Developmental Biology 434 (2018) 196–205

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

The switch from cAMP-independent to cAMP-dependent arrest of meiotic prophase is associated with coordinated GPR3 and CDK1 expression in mouse oocytes

Laura D. Firmani, Tracy F. Uliasz, Lisa M. Mehlmann*

Department of Cell Biology, UConn Health, Farmington, CT 06030, USA

ARTICLE INFO

Keywords:
Meiotic competence
cAMP
CDK1
GPR3

ABSTRACT

Mammalian oocytes are arrested in meiotic prophase from around the time of birth until just before ovulation. Following an extended period of growth, they are stimulated to mature to the metaphase II stage by a preovulatory luteinizing hormone (LH) surge that occurs with each reproductive cycle. Small, growing oocytes are not competent to mature into fertilizable eggs because they do not possess adequate amounts of cell cycle regulatory proteins, particularly cyclin-dependent kinase 1 (CDK1). As oocytes grow, they synthesize CDK1 and acquire the ability to mature. After oocytes achieve meiotic competence, meiotic arrest at the prophase stage is dependent on high levels of cAMP that are generated in the oocyte under the control of the constitutively active G_s-coupled receptor, GPR3. In this study, we examined the switch between GPR3-independent and GPR3-dependent meiotic arrest. We found that the ability of oocytes to mature, as well as oocyte CDK1 levels, were dependent on follicle size, but CDK1 expression in oocytes from preantral follicles was not acutely altered by the activity of follicle stimulating hormone (FSH). *Gpr3* was expressed and active in incompetent oocytes within early stage follicles, well before cAMP is required to maintain meiotic arrest. Oocytes from *Gpr3*$^{-/-}$ mice were less competent to mature than oocytes from *Gpr3*$^{+/+}$ mice, as assessed by the time course of germinal vesicle breakdown. Correspondingly, *Gpr3*$^{-/-}$ oocytes contained significantly lower CDK1 levels than their *Gpr3*$^{+/+}$ counterparts that were at the same stage of follicle development. These results demonstrate that GPR3 potentiates meiotic competence, most likely by raising cAMP.

1. Introduction

Mammalian oocytes are arrested in meiotic prophase for most of a female's reproductive lifespan and they remain arrested in prophase during a long period of oocyte and follicle growth. When follicles reach the preovulatory stage they become responsive to a surge in luteinizing hormone (LH) that occurs once per reproductive cycle. The LH surge signals the oocyte (via its action on follicle cells) to resume meiosis. Two mechanisms keep oocytes in meiotic arrest and prevent them from spontaneously resuming meiosis while in the ovary, one of which depends on cAMP and one that does not.

Meiotically arrested mouse oocytes progressively increase in size from ~20 to ~75 μm in diameter (Eppig and O'Brien, 1996; Picton et al., 1998). During most of this period of growth, prophase arrest is maintained because oocytes have not yet synthesized and accumulated mRNAs and proteins that are necessary for progression past prophase (Sorensen and Wassarman, 1976). Once the oocyte becomes competent to mature, meiotic arrest becomes dependent on high levels of cAMP, which is generated under the control of the constitutively active, G_s-coupled receptor, GPR3 (Hinckley et al., 2005; Ledent et al., 2005; Mehlmann, 2005b; Mehlmann et al., 2002, 2004). The elevated level of cAMP during meiotic arrest activates protein kinase A (PKA), which leads to the phosphorylation and inactivation of cyclin-dependent kinase 1 (CDK1; also known as $p34^{cdc2}$); the activation of CDK1 when cAMP levels are decreased is essential for meiotic resumption (Mehlmann, 2005a; Holt et al., 2013; Mehlmann, 2013). Cyclic AMP levels in the oocyte are kept high by cGMP contributed from the follicle cells; cGMP inhibits the activity of PDE3A, the phosphodiesterase in the oocyte that degrades cAMP when it is active (Mehlmann, 2005a). If meiotically competent oocytes are isolated from follicle cells, cAMP levels decrease (Schultz et al., 1983) and oocytes undergo spontaneous GVBD (Edwards, 1965); thus, both the oocyte and follicle cells contribute to cAMP-dependent meiotic arrest.

Oocytes reportedly achieve meiotic competence when they reach ~80% of their full-grown size (> 60 μm) (Hirao et al., 1993; Szybek, 1972; Wassarman and Josefowicz, 1978). This occurs concomitantly

* Corresponding author.
E-mail address: lmehlman@uchc.edu (L.M. Mehlmann).

https://doi.org/10.1016/j.ydbio.2017.12.014
Received 8 September 2017; Received in revised form 18 December 2017; Accepted 19 December 2017
Available online 21 December 2017
0012-1606/ Published by Elsevier Inc.

with folliculogenesis and coincides with the antrum formation in the follicle (Szybek, 1972). CDK1, as well as its binding partner Cyclin B, are important for this process (Adhikari et al., 2012; Chesnel and Eppig, 1995). The relative and absolute amounts of CDK1 and Cyclin B protein have been measured, and both increase significantly during oocyte growth, whereas the relative amount of Cyclin B is always in excess of CDK1 (Chesnel and Eppig, 1995; de Vantéry et al., 1996; Kanatsu-Shinohara et al., 2000; Mitra and Schultz, 1996); therefore, CDK1 is a limiting factor in the acquisition of meiotic competence. Thus far, CDK1 levels have only been measured in populations of oocytes removed from 10 to 12-day-old ovaries compared with fully-grown oocytes removed from 6-week-old mice that had previously been injected with hormones to stimulate follicular growth. Meiotic competence is not solely dependent on CDK1 levels, however, because CDK1 levels increase in cultured small, nongrowing, incompetent oocytes without inducing meiotic maturation (Chesnel and Eppig, 1995). In addition, overexpression of CDK1 in incompetent mouse oocytes is not sufficient to cause meiotic resumption (de Vantéry et al., 1997).

Because meiotic arrest begins in a cAMP-independent manner and progresses to GPR3 (and therefore cAMP)- dependent arrest, there must be a switch in maintaining meiotic arrest, at some point, during oogenesis. No information exists as to when the GPR3 system turns on to achieve this switch. In addition to its established role in maintaining meiotic arrest in meiotically competent oocytes, there is evidence that cAMP promotes the acquisition of meiotic competence in growing oocytes (Carroll et al., 1991; Chesnel et al., 1994). This is based on experiments demonstrating that incompetent oocytes can acquire meiotic competence after being cultured in the presence of the cAMP analog, dbcAMP (Chesnel et al., 1994). However, whether cAMP is produced within the oocyte to contribute to the acquisition of meiotic competence, as well as the source of this cAMP in growing oocytes, has not been investigated.

In this study, we examined the mechanisms of the acquisition of meiotic competence associated with the switch in meiotic regulation from cAMP-independent arrest to cAMP-dependent arrest. In addition, because of the evidence that cAMP contributes to the acquisition of meiotic competence, and given that GPR3 is the receptor that stimulates cAMP production in oocytes, we investigated the potential role of GPR3 in the acquisition of meiotic competence. We found that CDK1 levels increase during the period when an oocyte becomes competent to resume meiosis. In addition, *Gpr3* mRNA is expressed in incompetent oocytes very early on in follicle development and cAMP is generated prior to antral follicle development. Furthermore, the expression of *Gpr3* appears to potentiate meiotic competence in oocytes that are at the stage where meiotic competence is usually achieved. The data presented here demonstrate that the switch to the GPR3 system occurs before cAMP is required to maintain meiotic arrest, thus ensuring that this system will be in place when it is required.

2. Materials and methods

2.1. Media and reagents

Unless otherwise noted, all reagents were from Sigma Aldrich (St. Louis, MO). In most cases, the media used for oocyte and follicle isolation was MEMα (Life Technologies, Carlsbad, CA) supplemented with 20 mM HEPES, 75 μg/ml penicillin G, 50 μg/ml streptomycin, 0.1% polyvinyl alcohol (PVA), and 10 μM milrinone. For experiments in which we cultured follicles with follicle stimulating hormone (FSH), we used bicarbonate-buffered MEMα (Mehlmann, 2005b) supplemented with 5% fetal bovine serum (FBS; see below). For experiments in which we treated oocytes with dbcAMP, follicles were isolated in HEPES-buffered MEMα containing 10 μM milrinone, and oocytes were removed and cultured overnight in CZB medium (Chatot et al., 1989) supplemented with 5% FBS, 10 μM milrinone, and 250 μM dbcAMP.

