Published in final edited form as:

Steroids. 2007 February ; 72(2): 117–123.

Gβγ Signaling Reduces Intracellular cAMP to Promote Meiotic Progression in Mouse Oocytes

Arvind Gill^{*} and Stephen R Hammes^{*,†}

^{*} Department of Internal Medicine, Division of Endocrinology and Metabolism, Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75390-8857, USA

Abstract

In nearly every vertebrate species, elevated intracellular cAMP maintains oocytes in prophase I of meiosis. Prior to ovulation, gonadotropins trigger various intra-ovarian processes, including the breakdown of gap junctions, the activation of EGF receptors, and the secretion of steroids. These events in turn decrease intracellular cAMP levels in select oocytes to allow meiotic progression, or maturation, to resume. Studies suggest that cAMP levels are kept elevated in resting oocytes by constitutive G protein signaling, and that the drop in intracellular cAMP that accompanies maturation is due to attenuation of this inhibitory G protein-mediated signaling. Interestingly, one of these G protein regulators of meiotic arrest is the Gα_s protein, which stimulates adenylyl cyclase to raise intracellular cAMP in two important animal models of oocyte development: *Xenopus leavis* frogs and mice. In addition to Gα_s, constitutive Gβγ activity similarly stimulates adenylyl cyclase to raise cAMP and prevent maturation in *Xenopus* oocytes; however, the role of Gβγ in regulating meiosis in mouse oocytes has not been examined. Here we show that Gβγ does not contribute to the maintenance of murine oocyte meiotic arrest. In fact, contrary to observations in frog oocytes, Gβγ signaling in mouse oocytes reduces cAMP and promotes oocyte maturation, suggesting that Gβγ might in fact play a positive role in promoting oocyte maturation. These observations emphasize that, while many general concepts and components of meiotic regulation are conserved from frogs to mice, specific differences exist that may lead to important insights regarding ovarian development in vertebrates.

Keywords

steroid; oocyte; G protein; cAMP; adenylyl cyclase; ovary

Introduction

Meiosis in frog oocytes

Steroids trigger many transcription-independent, or nongenomic, effects by regulating G protein signaling [1,2]. These G protein-coupled signals occur in a wide variety of cell types, including endothelial cells, neurons, and breast epithelial cells [3–5]. One fascinating and biologically relevant model of steroid-mediated G protein signaling is steroid-triggered oocyte maturation, or meiotic progression, in *Xenopus leavis* oocytes [6,7]. In female *Xenopus* frogs, oocytes within the ovary are held in meiotic arrest at prophase I. Just prior to ovulation,

[†]Corresponding author: Phone: 214-648-3749, FAX: 214-648-7934, email: stephen.hammes@utsouthwestern.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

gonadotropins stimulate ovarian steroid production in an unusual mechanism that appears to involve both follicular cells and germ cells [8]. These newly synthesized steroids then trigger oocytes to re-enter the cell cycle and progress through to metaphase II, at which point the mature oocytes are ready for ovulation and subsequent fertilization.

Interestingly, evidence from many laboratories suggests that steroid-triggered oocyte maturation of *Xenopus laevis* oocytes occurs via a “release of inhibition” model whereby oocytes are held in meiotic arrest by constitutive G protein signaling. Steroids then attenuate this inhibitory G protein signaling to promote maturation. The G proteins regulating the inhibitory signal include $G\alpha_s$ and $G\beta\gamma$, both of which appear to act together to stimulate adenylyl cyclase and increase intracellular cAMP. Evidence supporting this model is plentiful, including the following: First, steroids trigger a rapid decrease in intracellular cAMP with a concomitant decrease in PKA activity [9–12]. Second, over-expression of either $G\alpha_s$ or $G\beta\gamma$ inhibits steroid-triggered oocyte maturation, while reduction of $G\alpha_s$ or $G\beta\gamma$ levels or activity leads to enhanced maturation in response to steroids [13–16]. Third, stimulation of over-expressed $G\alpha_s$ - or $G\beta\gamma$ -coupled receptors markedly inhibits steroid-triggered oocyte maturation [17,18]. Finally, by using over-expressed $G\beta\gamma$ -regulated inward rectifying potassium channels (GIRKs) as markers of endogenous $G\beta\gamma$ signaling in oocytes, constitutive $G\beta\gamma$ signaling is detected that is reduced within minutes after steroid stimulation (Hammes, *in press*).

