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Nongenomic Steroid-Triggered Oocyte Maturation: Of Mice and Frogs

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Abstract

Luteinizing hormone (LH) mediates many important processes in ovarian follicles, including cumulus cell expansion, changes in gap junction expression and activity, sterol and steroid production, and the release of paracrine signaling molecules. All of these functions work together to trigger oocyte maturation (meiotic progression) and subsequent ovulation. Many laboratories are interested in better understanding both the extra-oocyte follicular processes that trigger oocyte maturation, as well as the intra-oocyte molecules and signals that regulate meiosis. Multiple model systems have been used to study LH-effects in the ovary, including fish, frogs, mice, rats, pigs, and primates. Here we provide a brief summary of oocyte maturation, focusing primarily on steroid-triggered meiotic progression in frogs and mice. Furthermore, we present new studies that implicate classical steroid receptors rather than alternative non-classical membrane steroid receptors as the primary regulators of steroid-mediated oocyte maturation in both of these model systems.

Keywords

oocyte; maturation; steroid; nongenomic; testosterone; G protein

I. Introduction

In nearly all vertebrates, oocytes are arrested in prophase I of meiosis until just prior to ovulation, when the gonadotropin Luteinizing Hormone (LH) binds to G protein-coupled receptors in ovarian follicles to unleash a myriad of signals that ultimately trigger oocytes to re-enter the cell cycle in a process called maturation. Oocytes progress through meiosis to metaphase II, at which point they again arrest until after fertilization, when meiosis is completed [1]. A long-standing model system to study oocyte maturation has been *Xenopus laevis* [2-4]. *Xenopus* oocytes remain in meiotic arrest after removal from the ovary, but can be induced to re-enter the cell cycle in response to multiple steroids. Steroid-triggered oocyte maturation in *Xenopus laevis* oocytes occurs completely independent of transcription, because: 1) very little transcription occurs during the maturation process; 2) addition of transcriptional inhibitors has no effect on steroid-mediated maturation *in vitro*; and 3) removal of nuclei from oocytes has no effect on steroid-triggered cytoplasmic signals associated with maturation. Since

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transcription plays no role in the meiotic process, steroid-triggered *Xenopus* oocyte maturation serves as an ideal physiologic model for studying transcription-independent, or nongenomic, steroid signaling.

Importantly, while significant progress has been made in identifying the steroids, steroid receptors, and intracellular signaling pathways that regulate oocyte maturation in *Xenopus laevis*, the relevance of steroids in regulating mammalian oocyte maturation has remained controversial. Here we provide a brief overview of meiotic progression in both frogs and mouse oocytes, and present novel data implicating classical steroid receptors as important regulators of steroid-triggered maturation in both systems.

II. Oocyte Maturation in *Xenopus laevis*

1. Androgens are the Physiologic Mediators of *Xenopus* Oocyte Maturation

As mentioned in the introduction, *Xenopus laevis* has served as an excellent experimental model for studying maturation and cell cycle regulation. The advantage of the *Xenopus* model is the ease of isolating large numbers of oocytes for over-expression and knockdown studies, as well as for assaying signals associated with meiosis (e.g., changes in cAMP, activation of MAPK and CDK cascades) [2-5]. In addition, isolated *Xenopus* oocytes remain in meiotic arrest until stimulated by steroid [4], thus allowing characterization of early signals triggering meiotic progression.

In most studies, progesterone is used as the *in vitro* promoter of *Xenopus laevis* oocyte maturation. Because it works well *in vitro*, progesterone was assumed to be the *in vivo* mediator as well; however, significant evidence suggested otherwise. First, mifepristone (RU486), a potent inhibitor of *Xenopus* PR-mediated transcription, did not block progesterone-mediated maturation [6,7]. Second, reduction of endogenous PR levels or over-expression of exogenous PR in *Xenopus* oocytes only partially altered progesterone-induced maturation [8,9]. Finally, *in vitro* stimulation of *Xenopus* ovarian fragments or follicles with gonadotropin revealed that other steroids, such as testosterone (a more potent promoter of oocyte maturation than progesterone [6,10]), were secreted at significantly higher levels than progesterone [11,12].