2.2. Oocyte and follicle isolation and culture

23–26-day-old mice were used for most experiments. In a few cases, we also used 2-, 8-, and 11-day-old mice. *Gpr3*$^{-/-}$ mice were generated as described previously (Mehlmann et al., 2004) and the colony was maintained by breeding *Gpr3*$^{+/-}$ mice to generate *Gpr3*$^{-/-}$ and *Gpr3*$^{+/+}$ mice for littermate controls. If littermates were not available, age-matched wild type C57Bl/6 J mice were obtained from Jackson Labs (Bar Harbor, ME). Mice were genotyped as previously described (Mehlmann et al., 2004). Follicles were manually dissected from ovaries using 30 gauge needles. Oocytes were isolated from ovaries following antral follicle puncture. Cumulus cells were removed by repeated pipetting through a small-bore pipet.

For experiments in which follicles of different sizes were used, follicles were measured using a calibrated ocular reticle. Oocytes were released from the follicles by tearing a slit on one side. For the time course experiments, follicles were isolated from ovaries in medium containing 10 μM milrinone to prevent germinal vesicle breakdown (GVBD), were grouped according to size, and the oocytes removed from the follicles. The cumulus cells were removed and the isolated oocytes were then washed into fresh medium without milrinone to initiate meiotic resumption. Oocytes were scored hourly using a Zeiss Discovery V8 stereomicroscope for GVBD. Isolated oocytes were imaged using a 40× 1.4 NA lens and oocyte diameters were measured using ImageJ (NIH).

For experiments in which follicles were cultured with or without FSH, we isolated preantral follicles in bicarbonate-buffered MEMα without milrinone (Mehlmann, 2005b) and plated them individually on Millicell membranes (Millipore, Billerica, MA), plating a maximum of 20 follicles per Millicell. Half the follicles were incubated in medium containing 30 ng/ml ovine FSH (from A. F. Parlow, National Hormone and Peptide Program, Torrance, CA). Oocytes were removed from follicles following a 20 h incubation period. Only GV-intact oocytes within undamaged follicles were utilized for experiments.

2.3. Western Blotting

Oocytes were obtained from follicles of defined sizes, cumulus cells were removed, and oocytes were transferred to microfuge tubes in a small volume of medium. 15 μl of 1× sample buffer (Laemmli, 1970) containing 5% β-mercaptoethanol was added to each tube, samples were boiled for 5 min, and the whole volume was loaded into a 4–20% polyacrylamide gel (BioRad). Separated proteins were electrophoretically transferred onto nitrocellulose membranes and the membranes were blocked in 1% non-fat dry milk. Primary antibody (anti-CDK1 from Santa Cruz, catalog #sc-53219) was diluted to 2 μg/ml and was incubated on the blot overnight at 4 °C. Peroxidase-conjugated secondary antibody (Santa Cruz) was diluted 1:5000 in blocking buffer. Blots were developed using either ECL Prime (GE Healthcare, Chicago, IL) or WesternBright Sirius horseradish peroxidase substrate (Advansta, Menlo Park, CA), and imaged using a charge-coupled device camera (G:box Chemi XT4; Syngene). ImageJ software was used to perform the densitometry.

2.4. Salmon-gal staining

Whole ovaries were dissected and were either cut into smaller pieces, or follicles were separated manually. In some cases, isolated oocytes were used. Salmon-gal staining was performed as previously described (Sundararajan et al., 2012). Briefly, ovaries, follicles, and isolated oocytes were fixed for 5 min in PBS containing 2% formalin, 0.2% glutaraldehyde, 5 mM EGTA, 2 mM $MgCl_2$, and 0.1% PVA, then were washed 3 times in rinse solution containing 0.1% sodium deoxycholate, 0.2% Nonidet p40, and 2 mM $MgCl_2$. After the rinses, staining was conducted using 1 mg/ml salmon-gal and 0.4 mM 4-nitro blue tetrazolium chloride (NBT) for 10–20 min at 37 °C. Two final

washes of PBS with PVA were performed and all tissues were plated on Millicell culture plates (Millipore) and imaged using either a 10×, 0.3 N.A. or a 20×, 0.5 N.A. lens. Photographs were taken using a Canon EOS M digital camera. Stained and unstained follicles, excluding the stroma outside the basement membrane, were measured using ImageJ.

2.5. cAMP measurements

Oocytes obtained from *Gpr3*$^{+/+}$ and *Gpr3*$^{-/-}$ follicles that were ~140–180 μm in diameter were isolated in medium containing milrinone and cleared of all cumulus cells using a small-bore mouth pipet. Oocytes were microinjected with the cAMP sensor protein, Epac2-camps300 (Lowther et al., 2011; Nikolaev et al., 2004; Norris et al., 2009), as previously described (Kline, 2009; Norris et al., 2009), except that we used a final concentration in the oocyte of 2 μM. Fluorescence was excited at 435 nm and was detected at 535/50 nm (YFP) and 480/40 nm (CFP). Images were collected using a 40× 1.4 NA water immersion objective on a Zeiss Pascal confocal microscope. Following a scan in the presence of milrinone, milrinone was washed out of the medium and scans were performed 30 min after removing milrinone. YFP and CFP intensities were quantified within a circular fluorescent region of interest using the Zeiss Zen software. cAMP concentrations were calculated as described in Norris et al. (2009), using the standard curve that was generated by recording the basal YFP:CFP ratios before and after injecting cAMP to obtain the sensor maximum, and injecting PDE3A (to lower cAMP to a minimum level) to obtain the sensor minimum.

2.6. RT-PCR

Total RNA was extracted from isolated oocytes or whole ovaries using the RNeasy Plus Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. RNA was reversed transcribed to cDNA using the iSCRIPT cDNA synthesis kit (Bio-Rad, Hercules, CA), using random primers. The following primer sets were used: *Gpr3* forward 5'-TATCCACTCTCCAAGAACCATCTGG-3'; *Gpr3* reverse 5'-GAATTAAGCCCTGGTGGACCTAAC-3'; *Gapdh* forward 5'-TGTTC-CTACCCCCAATGTGT-3'; *Gapdh* reverse 5'-TGTGAGGGAGATG-CTCAGTG-3'; *Cdk1* forward 5'- GAACACCTTTCCCAAGTGGA-3'; *Cdk1* reverse 5'- CCATTTTGCCAGAGATTCGT-3'; *Rpl19* forward 5'-CGGGAATCCAAGAAGATTGA-3'; *Rpl19* reverse 5'-TTCAGCTTGTGG-ATGTGCTC-3'.

Cycling parameters for endpoint PCR (*Gpr3 and Gapdh*) were an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 60 °C for 45 s, 72 °C for 90 s, and a final extension at 72 °C for 7 min. PCR products were electrophoresed on a 2% agarose gel, visualized using ethidium bromide (Bio-Rad, Hercules, CA), and imaged using the G: box.

Real-time qPCR (*Cdk1* and *Rpl19*) was performed using a SYBR green kit, SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA) on a Bio-Rad CFX96 system (Bio-Rad, Hercules, CA). The cycling parameters included an initial denaturation at 95 °C for 3 min, followed by 50 cycles of 95 °C for 10 s and 55 °C for 30 s. A melt curve was performed, starting at 65 °C and increasing by 0.5 °C every 5 s up to 95 °C to determine primer specificity. *Cdk1* gene expression was normalized to *Rpl19* using the $2^{-\Delta\Delta Ct}$ method.

2.7. Statistical analysis

Statistical tests used for each experiment are described in the figure legends. GraphPad Prism6 was used for all statistical analyses.

3. Results and discussion

3.1. Oocyte meiotic competence correlates with follicle size

To systematically study when oocytes acquire meiotic competence with respect to follicle development, we examined the time course of oocyte maturation, as indicated by GVBD, in oocytes that were manually dissected from mouse ovaries and grouped according to follicle diameter. Previous studies examining the development of meiotic competence primarily utilized oocytes removed from ovaries of very young (~10-day-old) mice (Hirao et al., 1993; Szybek, 1972; Wassarman and Josefowicz, 1978). Although one study examined maturation rates in oocytes removed from defined follicle sizes (Erickson and Sorensen, 1974), this study utilized follicles from sexually mature, cycling mice irrespective of the stage in the estrous cycle and they did not carefully distinguish early antral from antral follicles, which is the stage at which meiotic competence is thought to be attained (Chesnel et al., 1994; Mehlmann et al., 2004). Here, we used prepubertal mice (~23–27 days old) that were not yet cycling. We considered follicles ~140–180 μm in diameter to be at the preantral stage, follicles ~220–250 μm in diameter to be at the early antral stage, and follicles ≥290 μm in diameter to be at the antral stage. After follicle isolation, oocytes were removed and scored every hour for GBVD, for up to 5 h.