What are the physiologic steroids that mediate *Xenopus laevis* oocyte maturation in ovulating frogs? While multiple steroids are equally potent promoters of oocyte maturation *in vitro*, including progesterone, testosterone, and androstenedione [19–21], hCG stimulation of ovaries both *in vitro* and *in vivo* demonstrates that the androgens androstenedione and testosterone are produced at 10-fold higher amounts than progesterone [19,20]. Furthermore, inhibition of androgen production downstream of progesterone in the steroidogenic pathway markedly inhibits both hCG-stimulated oocyte maturation and ovulation in live female frogs [22].

How do androgens suppress G protein signaling in frog oocytes? To start, androgens appear to activate extra-nuclear classical androgen receptors (ARs), as pharmacologic blockade of steroid binding to the AR, or reduction of AR expression by RNA interference, results in decreased steroid-triggered $G\beta\gamma$ signaling (Hammes, *in press*) and subsequent oocyte maturation [18,20,22]. While the detailed mechanisms are still not well-understood, androgen-activated ARs appear to attenuate G protein signaling through protein-protein complexes that may involve the scaffold molecule called the Modulator of Nongenomic Actions of steroid Receptors (MNAR) [23,24].

Meiosis in mouse oocytes

Similar to *Xenopus* oocytes, mouse oocytes are also held in meiotic arrest prior to ovulation [25,26]. In addition, under some conditions, steroids appear to be capable of promoting mouse oocyte maturation [27,28]. However, unlike frog oocytes, mouse oocytes do not require steroids to re-enter meiosis, as they mature spontaneously upon removal from follicles, and inhibition of steroidogenesis in mouse follicles does not appear to block gonadotropin-mediated maturation [29]. These observations indicate that the inhibitory signals maintaining meiotic arrest in mouse oocytes are derived at least in part from the surrounding follicle cells rather than being endogenous to the oocytes themselves. Furthermore, these results suggest, while steroids are the primary regulators of oocyte maturation in frogs, they are likely one of many signals capable of triggering mouse oocyte maturation.

Despite differences in signals outside the germ cell that regulate meiosis in mouse versus frog oocytes, intracellular constitutive G protein signaling is a shared mechanism for maintaining meiotic arrest in both species. For example, as in frog oocytes, meiotic arrest in mouse oocytes

is mediated by constitutive $G\alpha_s$ activity, as injection of antibodies against $G\alpha_s$ into follicle-enclosed oocytes enhances oocyte maturation [30]. Intriguingly, the constitutive inhibitory $G\alpha_s$ activity in mice may be mediated at least in part through a constitutively active G protein-coupled receptor called GPR3, as over-expression of GPR blocks oocyte maturation, while knockout mice lacking GPR3 have a higher incidence of spontaneous oocyte maturation [31]. Similarly, over-expression of mammalian GPR3 in frog oocytes inhibits steroid-triggered maturation, suggesting that a *Xenopus* isoform of GPR3 may also be regulating constitutive G protein signaling in *Xenopus* oocytes [32].

While the importance of $G\alpha_s$ in maintaining meiotic arrest is well established in mouse oocytes, the role of $G\beta\gamma$ in regulating meiosis has not been examined. Here we studied the effects of modulating $G\beta\gamma$ signaling on mouse oocyte maturation. We demonstrated that, in stark contrast to frog oocytes, $G\beta\gamma$ signaling in mouse oocytes decreased intracellular cAMP levels, and actually promoted maturation of follicle-enclosed oocytes. These studies underscore the concept that, while many of the general principals of meiotic regulation are conserved in vertebrates, species-specific differences exist that must be recognized.