To determine the true physiologic mediator of *Xenopus laevis* oocyte maturation, female frogs were injected with human chorionic gonadotropin (hCG) followed by measurement of serum and ovarian steroid levels [6]. At every time point progesterone was nearly undetectable, while concentrations of androgens androstenedione and testosterone were more than ten-fold that of progesterone. Furthermore, *in vivo* inhibition of androgen production using a CYP17 inhibitor markedly reduced hCG-induced oocyte maturation and significantly delayed ovulation [13]. Together with the aforementioned *in vitro* studies, these observations indicate that androgens rather than progesterone are the primary physiologic mediators of oocyte maturation.

Interestingly, *Xenopus* oocytes express high levels of CYP17, the enzyme that converts progestins to androgens [6,14-16]. In fact, nearly all CYP17 in the frog ovary is localized to the oocytes rather than follicular cells [14], suggesting an unusual paradigm whereby oocytes are regulating production of the steroid that then promotes its own maturation. Furthermore, expression of CYP17 in oocytes means that addition of progesterone to oocytes *in vitro* actually results in the presence of two equally potent promoters of oocyte maturation: progesterone and androstenedione. This metabolism likely explains why alteration of PR levels or the use of PR antagonists only partially affects progesterone-mediated maturation.

2. The Classical Androgen Receptor Regulates Androgen-Induced Oocyte Maturation

By focusing on testosterone, concerns about steroid metabolism were eliminated, and the receptor regulating testosterone-induced meiotic progression was identified as the classical

androgen receptor (AR). Knockdown of AR expression or treatment with the AR antagonist flutamide markedly reduced testosterone-mediated maturation and activation of MAPK and CDK1 [6,14,17]. In fact, Selective Androgen Receptor Modulators (SARMs) have been described that specifically promote only nongenomic AR-mediated signaling (maturation and kinase activation) versus genomic AR-mediated signaling (transcription) [13,17]. Interestingly, approximately 5% of AR was expressed on oocyte cells surface, suggesting that this membrane-localized AR might be regulating nongenomic androgen effects. However, whether these membrane-associated receptors are solely responsible for AR-mediated oocyte maturation has yet to be determined.

3. Alternative Potential Steroid Receptors in *Xenopus* Oocytes

While pharmacologic and knockdown experiments implicate the classical AR as the regulator of testosterone-induced *Xenopus* oocyte maturation, the identity of the receptor mediating progesterone-triggered maturation remains controversial. In favor of the classical progesterone receptor (PR), over-expression of membrane-targeted PR significantly enhanced progesterone-mediated oocyte maturation, and the PR co-precipitated with G proteins in somatic cells and oocytes [18,19]. Furthermore, knockdown of the classical *Xenopus* PR slightly reduced progesterone-induced oocyte maturation [8,9]. However, evidence against the classical PR regulating progesterone-induced oocyte maturation is that these aforementioned effects of altering PR expression are only partial, and the pharmacology of progestin-mediated maturation is inconsistent with classical PR involvement.

In fact, similar to androgen actions via the classical AR, the classical PR very likely does regulate maturation in response to some progestins. However, these studies are difficult to interpret due to complexities of using progesterone as an *in vitro* agonist of *Xenopus* oocyte maturation. First, as mentioned, progesterone is rapidly converted to androstenedione by CYP17 [6,14]; thus, two maturation-inducing ligands are introduced to oocytes incubated with progesterone. Second, progesterone binds to the classical *Xenopus* AR with almost equal affinity to the PR [20], indicating that multiple classical receptors likely regulate progesterone-mediated maturation, even in the absence of steroid metabolism.

Notably, a novel membrane-localized progesterone receptor termed mPR has recently been implicated as a potential regulator of progesterone-induced *Xenopus* oocyte maturation [21]. The mPR family had been reported to regulate G protein signaling, and appears to regulate progestin-mediated oocyte maturation in fish [22]. *Xenopus* oocytes express an isoform of mPR β , and injection of an antibody against this mPR into *Xenopus* oocytes inhibited progesterone-mediated oocyte maturation [21].