As expected, nearly all of the oocytes retrieved from antral follicles underwent GVBD within 2 h after isolation (Fig. 1A). Similar rates of GVBD were observed in oocytes retrieved from the 220–250 μm follicle group. In contrast, we only observed ~40% GVBD in oocytes obtained from preantral stage follicles after 5 h. We found that 30% of oocytes removed from follicles 220–250 μm in diameter had undergone GVBD at the time of follicle isolation, whereas none of the oocytes removed from the ≥290 μm group had undergone GVBD at the time of follicle isolation. This is likely because some of the 220–250 μm follicles were atretic since we did not prime the mice with PMSG before follicle isolation; hormonal priming would be expected to rescue such follicles destined for atresia. Atretic follicles often contain oocytes that have undergone GVBD (Gougeon and Testart, 1986).

Because the ability to mature has been linked to oocyte size, we measured oocyte diameters in oocytes retrieved from preantral and antral follicles. The average diameter of preantral oocytes was significantly smaller than antral oocytes (71 ± 0.4 μm vs 74 ± 0.3 μm respectively), and the percentage of preantral oocytes that underwent GVBD in our study was lower than that reported by Hirao et al. (1993), who found that 90% of oocytes 65–70 μm in diameter underwent GVBD in culture conditions similar to those used in our study. This discrepancy is likely due to the length of time in culture, as we only monitored GVBD for 5 h, whereas Hirao et al. (1993) examined oocytes after they had been cultured for 24 h. Previous studies have shown that oocytes within ovaries of ≥15-day-old mice can undergo spontaneous GVBD when removed from ovaries (Sorensen and Wassarman, 1976; Wickramasinghe et al., 1991). However, these previous studies retrieved oocytes from ovaries irrespective of follicle size. Our study systematically correlates GVBD competence with follicle size, and the results clearly show that oocytes acquire meiotic competence as follicles reach a diameter of ~220 μm.

3.2. CDK1 levels increase in oocytes from the preantral to the antral follicle stage

Several previous studies have identified CDK1 as a key factor that contributes to the acquisition of meiotic competence in mouse oocytes (Chesnel and Eppig, 1995; de Vantéry et al., 1996; Kanatsu-Shinohara et al., 2000; Mitra and Schultz, 1996). Levels of CDK1 increase during oocyte growth, as determined by measuring proteins using western blots. However, in these previous studies, CDK1 measurements were done using oocytes removed from 10 to 12-day-old ovaries compared

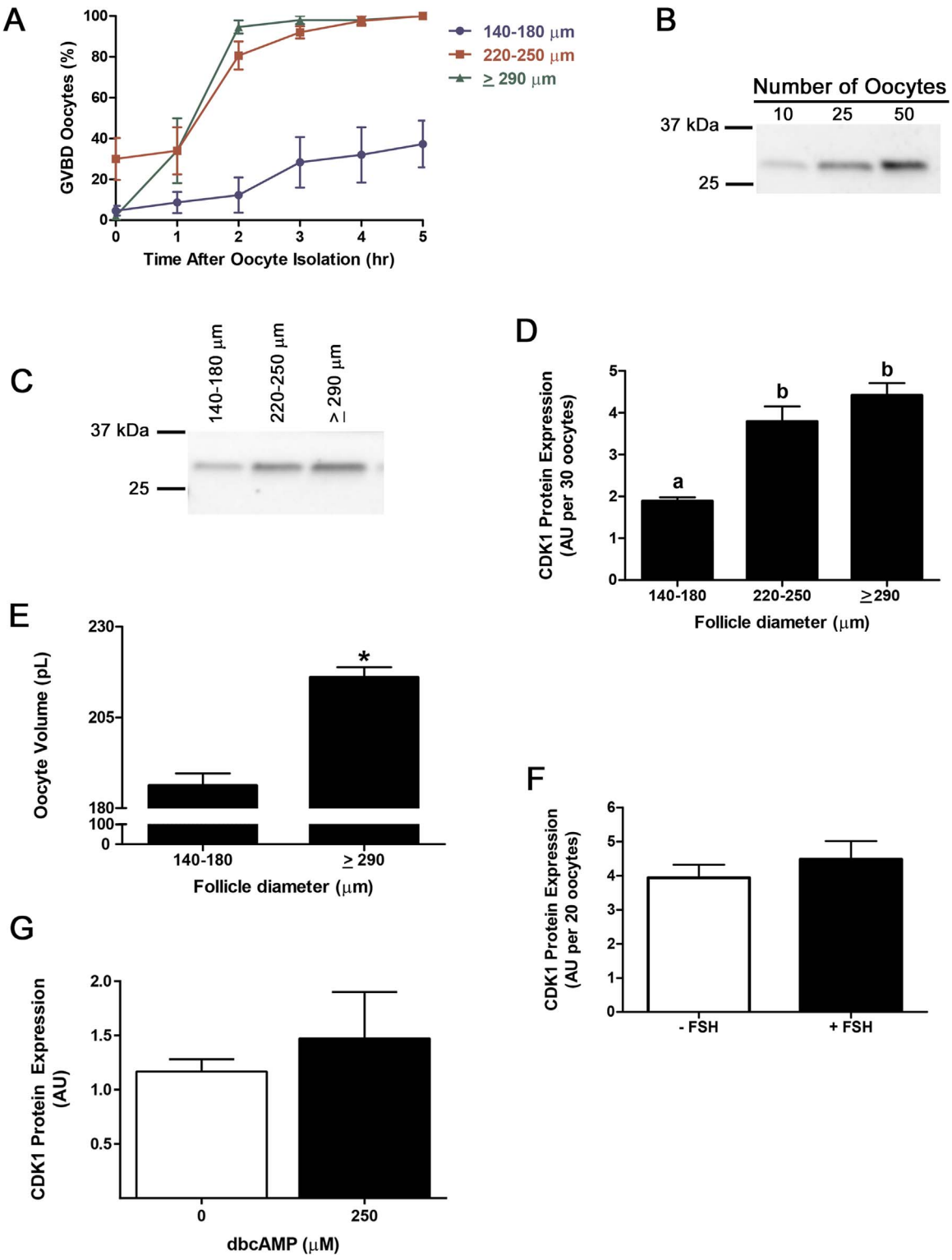

Fig. 1. Meiotic competence increases during oocyte and follicle growth, and CDK1 levels increase as oocytes become meiotically competent. A) GVBD time course using oocytes isolated from follicles of different size ranges. The graph demonstrates that oocytes become meiotically competent within follicles ~220–250 μm in diameter. B) Defined numbers of full-grown, prophase arrested oocytes were probed for CDK1 with western blot to test the linearity of ECL signal. C) Western blot for CDK1 in oocytes isolated from follicles of defined diameters, from the same ovary. D) CDK1 densitometry, showing that the amount of CDK1 increases significantly in the 220–250 μm group, which corresponds to the time that oocytes acquire meiotic competence. Data were compiled from 6 independent experiments. Bars with different letters are significantly different ($P < 0.05$). E) Volumes in oocytes retrieved from 140 to 180 μm diameter follicles compared to ≥290 μm diameter follicles. F, G) CDK1 levels are not acutely regulated by FSH or cAMP. For (F), oocytes from 140 to 180 μm diameter follicles were cultured for 20 h in medium with or without 30 ng/ml FSH and the blot was run after removing oocytes from follicles. For (G), isolated oocytes from 140 to 180 μm diameter follicles were treated for 20 h with dbcAMP before running western blots. Significance was determined by Student's *t*-test; $P < 0.05$ was considered significant. Bars are mean ± SEM.

with fully-grown oocytes removed from 6-week-old, hormonally-primed ovaries. To determine if CDK1 levels increase during the period of oocyte growth from the preantral to the antral stage, we isolated follicles of different sizes from the same ovaries, grouped the follicles according to size, removed the oocytes, and performed western blots for CDK1. In an initial experiment, we ran a CDK1 blot using defined numbers of fully-grown oocytes to estimate the number of oocytes to use for subsequent experiments and to confirm the linearity of our western blotting detection method (Fig. 1B). We found that our CDK1 antibody produced a clean band and the signal increased linearly. We observed a strong band using 25–50 oocytes, so unless noted, we used 30 oocytes per lane in subsequent experiments. The amount of CDK1 increased significantly in oocytes during the period from the preantral to the antral stage, particularly from the preantral to the early antral stage (Fig. 1C,D). This increase was not due solely to an increase in oocyte volume which also occurred during the period of follicle growth because the amount of CDK1 increased approximately 2-fold, whereas the oocyte volume increased only ~1.25-fold (Fig. 1E). This result correlates with the increased ability of oocytes within early antral follicles (220–250 μm in diameter) to undergo GVBD, and supports the hypothesis that the level of CDK1 is important for the acquisition of meiotic competence.