Experimental

Materials

Most of the plasmids used for this study are described elsewhere [14]. The cDNA encoding transducin in the pFROG vector was a gift from S. Coughlin (UC San Francisco). The cDNAs encoding the bovine $G\beta 1$ in pGEM-HE, $G\gamma 2$ in pFROG, and the G protein receptor kinase minigene (GRK) in pGEM-HE were gifts from L. Jan (UC San Francisco). The wild-type and constitutively active bovine $G\alpha_s$ cDNAs were gifts from S. Mumby (UT Southwestern), and were cloned into the pGEM-HE vector. The anti- $G\alpha_s$ antibody was also a gift from S. Mumby. The muscarinic-2 receptor (M2R) in pcDNA3.1 was purchased from UMR cDNA Resource Center (Rolla, MO), and the cDNA was cloned into the pGEM-HE vector. Finally, CL57BL/6J mice were purchased from Jackson laboratories (Bar Harbor, ME).

RNA preparation

Plasmids containing the indicated cDNAs were linearized and transcribed *in vitro* using either SP6 or T7 as described [14]. The purified and precipitated cRNA was re-suspended in 10 mM Hepes, pH 7.4, and quantified by spectrophotometry. Each cRNA was diluted to a final concentration of 200 ng/ μ l prior to injection.

Oocyte Isolation and Injection

Follicles were isolated from twenty-one day old C57BL/6J mice by puncturing isolated ovaries with 30 gauge needles. Intact follicles greater than 400 microns were collected and washed two times in M-2 media (Specialty Media, Phillipsburg, NJ). Using a Nikon PLI-188 microinjector and sterile injecting pipets (Humagen, Charlottesville, VA.), 10–15 picoliters of mRNA was microinjected into the cytoplasm of the follicle-enclosed oocyte in M-2 medium warmed to 37° C. Following injections, follicles were transferred to M-16 medium (Specialty Media) for overnight incubation at 37° C in 5% CO₂. The next morning, follicles were punctured carefully to release the denuded oocytes, and meiotic progression was scored every 30 minutes for 4–5 hours using an inverted Nikon SMZ-1000 microscope. The zero time point was defined as the moment of follicle puncture. Oocyte maturation was scored by monitoring Germinal Vesicle Breakdown (GVBD). Oocytes showing clear nuclear membranes and nucleoli were classified as GV stage; those without visible nuclear structure were classified as GVBD.

For the frog oocyte injection, stage V - VI *Xenopus laevis* oocytes were isolated from frog ovaries, denuded by incubation with collagenase, and injected with 50 nanoliters of the indicated cRNAs (200 ng/μl) as described [14].

Measurement of cAMP

Injected oocytes from mouse follicles were gently denuded. Those expressing the M2R were treated for 10 minutes with or without 30 μM carbachol, followed by lysis in 100 μl of 0.1M HCl/1% Triton-X-100 (20 oocytes for each point). Oocytes expressing all other proteins were denuded and put directly in lysis buffer. All samples were then centrifuged to remove debris, and cAMP levels in the supernatants were measured in duplicate using an acetylated cAMP competitive ELISA kit (Endogen, Rockford, IL) per the manufacturer's protocol.

For *Xenopus laevis*, five oocytes were lysed in 500 μl of the aforementioned buffer. Samples were then diluted 1:10 and analyzed using the acetylated cAMP assay.

Gα_s Western blot

For each condition, twenty injected mouse oocytes were permeabilized in 20 μl of 2x SDS-sample buffer [18,23]. The entire lysate was then loaded in an individual lane for SDS-PAGE, transferred to Immobilon-P membranes (Millipore, Billerica, MA), blocked in 5% TBST-milk for 1 hour, then incubated with our anti-Gα_s at 1:10,000 overnight at 4°C. Membranes were then washed, incubated with HRP-conjugated secondary antibody (BioRad, Hercules, CA) for 1 hour, and signal detected by ECL-Plus (Amersham, Piscataway, NJ).

