacek R, Svoboda P. An oocyte-specific ELAVL2 isoform is a translational repressor ablated from meiotically competent antral oocytes. Cell Cycle 2014; 13:1187–1200.