3.3. FSH does not acutely alter oocyte CDK1 levels

Follicle stimulating hormone (FSH) is essential for follicle growth, as follicle development past the preantral stage does not occur in FSH receptor-deficient mice (Abel et al., 2000). Although follicle cells express RNA encoding FSH receptors in follicles as small as ~75 μm in diameter (Hardy et al., 2017), they generally start becoming responsive to FSH when they are ~130 μm in diameter (Hardy et al., 2017). To investigate if this responsiveness to FSH can be a signal to stimulate CDK1 expression, we isolated oocytes from FSH-responsive, preantral stage follicles (140–180 μm in diameter) after culturing follicles for ~24 h with or without FSH. We chose a 24 h incubation period rather than a longer culture time to determine if FSH acutely regulates CDK1 expression. There was no difference in CDK1 levels in oocytes treated with FSH compared to untreated follicles (Fig. 1F). This result indicates that FSH does not acutely regulate CDK1 levels, but chronic stimulation probably contributes to CDK1 levels by stimulating follicle growth, which is associated with the acquisition of meiotic competence.

FSH acts through the FSH receptor, a G_s-coupled receptor on the follicle cells, stimulating the production of cAMP (Hunzicker-Dunn and Mayo, 2015; Simoni et al., 1997). A popular hypothesis for many years was that cAMP produced in follicle cells diffuses through gap junctions into the oocyte to elevate cAMP levels and inhibit premature meiotic resumption (Dekel, 2005; Edry et al., 2006). Dekel et al. (1984) demonstrated that elevated cAMP levels in cumulus cells in response to forskolin treatment was required to inhibit spontaneous GVBD in rat oocytes, suggesting that cAMP is produced in follicle cells and diffuses through gap junctions into the oocyte. However, they did not measure cAMP levels in isolated oocytes following forskolin treatment. Likewise, inducing gap junction closure with carbenoxelone in follicle-enclosed oocytes causes GVBD in mouse and rat oocytes (Norris et al., 2009; Sela-Abramovich et al., 2006), but no oocyte cAMP measurements were done before 45 min after treating follicles with carbenoxolone. Webb et al. (2002) showed that FSH stimulation of the cumulus cells causes a transient increase in cAMP in the oocyte. However, this is possibly a delayed response, not consistent with cAMP transfer through gap junctions. Finally, Li et al. (2012) demonstrated a relatively small increase in oocyte cAMP levels in response to treatment of cumulus-enclosed mouse oocytes with FSH, but the cAMP levels did not equilibrate in cumulus cells and oocytes, which is inconsistent with cAMP diffusion through gap junctions. In any case, any cAMP increase that might occur in the oocyte in response to FSH stimulation of the cumulus cells did not affect CDK1 expression in the oocyte (Fig. 1F). Consistent with this conclusion, treating isolated oocytes obtained from 140 to 180 μm follicles with dbcAMP for 20 h did not stimulate expression of CDK1 (Fig. 1G), indicating that cAMP does not acutely regulate CDK1 expression.

3.4. The constitutively active G-protein coupled receptor, Gpr3, is expressed in oocytes early in follicle development

Once meiotic competence has been achieved, arrest at the prophase stage becomes dependent on cAMP that is produced in oocytes through the constitutive activity of the G_s-coupled receptor, GPR3 (Hinckley et al., 2005; Ledent et al., 2005; Mehlmann, 2005b; Mehlmann et al., 2004). There is some evidence that GPR3 may be active in oocytes within preantral follicles (Freudzon et al., 2005; Hinckley et al., 2005; Vaccari et al., 2008), suggesting that the cAMP-producing system is functional before it is needed to maintain meiotic arrest; however, it has not been determined when *Gpr3* expression begins. cAMP may also promote the acquisition of meiotic competence in mouse oocytes (Chesnel et al., 1994). We examined when the *Gpr3* promoter becomes active during follicle development using a *Gpr3* knockout mouse in which the single exon of the *Gpr3* gene was replaced with a lacZ cassette under the control of the *Gpr3* promoter (Mehlmann et al., 2004; Tanaka et al., 2009). When the *Gpr3* promoter is active, the lacZ cassette stimulates the production of β-galactosidase, the activity of which can be detected by staining with 6-chloro-3-indolyl-β-D-galactopyranoside (salmon-gal), a more sensitive substrate than the commonly used X-gal (Sundararajan et al., 2012). We stained whole ovaries and isolated follicles from 17 to 23-day-old $Gpr3^{-/-}$ mice and found that β-galactosidase was present in virtually all oocytes from preantral follicles, indicating active transcription of *Gpr3* (Fig. 2A). β-galactosidase was expressed only within the oocytes rather than the follicle cells, and staining was restricted to the GV, possibly due to a nuclear localization signal commonly associated with β-galactosidase. As expected, $Gpr3^{+/+}$ oocytes, which did not contain the lacZ cassette, did not stain when incubated with salmon-gal (Fig. 2B).

Because essentially all the oocytes in follicles from 3-week-old mice stained positive for β-galactosidase, we examined β-galactosidase expression in 11-day-old $Gpr3^{-/-}$ mice to determine when the *Gpr3* promoter becomes active. As in the older mice, almost all oocytes within small, preantral follicles stained, although we occasionally found an oocyte from the smallest follicles that did not stain (Fig. 2C). We then tested even younger (8-day-old) $Gpr3^{-/-}$ mice and found that 61% (19/31) of oocytes within follicles from these mice were also positive for β-galactosidase (Fig. 2D). We could not detect positive staining in ovaries from newborn (4-day-old) mice using salmon-gal (not shown). To confirm the absence of detectable *Gpr3* mRNA in newborns, we examined *Gpr3* expression in the ovaries of 2-day-old mice using RT-PCR. As shown in Fig. 2E, ovaries from four separate mice did not express detectable *Gpr3*, whereas 2 oocyte equivalents from a 25-day-old mouse showed a strong band positive for *Gpr3*. The newborn ovaries had similar or higher amounts of *Gapdh* compared to the oocytes from the 25-day-old mouse. Because newborn ovaries only contain primordial follicles consisting of an oocyte surrounded by a single layer of squamous epithelial cells, the majority of the ovary is composed of oocytes rather than somatic cells. Therefore, the lack of *Gpr3* signal in the newborn ovaries strongly suggests that *Gpr3* is not expressed in mouse oocytes at this age.

The average diameter of *Gpr3*-positive oocytes from 8-day-old mice was significantly greater than that of unstained oocytes (36 ± 1 μm S.E.M., vs 27 ± 1 μm). Likewise, follicles containing *Gpr3*-positive oocytes had significantly larger diameters than *Gpr3*-negative follicles (53 ± 1 μm vs 45 ± 3 μm). We did not observe any *Gpr3*-positive oocytes smaller than 31 μm, while 75% (n=12) of the *Gpr3*-negative oocytes we measured were 30 μm or smaller. Therefore, *Gpr3* RNA starts to be expressed in oocytes when they are ~30 μm in diameter.