Results and Discussion

Endogenous Gβγ signaling does not contribute to the maintenance of meiotic arrest in mouse oocytes

As mentioned in the introduction, constitutive Gα_s-mediated stimulation of adenylyl cyclase plays a critical role in maintaining meiotic arrest in both *Xenopus laevis* and in mice. In addition, constitutive Gβγ-mediated signaling is important for maintaining meiotic arrest in *Xenopus* oocytes, perhaps by working in conjunction with Gα_s to enhance adenylyl cyclase activity. To determine whether constitutive Gβγ signaling is also important for maintaining meiotic arrest in mice, follicle-enclosed mouse oocytes were injected with cRNAs encoding either transducin or the carboxyl tail of the G protein-coupled receptor kinase 1 (GRK-1), both of which are known to sequester Gβγ heterodimers and reduce Gβγ signaling [14]. Unlike in *Xenopus* oocytes, where injection of cRNAs generated from these same plasmids resulted in enhanced steroid-triggered oocyte maturation [14], expression of transducin or the carboxyl GRK protein in mouse oocytes had no effect on the rate or magnitude of spontaneous GVBD once oocytes were removed from follicles (Fig. 1A and 1B). These results indicate that, unlike in frogs, constitutive Gβγ signaling is not likely to be involved in maintaining meiotic arrest in mouse oocytes.

Gβγ signaling reduces intracellular cAMP and promotes spontaneous mouse oocyte maturation in pre-ovulatory follicles

The observation that constitutive Gβγ signaling was not involved in maintaining mouse oocytes in meiotic arrest suggested that either constitutive Gβγ signaling simply does not occur in mouse oocytes, or that constitutive Gβγ signaling is present, but does not enhance adenylyl cyclase activity to increase cAMP and maintain meiotic arrest. To differentiate between these two possibilities, follicle-enclosed oocytes were injected with cRNAs encoding both Gβ and Gγ. Notably, when Gβγ is over-expressed in *Xenopus* oocytes, steroid-triggered oocyte maturation is markedly reduced [14]. In contrast, over-expression of Gβγ in follicle-enclosed

mouse oocytes resulted in 70% spontaneous GVBD, even while the oocytes were still enclosed in follicles (Fig. 2A). As a comparison, the rate of spontaneous GVBD of mock-injected oocytes measured directly after removal from follicles was only 30%. Even more remarkably, intracellular cAMP in mouse oocytes over-expressing G $\beta\gamma$ was reduced by nearly 10-fold (Fig. 2B). This contrasted with over-expression of G $\beta\gamma$ in *Xenopus laevis* oocytes, which led to nearly a five-fold induction of cAMP (Fig. 2B). Together, these data demonstrate that G $\beta\gamma$ signaling has completely opposite effects on cAMP levels in mouse versus *Xenopus laevis* oocytes. Furthermore, since G $\beta\gamma$ signaling actually promotes oocyte maturation in mouse oocytes, these results confirm that little to no constitutive G $\beta\gamma$ signaling is likely occurring in immature oocytes.

Stimulation of the G $\beta\gamma$ -coupled muscarinic 2 receptor (M2R) promotes mouse oocyte maturation

To confirm that G $\beta\gamma$ signaling increases intracellular cAMP levels and promotes mouse oocyte maturation, follicle-enclosed oocytes were injected with cRNA encoding the G $\beta\gamma$ -coupled M2R receptor. Stimulation of the M2R receptor with its agonist carbachol should activate endogenous G $\beta\gamma$ signaling, thus eliminating concerns related to over-expression of exogenous G proteins. As mentioned, activation of over-expressed M2R in *Xenopus laevis* oocytes markedly inhibits steroid-triggered oocyte maturation, most likely due to G $\beta\gamma$ -mediated stimulation adenylyl cyclase [18]. As anticipated, activation of the M2R had the opposite effect in mouse oocytes. Follicle-enclosed mouse oocytes were injected with cRNA encoding the M2R. Twenty-four hours after injection, oocytes were removed from follicles and treated with either saline or 30 μ M carbachol, and GVBD was measured. Interestingly, denuded M2R-expressing mouse oocytes treated with carbachol consistently progressed through GVBD more rapidly than mock-treated cells (Fig. 3A). In addition, carbachol stimulation of mouse oocytes over-expressing the M2R decreased intracellular cAMP by approximately twofold (Fig. 3B). In contrast, stimulation of the M2R in *Xenopus* oocytes markedly increased intracellular cAMP by approximately six-fold.

Notably, stimulation of the M2R will also activate G α_i , which could then contribute to the observed decrease in intracellular cAMP and enhanced maturation associated with carbachol treatment. However, pertussis toxin does not affect LH-induced oocyte maturation of mouse oocytes [33], nor does over-expression of either wild type or constitutively active G α_i (data not shown); thus, as in frog oocytes [14], G α_i -mediated signaling is not likely a major regulator of meiosis in mouse oocytes.

Together, these studies using G $\beta\gamma$ over-expression and M2R stimulation confirm that G $\beta\gamma$ functions very differently in mouse versus frog oocytes. In frogs, evidence suggests that G $\beta\gamma$ directly activates adenylyl cyclase activity to increase intracellular cAMP [34]. In fact, certain isoforms of adenylyl cyclase, including ACII and ACVII, are activated in concert by both G $\beta\gamma$ and G α_s [35], and ACVII has been identified in *Xenopus laevis* oocytes [34].

In contrast, we show here that G $\beta\gamma$ signaling in mouse oocytes decreases intracellular cAMP and promotes spontaneous maturation. These observations suggest that either mouse oocytes do not express ACII or ACVII, or that these cyclases cannot be activated by G $\beta\gamma$ in these cells. Furthermore, the ability of G $\beta\gamma$ to reduce intracellular cAMP indicates that G $\beta\gamma$ and G α_s signaling are directly antagonistic in mouse oocytes.

G $\beta\gamma$ attenuates the rise in cAMP due to G α_s -mediated stimulation of adenylyl cyclase

To determine whether G $\beta\gamma$ can block G α_s -mediated stimulation of adenylyl cyclase and inhibition of maturation, follicle-enclosed mouse oocytes were injected with cRNA encoding G α_s with or without G $\beta\gamma$. As expected, over-expression of G α_s significantly inhibited

spontaneous germinal vesicle breakdown after oocytes were removed from follicles (Fig. 4A). Furthermore, intracellular cAMP was increased in these $G\alpha_s$ -injected oocytes relative to mock-injected oocytes (Fig. 4A). Interestingly, over-expression of $G\beta\gamma$ rescued the inhibitory effects of $G\alpha_s$ on oocyte maturation, as well as the stimulatory effect on cAMP production. Notably, co-expression of $G\beta\gamma$ with $G\alpha_s$ did not alter $G\alpha_s$ expression levels, as determined by Western blot (Fig. 4B). These results suggest that the over-expressed $G\beta\gamma$ might directly bind to over-expressed $G\alpha_s$ to prevent activation of adenylyl cyclase

To determine whether $G\beta\gamma$ was in fact suppressing $G\alpha_s$ activity through binding and sequestration, similar studies were performed using a constitutively activated form of $G\alpha_s$, which presumably binds poorly to $G\beta\gamma$. As expected, over-expression of constitutively activated $G\alpha_s$ significantly raised intracellular cAMP (10-fold) and inhibited spontaneous oocyte maturation relative to mock-injected oocytes (Fig. 4C). Co-expression of $G\beta\gamma$ with constitutively activated $G\alpha_s$ did not increase the rate or extent of oocyte maturation relative to oocytes only expressing constitutively activated $G\alpha_s$, most likely due to continued elevation of intracellular cAMP that was still four-fold higher than in mock-injected oocytes (Fig. 4C). However, co-expression of $G\beta\gamma$ still reduced constitutively activated $G\alpha_s$ -stimulated cAMP by approximately three-fold (Fig. 4C). Since $G\beta\gamma$ presumably binds poorly to constitutively activated $G\alpha_s$, this result suggests that some of $G\beta\gamma$'s ability to lower intracellular cAMP may be mediated independent of $G\alpha_s$ sequestration. Other possibilities include direct inhibition of adenylyl cyclase or activation of phosphodiesterases by $G\beta\gamma$. Further biochemical studies will be necessary to definitively differentiate between these possibilities; however, it is intriguing to postulate that activation of $G\beta\gamma$ signaling in mouse oocytes, perhaps via a $G\beta\gamma$ -coupled receptor, may contribute to the decrease in intracellular cAMP that regulates maturation.