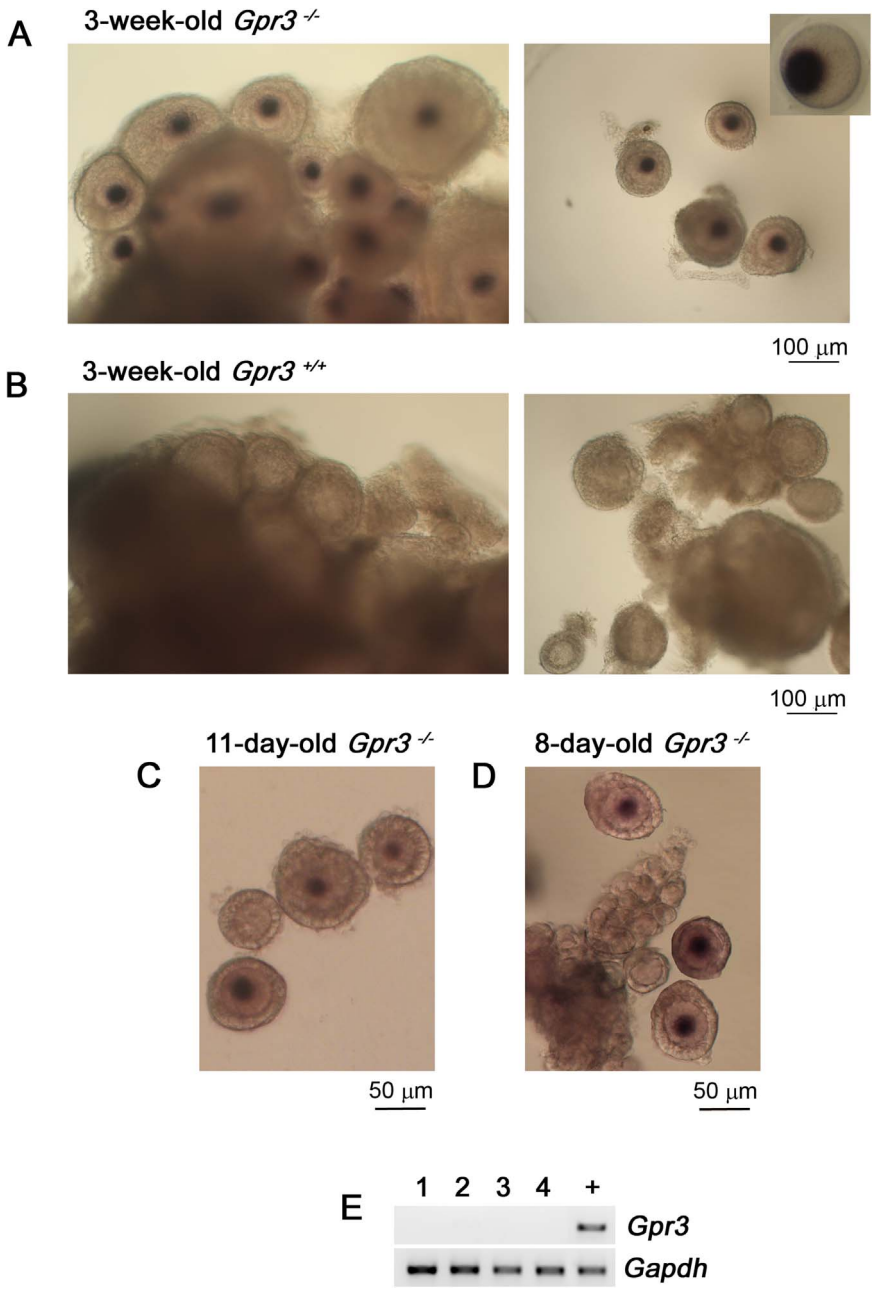

Fig. 2. *Gpr3* mRNA is present in oocytes early in follicle development. A, B) Salmon-gal staining in whole ovaries or in isolated follicles obtained from 3-week-old $Gpr3^{-/-}$ (A) or $Gpr3^{+/+}$ (B) mice. The inset in (A) shows staining within the GV of an isolated oocyte. C, D) Salmon-gal staining in follicles from 11-day-old (C) and 8-day-old (D) $Gpr3^{-/-}$ mice. E) RT-PCR amplifying *Gpr3* in ovaries from four different 2-day-old mice (lanes 1–4). 2 oocytes from a 25-day-old mouse (+) were used as a positive control for *Gpr3*. *Gapdh* was used a positive control for the newborn ovary cDNA.

Due to the unavailability of antibodies that can effectively detect endogenous GPR3 in mouse oocytes, we were unable to examine when GPR3 protein becomes expressed.

This result demonstrates that oocytes express the machinery necessary to generate cAMP within small, growing oocytes. In newborn mouse ovaries, cAMP has been linked to meiotic progression to diplotene as well as the formation of primordial follicles (Wang et al., 2015). This study showed that cAMP levels increase significantly in mouse ovaries just before birth; cAMP controls the disassembly and degradation of synaptonemal complex protein 1 (SYCP1) in the oocyte, thereby permitting the oocyte to reach the dictyate stage of prophase. Inhibiting cAMP production in newborn ovaries causes an increase in SYCP1 and leads to a delay in meiotic prophase as well as a disruption in germline cyst breakdown and primordial follicle formation (Wang et al., 2015). The effects of cAMP at these early stages appear to be due to the activity of adenylate cyclase type 2 in the oocyte, whereas meiotic arrest in meiotically competent oocytes is due to the activity of adenylate cyclase type 3 (Horner et al., 2003). Because we could not detect *Gpr3* in newborn ovaries using salmon-gal staining and PCR, it is unlikely that GPR3 provides the source of cAMP in newborn ovaries. In addition, oocytes from $Gpr3^{-/-}$ mice clearly reach the diplotene stage and follicles form normally (this study; Mehlmann et al., 2004), so it is likely that newborn oocytes produce cAMP through a different system. Our results demonstrate that *Gpr3* is expressed in small, growing oocytes, suggesting that cAMP could be produced before the fully-grown stage, in which meiotic arrest at prophase depends on cAMP.

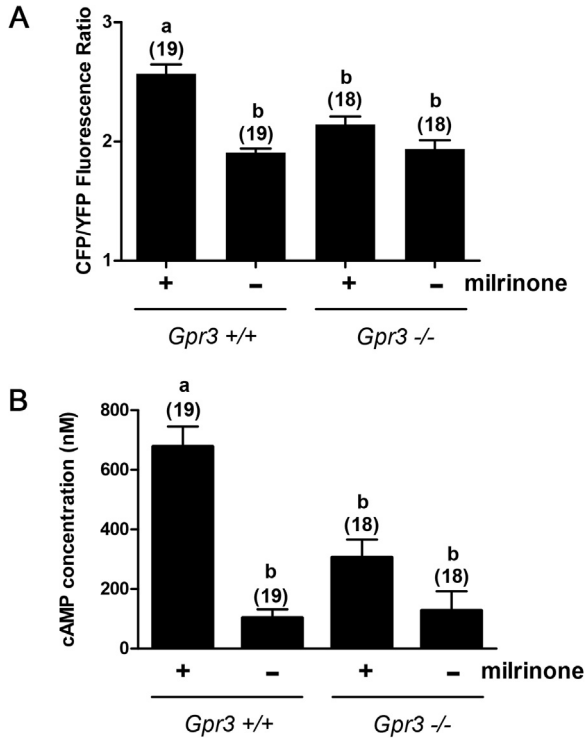

Fig. 3. GPR3 is active and stimulates cAMP production in oocytes from follicles that are 140–180 μm in diameter. Measurement of cAMP concentrations in *Gpr3*$^{-/-}$ and *Gpr3*$^{+/+}$ oocytes using the Epac2-camps300 FRET sensor. cAMP concentrations were first measured in oocytes from follicles 140–180 μm in diameter in the presence of the PDE3A inhibitor, milrinone, then milrinone was washed out, stimulating cAMP degradation, and cAMP was measured 30 min later. A) Data presented as ratios of CFP/YFP fluorescence. B) Data presented as cAMP concentration. Bars are mean ± SEM and significance was determined by one-way ANOVA; $P < 0.05$. Bars with different letters are significantly different.