Summary

These studies and others highlight the many differences between G protein-mediated signaling in oocytes from different vertebrate species. For example, in fish, oocyte maturation is pertussis toxin sensitive, and $G\alpha_i$ appears to be the primary G protein triggering oocyte maturation. In contrast, $G\alpha_i$ does not appear to be playing a significant role in mediating oocyte maturation in frogs and mice [14,33]. Instead, both $G\alpha_s$ and $G\beta\gamma$ play critical roles in maintaining meiotic arrest in frogs oocytes, while only $G\alpha_s$ seems to maintain meiotic arrest in mouse oocytes. In fact, we find that $G\beta\gamma$ signaling can actually lower intracellular cAMP and promote maturation in mouse oocytes, suggesting that $G\beta\gamma$ may be a positive regulator of meiosis in mice. Further studies using intact FSH-primed follicles will be necessary to address whether activation of $G\beta\gamma$ signaling is a true physiologic mediator of LH-induced mouse oocyte maturation.

Acknowledgements

We thank Lisa Halvorson and Bruce Carr at UT Southwestern for generously allowing us to use their injection equipment. S.R.H. is a W. W. Caruth, Jr. Endowed Scholar in Biomedical Research. Work from our laboratory was supported by the NIH (DK59913) and the March of Dimes (FY05-78).

References

1. Cato AC, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. *Sci STKE* 2002;2002(138):9.
2. Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol* 2005;67:335–76. [PubMed: 15709962]
3. Levin ER. Integration of the extranuclear and nuclear actions of estrogen. *Mol Endocrinol* 2005;19(8):1951–9. [PubMed: 15705661]
4. Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. *Annu Rev Physiol* 2002;64:749–74. [PubMed: 11826287]

5. Kelly MJ, Qiu J, Ronnekleiv OK. Estrogen signaling in the hypothalamus. *Vitam Horm* 2005;71:123–45. [PubMed: 16112267]
6. Hammes SR. Steroids and oocyte maturation--a new look at an old story. *Mol Endocrinol* 2004;18(4):769–75. [PubMed: 14630999]
7. Maller JL, Krebs EG. Regulation of oocyte maturation. *Curr Top Cell Regul* 1980;16:271–311. [PubMed: 6249544]
8. Yang WH, Lutz LB, Hammes SR. *Xenopus laevis* Ovarian CYP17 Is a Highly Potent Enzyme Expressed Exclusively in Oocytes. Evidence that Oocytes Play a Critical Role in *Xenopus* Ovarian Androgen Production. *J Biol Chem* 2003;278(11):9552–9. [PubMed: 12522215]
9. Sadler SE, Maller JL. Progesterone inhibits adenylate cyclase in *Xenopus* oocytes. Action on the guanine nucleotide regulatory protein. *J Biol Chem* 1981;256(12):6368–73. [PubMed: 7240211]
10. Sadler SE, Maller JL. Inhibition of *Xenopus* oocyte adenylate cyclase by progesterone: a novel mechanism of action. *Adv Cyclic Nucleotide Protein Phosphorylation Res* 1985;19:179–94. [PubMed: 3159186]
11. Wang J, Cao WL, Liu XJ. Protein kinase A(PKA)-restrictive and PKA-permissive phases of oocyte maturation. *Cell Cycle* 2006;5(2):213–7. [PubMed: 16397412]
12. Wang J, Liu XJ. Progesterone inhibits protein kinase A (PKA) in *Xenopus* oocytes: demonstration of endogenous PKA activities using an expressed substrate. *J Cell Sci* 2004;117(Pt 21):5107–16. [PubMed: 15456849]
13. Gallo CJ, et al. Stimulation of *Xenopus* oocyte maturation by inhibition of the G-protein alpha S subunit, a component of the plasma membrane and yolk platelet membranes. *J Cell Biol* 1995;130(2):275–84. [PubMed: 7615631]
14. Lutz LB, et al. G protein beta gamma subunits inhibit nongenomic progesterone-induced signaling and maturation in *Xenopus laevis* oocytes. Evidence for a release of inhibition mechanism for cell cycle progression. *J Biol Chem* 2000;275(52):41512–20. [PubMed: 11018039]
15. Sheng Y, et al. Regulation of *Xenopus* oocyte meiosis arrest by G protein betagamma subunits. *Curr Biol* 2001;11(6):405–16. [PubMed: 11301251]
16. Romo X, et al. G(alpha)s levels regulate *Xenopus laevis* oocyte maturation. *Mol Reprod Dev* 2002;63(1):104–9. [PubMed: 12211067]
17. Guzman L, et al. A Gbetagamma stimulated adenylyl cyclase is involved in *Xenopus laevis* oocyte maturation. *J Cell Physiol* 2005;202(1):223–9. [PubMed: 15389534]
18. Lutz LB, et al. Selective modulation of genomic and nongenomic androgen responses by androgen receptor ligands. *Mol Endocrinol* 2003;17(6):1106–16. [PubMed: 12637588]
19. Fortune JE. Steroid production by *Xenopus* ovarian follicles at different developmental stages. *Dev Biol* 1983;99(2):502–9. [PubMed: 6413284]
20. Lutz LB, et al. Evidence that androgens are the primary steroids produced by *Xenopus laevis* ovaries and may signal through the classical androgen receptor to promote oocyte maturation. *Proc Natl Acad Sci U S A* 2001;98(24):13728–33. [PubMed: 11707587]
21. Smith LD, Ecker RE. The interaction of steroids with *Rana pipiens* Oocytes in the induction of maturation. *Dev Biol* 1971;25(2):232–47. [PubMed: 5562852]
22. White SN, et al. Specific modulation of nongenomic androgen signaling in the ovary. *Steroids* 2005;70(5–7):352–60. [PubMed: 15862817]
23. Haas D, et al. The modulator of nongenomic actions of the estrogen receptor (MNAR) regulates transcription-independent androgen receptor-mediated signaling: evidence that MNAR participates in G protein-regulated meiosis in *Xenopus laevis* oocytes. *Mol Endocrinol* 2005;19(8):2035–46. [PubMed: 15831520]
24. Wong CW, et al. Estrogen receptor-interacting protein that modulates its nongenomic activity-crosstalk with Src/Erk phosphorylation cascade. *Proc Natl Acad Sci U S A* 2002;99(23):14783–8. [PubMed: 12415108]
25. Albertini DF, Carabatsos MJ. Comparative aspects of meiotic cell cycle control in mammals. *J Mol Med* 1998;76(12):795–9. [PubMed: 9846949]
26. Albertini DF, et al. Cellular basis for paracrine regulation of ovarian follicle development. *Reproduction* 2001;121(5):647–53. [PubMed: 11427152]

27. Gill A, Jamnongjit M, Hammes SR. Androgens promote maturation and signaling in mouse oocytes independent of transcription: a release of inhibition model for mammalian oocyte meiosis. *Mol Endocrinol* 2004;18(1):97–104. [PubMed: 14576339]
28. Jamnongjit M, Gill A, Hammes SR. Epidermal Growth Factor Receptor signaling is required for normal ovarian steroidogenesis and oocyte maturation. *Proc Natl Acad Sci U S A* 2005;102:16257–16261. [PubMed: 16260720]
29. Lieberman ME, et al. Oocytic meiosis in cultured rat follicles during inhibition of steroidogenesis. *Acta Endocrinol (Copenh)* 1976;83(1):151–7. [PubMed: 989220]
30. Mehlmann LM, Jones TL, Jaffe LA. Meiotic arrest in the mouse follicle maintained by a Gs protein in the oocyte. *Science* 2002;297(5585):1343–5. [PubMed: 12193786]
31. Mehlmann LM, et al. The Gs-linked receptor GPR3 maintains meiotic arrest in mammalian oocytes. *Science* 2004;306(5703):1947–50. [PubMed: 15591206]
32. Hinckley M, et al. The G-protein-coupled receptors GPR3 and GPR12 are involved in cAMP signaling and maintenance of meiotic arrest in rodent oocytes. *Dev Biol.* 2005
33. Mehlmann LM, et al. Meiotic resumption in response to luteinizing hormone is independent of a G (i) family G protein or calcium in the mouse oocyte. *Dev Biol.* 2006
34. Sheng Y, Montplaisir V, Liu XJ. Co-operation of G α and G $\beta\gamma$ in maintaining G2 arrest in *Xenopus* oocytes. *J Cell Physiol* 2005;202(1):32–40. [PubMed: 15389551]
35. Federman AD, et al. Hormonal stimulation of adenylyl cyclase through Gi-protein beta gamma subunits. *Nature* 1992;356(6365):159–61. [PubMed: 1312225]

Figure 1.