3.5. GPR3 is active in oocytes from preantral follicles (140–180 μm diameter)

To confirm that GPR3 is functional in the oocytes within preantral follicles, we used a FRET-based assay to measure free cAMP in oocytes removed from follicles that were 140–180 μm in diameter. We included milrinone in the culture medium to prevent phosphodiesterase 3 A (PDE3A) activation during oocyte isolation, thus preventing cAMP hydrolysis. Isolated oocytes from *Gpr3*$^{+/+}$ and *Gpr3*$^{-/-}$ mice were injected with a sensor (Epac2-camps300) consisting of the cAMP binding domain of EPAC2, fused to CFP and YFP (Lowther et al., 2011; Nikolaev et al., 2004; Norris et al., 2009). FRET measurements showed significantly higher CFP/YFP fluorescence ratios in *Gpr3*$^{+/+}$ oocytes compared to *Gpr3*$^{-/-}$ oocytes (Fig. 3A). These ratios corresponded to cAMP concentrations of ~700 nM vs ~300 nM, respectively (Fig. 3B). 700 nM cAMP in *Gpr3*$^{+/+}$ oocytes is similar to the ~660 nM that was reported in full-grown oocytes within antral follicles (Norris et al., 2009), using the same FRET sensor that we used here. When milrinone was washed out of the culture medium, the CFP/YFP fluorescence ratios decreased and cAMP levels in the *Gpr3*$^{+/+}$ oocytes fell to ~100 nM within 30 min (Fig. 3). Cyclic AMP levels also fell to ~100 nM in the *Gpr3*$^{-/-}$ oocytes (Fig. 3B), although this change was not statistically significant. It was somewhat surprising that there was measurable cAMP in the *Gpr3*$^{-/-}$ oocytes. This suggests that another mechanism other than GPR3 can contribute to cAMP production in the oocyte. This small amount of cAMP, however, is clearly not sufficient to maintain meiotic arrest, as the depletion of *Gpr3*$^{-/-}$ in oocytes renders them incapable of maintaining meiotic arrest (Mehlmann, 2005b; Mehlmann et al., 2004). Overall, this result demonstrates that GPR3 is active in oocytes within small follicles, and shows that PDE3A is also present and capable of being activated in these oocytes.

3.6. Fewer oocytes from *Gpr3*$^{-/-}$ mice are competent to mature than *Gpr3*$^{+/+}$ oocytes

Two earlier studies showed that cAMP promotes the acquisition of meiotic competence. Both isolated as well as follicle-enclosed small, growing, incompetent oocytes become competent when cultured in the presence of dbcAMP (Carroll et al., 1991; Chesnel et al., 1994). As it has been found that oocytes within preantral follicles express *Gpr3* and produce cAMP, our data are consistent with the possibility that GPR3 may contribute to the acquisition of meiotic competence. To examine this, we analyzed the time course of GVBD using *Gpr3*$^{+/+}$ and *Gpr3*$^{-/-}$ oocytes removed from follicles of different sizes, as in the experiments described for Fig. 1. Fig. 4A shows that *Gpr3*$^{-/-}$ oocytes took longer to undergo GVBD, and fewer completed GVBD. The difference was particularly striking in oocytes retrieved from early antral follicles, the stage at which oocytes are beginning to acquire meiotic competence (Chesnel et al., 1994; Mehlmann et al., 2004). This result shows that, although *Gpr3*$^{-/-}$ oocytes eventually acquire the ability to undergo GVBD, they do so at a slower rate than their *Gpr3*$^{+/+}$ counterparts.

That *Gpr3*$^{-/-}$ oocytes undergo GVBD at a slower rate than *Gpr3*$^{+/+}$ oocytes is in agreement with results published by Ledent et al. (2005), who showed that fewer oocytes from 3.5-week-old and 6-month old *Gpr3*$^{-/-}$ mice undergo GVBD and first polar body formation than *Gpr3*$^{+/+}$ oocytes. The higher percentage of *Gpr3*$^{-/-}$ oocytes from ≥290 μm follicles that underwent GVBD within 5 h in our study is likely due to the fact that Ledent et al. (2005) did not distinguish antral follicle sizes; rather, they obtained oocytes from all follicles ≥190 μm in diameter. It should also be noted that we saw ~20% GVBD at the time of isolation of *Gpr3*$^{-/-}$ oocytes from antral follicles. This value is much lower than the ~85–90% GVBD rate observed previously in *Gpr3*$^{-/-}$ antral follicles (Mehlmann et al., 2004), and also lower than the ~50% GVBD observed by Ledent et al. (2005). The difference could be due to hormonal priming; Mehlmann et al. (2004) and Ledent et al. (2005) primed mice with PMSG prior to collecting ovaries or isolating follicles, which yielded follicles ≥350 μm in diameter, whereas here we used follicles from unprimed prepubertal mice that generally did not exceed 320 μm in diameter, so follicle size could be a factor in our results. It is also possible that priming could cause the synthesis of other proteins that are important for meiotic competence. In addition, previous studies counted all antral follicles, including follicles that may have been atretic, whereas here we isolated only follicles that were clear and presumably not atretic. It might be expected that oocytes within atretic follicles are either GVBD or degenerated, which could be reflected in the higher percentage of GVBD observed in the previous studies. Our results demonstrate that *Gpr3*$^{-/-}$ oocytes undergo spontaneous GVBD even within follicles that are apparently healthy and viable.

3.7. CDK1 levels increase in oocytes from *Gpr3*$^{-/-}$ oocytes but the levels are lower than in *Gpr3*$^{+/+}$ oocytes

To examine if GPR3 activity could be associated with CDK1 levels, we isolated follicles of various sizes from ovaries of *Gpr3*$^{-/-}$ mice and grouped them according to size, as above. We removed oocytes from these follicles and compared the amount of CDK1 in oocytes using western blot. We found that, as in *Gpr3*$^{+/+}$ oocytes, the amount of CDK1 increased during oocyte growth in *Gpr3*$^{-/-}$ oocytes (Fig. 4B,C). However, the amount of CDK1 was significantly lower in *Gpr3*$^{-/-}$ oocytes in the 140–180 μm and 220–250 μm groups. This difference was not due to differences in total protein among the *Gpr3*$^{+/+}$ and *Gpr3*$^{-/-}$ groups, as probing blots for the highly expressed IP_3 receptor showed no differences (Fig. 4D).

3.8. CDK1 expression is regulated translationally during the acquisition of meiotic competence

It is not clear how the expression of GPR3 leads to a higher amount

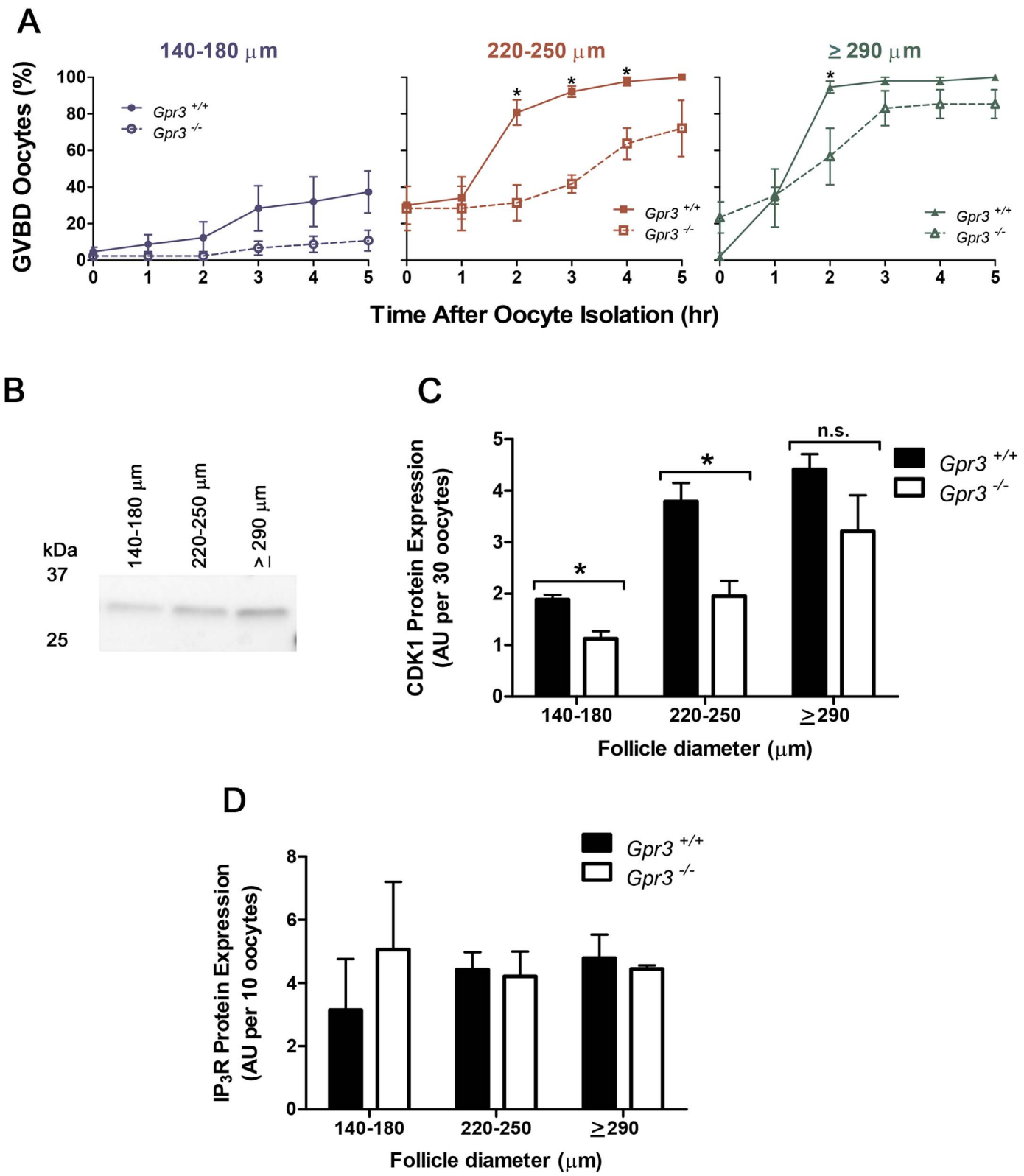

Fig. 4. Oocytes deficient in *Gpr3* are slower to achieve meiotic competence and have less CDK1 than $Gpr3^{+/+}$ oocytes. A) Time course of GVBD in $Gpr3^{-/-}$ and $Gpr3^{+/+}$ oocytes removed from follicles of defined sizes. $Gpr3^{+/+}$ data are the same as in Fig. 1. Significance was determined using two-way analysis of variance after arc-sin transformation to normalize data. ($P < 0.05$). B) Western blot for CDK1 in $Gpr3^{-/-}$ oocytes removed from follicles of defined diameters. C) The amount of CDK1 is significantly lower in oocytes retrieved from preantral and early antral follicles of $Gpr3^{-/-}$ mice compared with $Gpr3^{+/+}$ mice. The $Gpr3^{+/+}$ graph shown here is the same as in Fig. 1. CDK1 amounts for $Gpr3^{+/+}$ vs $Gpr3^{-/-}$ were compared by *t*-tests with the Holm-Sidak correction for multiple comparisons. *=$P < 0.03$. D) IP_3 receptor expression, as determined by western blot, in oocytes from both genotypes and removed from follicles of defined diameters.

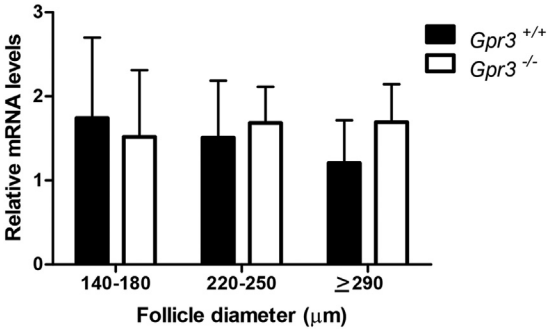

Fig. 5. *Cdk1* mRNA levels in *Gpr3*$^{+/+}$ and *Gpr3*$^{-/-}$ oocytes removed from follicles of defined diameters, as assessed by qRT-PCR.

of CDK1 in *Gpr3*$^{+/+}$ oocytes compared to *Gp3*$^{-/-}$ oocytes. The simplest explanation is that *Gpr3*$^{+/+}$ oocytes contain more mRNA encoding CDK1 than *Gpr3*$^{-/-}$ oocytes. However, real-time PCR using oocytes retrieved from follicles of different sizes and from both genotypes showed that the levels of *Cdk1* RNA were similar in all groups (Fig. 5), indicating that increased transcription is unlikely to account for higher amounts of CDK1 protein in the *Gpr3*$^{+/+}$ oocytes. Our results suggest that the increase in the amount of CDK1 protein during oocyte maturation occurs at the translational level. There is at least one example of cAMP increasing translation independently of stimulating gene transcription. Synthesis of tyrosine hydroxylase (TH) protein is stimulated by cAMP in midbrain dopaminergic neurons without an increase in mRNA levels (Chen et al., 2008). In these cells, cAMP treatment increases the association of TH mRNA with polysomes through proteins that bind to *cis*-acting sequences within the 3'UTR of the TH RNA (Chen et al., 2008; Xu et al., 2009). It is possible that cAMP has a similar stimulatory effect in oocytes, but this needs to be further examined.

4. Conclusions

This study shows that CDK1 levels increase in mouse oocytes as they progress from meiotically incompetent to meiotically competent stages, as follicles transition between preantral (140–180 μm in diameter) and early antral (220–250 μm in diameter) stages. *Gpr3* is expressed and active early in oocyte development, prior to the acquisition of meiotic competence and subsequent dependence on cAMP for meiotic arrest. The presence and activity of GPR3 protein are likely to precede the synthesis of CDK1, but are not required for it. Our results suggest that the production of cAMP through GPR3 signaling early on in development potentiates the acquisition of meiotic competence in oocytes. In support of this, fewer *Gpr3*$^{-/-}$ oocytes within 140–180 μm diameter follicles underwent GVBD than oocytes containing an intact GPR3 system. Lower amounts of CDK1 in *Gpr3*$^{-/-}$ oocytes could contribute to this decrease in meiotic competence observed in *Gpr3*$^{-/-}$ oocytes. Overall, these results indicate that GPR3 has a role in the acquisition of oocyte meiotic competence, but the mechanism through which it affects meiotic competence needs further study.

Acknowledgments

We thank Viacheslav Nikolaev for providing the EPAC2-camps300 protein, and Laurinda Jaffe for helpful discussions. This work was supported by the University of Connecticut Health Center Research Advisory Council and The Fund for Science.