Sequestration of $G\beta\gamma$ in mouse oocytes does not alter spontaneous maturation. Follicle enclosed oocytes were injected with buffer (Mock) or cRNAs encoding either transducin (A) or the carboxyl tail of GRK-1 (GRK, B). After an overnight incubation, oocytes were gently denuded from the follicles and GBVD scored at the indicated times as described in the methods section. The zero time point represents the time of oocyte release from follicles. Approximately twenty oocytes were used for each condition, and both experiments were repeated with similar results.

Figure 2.

Over-expressed Gβγ increases intracellular cAMP and promotes mouse oocyte maturation. (A) Follicle-enclosed mouse oocytes were injected with either buffer (Mock) or equal amounts of cRNAs encoding both Gβ and Gγ. After an overnight incubation, oocytes were gently denuded and GVBD immediately assessed. These results represent the average of five experiments ± S.E.M. (B) Follicle-enclosed mouse oocytes or denuded *Xenopus* oocytes were injected with buffer (Mock) or cRNAs encoding Gβ and Gγ as described in the methods. After an overnight incubation, mouse oocytes were gently denuded and cAMP levels in both the mouse and frog oocytes were determined by ELISA. Relative cAMP levels are indicated on the y-axis. Twenty

mouse and five *Xenopus* oocytes were used for each measurement (see methods). Each bar represent the average of three experiments $\pm$ S.E.M.

Figure 3.

Activation of endogenous $G\beta\gamma$ increases intracellular cAMP and promotes mouse oocyte maturation. (A) Follicle-enclosed mouse oocytes were injected with cRNA encoding the muscarinic 2 receptor (M2R). After overnight incubation, oocytes were gently denuded and incubated in either buffer alone or buffer plus 30 μ M carbachol. GVBD was measured at the indicated time points. (B) Follicle-enclosed mouse oocytes or denuded *Xenopus* oocytes were injected with buffer (Mock) or cRNA encoding the M2R. After an overnight incubation, mouse oocytes were gently denuded and both the frog and mouse oocytes were treated for 10 minutes with 30 μ M carbachol. The cAMP levels in both the mouse and frog oocytes were then determined by ELISA. Relative cAMP levels are indicated on the y-axis. Twenty mouse and

five *Xenopus* oocytes were used for each measurement (see methods). Each bar represent the average of three experiments $\pm$ S.E.M.

Figure 4.

$G\beta\gamma$ and $G\alpha_s$ have opposite effects on intracellular cAMP in mouse oocytes. (A) Follicle-enclosed mouse oocytes were injected with either buffer (Mock, circle) or cRNAs encoding either $G\alpha_s$ by itself (square) or $G\alpha_s$ plus $G\beta$ and $G\gamma$ (triangle). After an overnight incubation, oocytes were gently denuded and GVBD determined at the indicated times. Approximately twenty oocytes from each set of injections were used. Percent GVBD is shown in the graphs, and the relative intracellular cAMP levels from the same batch of oocytes at five hours are indicated on the right. Western blot analysis of injected oocytes demonstrates equal expression of $G\alpha_s$ (B). (C & D) The same experiments as shown in A & B, only oocytes were injected with cRNA encoding constitutively activated $G\alpha_s$ ($G\alpha_s - ca$) instead of wild-type $G\alpha_s$. Notably, here $G\alpha_s - ca$ increased cAMP 12-fold above mock-injected cells, while cAMP in oocytes co-expressing $G\beta\gamma$ with $G\alpha_s - ca$ were only 3.8-fold above mock-injected cells. All experiments were repeated at least twice with similar results.