References

Abel, M.H., Wootton, A.N., Wilkins, V., Huhtaniemi, I., Knight, P.G., Charlton, H.M., 2000. The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. Endocrinology 141, 1795–1803.
Adhikari, D., Zheng, W., Shen, Y., Gorre, N., Ning, Y., Halet, G., Kaldis, P., Liu, K., 2012. Cdk1, but not Cdk2, is the sole Cdk that is essential and sufficient to drive resumption of meiosis in mouse oocytes. Hum. Mol. Genet. 21, 2476–2484.
Carroll, J., Whittingham, D.G., Wood, M.J., 1991. Effect of dibutyryl cyclic adenosine monophosphate on granulosa cell proliferation, oocyte growth and meiotic maturation in isolated mouse primary ovarian follicles cultured in collagen gels. J. Reprod. Fertil. 92, 197–207.
Chatot, C.L., Ziomek, C.A., Bavister, B.D., Lewis, J.L., Torres, I., 1989. An improved culture medium supports development of random-bred 1-cell mouse embryos in vitro. J. Reprod. Fertil. 86, 679–688.
Chen, X., Xu, L., Radcliffe, P., Sun, B., Tank, A.W., 2008. Activation of tyrosine hydroxylase mRNA translation by cAMP in midbrain dopaminergic neurons. Mol. Pharmacol. 73, 1816–1828.
Chesnel, F., Eppig, J.J., 1995. Synthesis and accumulation of p34cdc2 and cyclin B in mouse oocytes during acquisition of competence to resume meiosis. Mol. Reprod. Dev. 40, 503–508.
Chesnel, F., Wigglesworth, K., Eppig, J.J., 1994. Acquisition of meiotic competence by denuded mouse oocytes: participation of somatic-cell product(s) and cAMP. Dev. Biol. 161, 285–295.
Dekel, N., 2005. Cellular, biochemical and molecular mechanisms regulating oocyte maturation. Mol. Cell Endocrinol. 234, 19–25.
Dekel, N., Aberdam, E., Sherizly, I., 1984. Spontaneous maturation in vitro of cumulus-enclosed rat oocytes is inhibited by forskolin. Biol. Reprod. 31, 244–250.
Edry, I., Sela-Abramovich, S., Dekel, N., 2006. Meiotic arrest of oocytes depends on cell-to-cell communication in the ovarian follicle. Mol. Cell Endocrinol. 252, 102–106.
Edwards, R.G., 1965. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. Nature 208, 349–351.
Eppig, J.J., O'Brien, M.J., 1996. Development in vitro of mouse oocytes from primordial follicles. Biol. Reprod. 54, 197–207.
Erickson, G.F., Sorensen, R.A., 1974. In vitro maturation of mouse oocytes isolated from late, middle, and pre-antral graafian follicles. J. Exp. Zool. 190, 123–127.
Freudzon, L., Norris, R.P., Hand, A.R., Tanaka, S., Saeki, Y., Jones, T.L., Rasenick, M.M., Berlot, C.H., Mehlmann, L.M., Jaffe, L.A., 2005. Regulation of meiotic prophase arrest in mouse oocytes by GPR3, a constitutive activator of the Gs G protein. J. Cell Biol. 171, 255–265.
Gougeon, A., Testart, J., 1986. Germinal vesicle breakdown in oocytes of human atretic follicles during the menstrual cycle. J. Reprod. Fertil. 78, 389–401.
Hardy, K., Fenwick, M., Mora, J., Laird, M., Thomson, K., Franks, S., 2017. Onset and Heterogeneity of Responsiveness to FSH in Mouse Preantral Follicles in Culture. Endocrinology 158, 134–147.
Hinckley, M., Vaccari, S., Horner, K., Chen, R., Conti, M., 2005. The G-protein-coupled receptors GPR3 and GPR12 are involved in cAMP signaling and maintenance of meiotic arrest in rodent oocytes. Dev. Biol. 287, 249–261.
Hirao, Y., Miyano, T., Kato, S., 1993. Acquisition of maturational competence in in vitro grown mouse oocytes. J. Exp. Zool. 267, 543–547.
Holt, J.E., Lane, S.I., Jones, K.T., 2013. The control of meiotic maturation in mammalian oocytes. Curr. Top Dev. Biol. 102, 207–226.
Horner, K., Livera, G., Hinckley, M., Trinh, K., Storm, D., Conti, M., 2003. Rodent oocytes express an active adenylyl cyclase required for meiotic arrest. Dev. Biol. 258, 385–396.
Hunzicker-Dunn, M., Mayo, K., 2015. Gonadotropin signaling in the ovary. In: Zeleznik, Pa (Ed.), Knobil and Neill's Physiology of ReproductionFourth edition. Elsevier Inc, 895–945.
Kanatsu-Shinohara, M., Schultz, R.M., Kopf, G.S., 2000. Acquisition of meiotic competence in mouse oocytes: absolute amounts ofp34(cdc2), cyclin B1, cdc25C, and wee1 in meiotically incompetent and competent oocytes. Biol. Reprod. 63, 1610–1616.
Kline, D., 2009. Quantitative microinjection of mouse oocytes and eggs. Methods Mol. Biol. 518, 135–156.
Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
Ledent, C., Demeestere, I., Blum, D., Petermans, J., Hamalainen, T., Smits, G., Vassart, G., 2005. Premature ovarian aging in mice deficient for Gpr3. Proc. Natl. Acad. Sci. USA 102, 8922–8926.
Li, J., Mao, G., Xia, G., 2012. FSH modulates PKAI and GPR3 activities in mouse oocyte of COC in a gap junctional communication (GJC)-dependent manner to initiate meiotic resumption. PLoS One 7, e37835.
Lowther, K.M., Nikolaev, V.O., Mehlmann, L.M., 2011. Endocytosis in the mouse oocyte and its contribution to cAMP signaling during meiotic arrest. Reproduction 141, 737–747.
Mehlmann, L.M., 2005a. Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. Reproduction 130, 791–799.

Mehlmann, L.M., 2005b. Oocyte-specific expression of Gpr3 is required for the maintenance of meiotic arrest in mouse oocytes. Dev. Biol. 288, 397–404.
Mehlmann, L.M., 2013. Signalling for meiotic resumption in granulosa cells, cumulus cells, and oocyte. In: Coticchio, De Santis, Albertini (Eds.), Mammalian Oogenesis. Springer Publishing Company, New York, NY, 171–182.
Mehlmann, L.M., Jones, T.L., Jaffe, L.A., 2002. Meiotic arrest in the mouse follicle maintained by a Gs protein in the oocyte. Science 297, 1343–1345.
Mehlmann, L.M., Saeki, Y., Tanaka, S., Brennan, T.J., Evsikov, A.V., Pendola, F.L., Knowles, B.B., Eppig, J.J., Jaffe, L.A., 2004. The Gs-linked receptor GPR3 maintains meiotic arrest in mammalian oocytes. Science 306, 1947–1950.
Mitra, J., Schultz, R.M., 1996. Regulation of the acquisition of meiotic competence in the mouse: changes in the subcellular localization ofcdc2, cyclin B1, cdc25C and wee1, and in the concentration of these proteins and their transcripts. J. *Cell Sci.* 109 (Pt 9), 2407–2415.
Nikolaev, V.O., Bunemann, M., Hein, L., Hannawacker, A., Lohse, M.J., 2004. Novel single chain cAMP sensors for receptor-induced signal propagation. J. Biol. Chem. 279, 37215–37218.
Norris, R.P., Ratzan, W.J., Freudzon, M., Mehlmann, L.M., Krall, J., Movsesian, M.A., Wang, H., Ke, H., Nikolaev, V.O., Jaffe, L.A., 2009. Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. Development 136, 1869–1878.
Picton, H., Briggs, D., Gosden, R., 1998. The molecular basis of oocyte growth and development. Mol. Cell Endocrinol. 145, 27–37.
Schultz, R.M., Montgomery, R.R., Belanoff, J.R., 1983. Regulation of mouse oocyte meiotic maturation: implication of a decrease in oocyte cAMP and protein dephosphorylation in commitment to resume meiosis. Dev. Biol. 97, 264–273.
Sela-Abramovich, S., Edry, I., Galiani, D., Nevo, N., Dekel, N., 2006. Disruption of gap junctional communication within the ovarian follicle induces oocyte maturation. Endocrinology 147, 2280–2286.
Simoni, M., Gromoll, J., Nieschlag, E., 1997. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. Endocr. Rev. 18, 739–773.
Sorensen, R.A., Wassarman, P.M., 1976. Relationship between growth and meiotic maturation of the mouse oocyte. Dev. Biol. 50, 531–536.
Sundararajan, S., Wakamiya, M., Behringer, R.R., Rivera-Perez, J.A., 2012. A fast and sensitive alternative for beta-galactosidase detection in mouse embryos. Development 139, 4484–4490.
Szybek, K., 1972. In-vitro maturation of oocytes from sexually immature mice. J. Endocrinol. 54, 527–528.
Tanaka, S., Shaikh, I.M., Chiocca, E.A., Saeki, Y., 2009. The Gs-linked receptor GPR3 inhibits the proliferation of cerebellar granule cells during postnatal development. PLoS One 4, e5922.
Vaccari, S., Horner, K., Mehlmann, L.M., Conti, M., 2008. Generation of mouse oocytes defective in cAMP synthesis and degradation: endogenous cyclic AMP is essential for meiotic arrest. Dev. Biol. 316, 124–134.
de Vantéry, C., Gavin, A.C., Vassalli, J.D., Schorderet-Slatkine, S., 1996. An accumulation of p34cdc2 at the end of mouse oocyte growth correlates with the acquisition of meiotic competence. Dev. Biol. 174, 335–344.
de Vantéry, C., Stutz, A., Vassalli, J.D., Schorderet-Slatkine, S., 1997. Acquisition of meiotic competence in growing mouse oocytes is controlled at both translational and posttranslational levels. Dev. Biol. 187, 43–54.
Wang, Y., Teng, Z., Li, G., Mu, X., Wang, Z., Feng, L., Niu, W., Huang, K., Xiang, X., Wang, C., Zhang, H., Xia, G., 2015. Cyclic AMP in oocytes controls meiotic prophase I and primordial folliculogenesis in the perinatal mouse ovary. Development 142, 343–351.
Wassarman, P.M., Josefowicz, W.J., 1978. Oocyte development in the mouse: an ultrastructural comparison of oocytes isolated at various stages of growth and meiotic competence. J. Morphol. 156, 209–235.
Webb, R.J., Marshall, F., Swann, K., Carroll, J., 2002. Follicle-stimulating hormone induces a gap junction-dependent dynamic change in [cAMP] and protein kinase a in mammalian oocytes. Dev. Biol. 246, 441–454.
Wickramasinghe, D., Ebert, K.M., Albertini, D.F., 1991. Meiotic competence acquisition is associated with the appearance of M-phase characteristics in growing mouse oocytes. Dev. Biol. 143, 162–172.
Xu, L., Sterling, C.R., Tank, A.W., 2009. cAMP-mediated stimulation of tyrosine hydroxylase mRNA translation is mediated by polypyrimidine-rich sequences within its 3'-untranslated region and poly(C)-binding protein 2. Mol. Pharmacol. 76, 872–883.