To further address the role of mPR β in regulating *Xenopus* oocyte maturation, we examined the effect of the anti-mPR antibody on testosterone-induced oocyte maturation. First, we confirmed that, as reported, the *Xenopus* mPR β did not bind testosterone [21]. Both *Xenopus* AR and mPR β were expressed independently in COS cells (Fig. 2A). Specific testosterone binding was observed in cells over-expressing the AR relative to mock-transfected cells. In contrast, testosterone binding to cells over-expressing mPR β was no higher than binding to mock-transfected cells. Remarkably, injection of the anti-mPR antibody profoundly reduced both progesterone- and testosterone-induced oocyte maturation (Fig. 2B), as well as testosterone-mediated activation of MAPK and CDK1 (data not shown). The ability of the anti-mPR antibody to block testosterone-mediated maturation in the absence of testosterone binding suggests that mPR β may be important for oocyte maturation, but is likely not functioning as an independent steroid receptor. Alternatively, the antibody may simply be toxic to oocytes, thus preventing oocyte maturation in response to any signal. Further studies, including mPR knockdown experiments, will be necessary to determine the true importance of mPR β in regulating steroid-triggered oocyte maturation in *Xenopus laevis*.

4. G Protein Signaling Regulates *Xenopus* Oocyte Maturation

How does steroid signaling through classical steroid receptors regulate oocyte maturation? Most studies suggest a “release of inhibition” model whereby oocytes are held in meiotic arrest by constitutive G protein signals that stimulate adenylyl cyclase to increase intracellular cAMP (Fig. 1). Steroids act to overcome or inhibit these signals, thus reducing intracellular cAMP and allowing meiotic progression to occur. Although cAMP is clearly a critical regulator of meiosis, whether acutely reducing intracellular cAMP levels is either necessary or sufficient to promote oocyte maturation remains divisive [23-25].

In *Xenopus laevis*, both constitutive $G\alpha_s$ and $G\beta\gamma$ signaling are critical for maintaining elevated cAMP levels and meiotic arrest [26-29]. Suppression of G protein signaling by inhibition of $G\alpha_s$ or sequestration of $G\beta\gamma$, enhanced steroid-triggered oocyte maturation. Furthermore, studies using GIRK channel activity as a surrogate for $G\beta\gamma$ signaling confirmed the presence of constitutive $G\beta\gamma$ signaling that is rapidly suppressed (within one minute) upon addition of testosterone [20]. Knockdown of AR expression blocked the inhibitory effects of testosterone on $G\beta\gamma$ signaling, reiterating the importance of the classical AR in regulating nongenomic testosterone-triggered signaling.

The question as to what proteins promote this constitutive $G\alpha_s$ and $G\beta\gamma$ signaling to maintain meiotic arrest has recently been addressed, and has implicated a constitutively activated G protein-coupled receptor, GPR3 [25,30-32]. GPR3 is expressed in mouse and frog oocytes, and mice lacking GPR3 have reduced fertility with increased spontaneous oocyte maturation in ovarian follicles. Using this mouse model, as well as other *in vitro* techniques, studies have suggested that GPR3 is an important, but not exclusive, regulator of meiotic arrest, signaling primarily via $G\alpha_s$.

GPR3 also regulates meiosis in *Xenopus laevis* oocytes. Over-expressed *Xenopus* GPR3 elevated intra-oocyte cAMP levels and inhibited steroid-triggered oocyte maturation and MAPK/CDK1 activation [25,30]. Reduction of endogenous GPR3 expression had the opposite effect, resulting in decreased intracellular cAMP levels and enhanced steroid-mediated maturation and intracellular signaling. Unlike in mice, GPR3 effects were regulated in large part via $G\beta\gamma$ signaling in *Xenopus* oocytes [25].

Interestingly, GPR3 may be inactivated during gonadotropin-mediated oocyte maturation, as hCG treatment of *Xenopus* ovarian follicles triggered metalloproteinase-mediated cleavage and inactivation of GPR3 at the oocyte cell surface [25]. However, inactivation or loss of GPR3 signaling in *Xenopus* oocytes is not sufficient to promote spontaneous maturation, suggesting that alternative signaling pathways are utilized to maintain meiotic arrest, and that the primary *in-vivo* trigger of oocyte maturation in *Xenopus* is still steroids.

How are classical steroid receptors interacting with G proteins to alter their signaling? Co-precipitation studies indicate that the classical PR can complex with $G\beta\gamma$, although direct binding between these proteins has not been demonstrated. However, the scaffold molecule called the modulator of nongenomic steroid responses (MNAR) [33-35] contains specific domains that interact with the *Xenopus* AR as well as with $G\beta\gamma$, and may be serving as an intermediary during steroid-triggered oocyte maturation. In fact, knockdown MNAR expression in *Xenopus* oocytes increased testosterone-mediated maturation and attenuated $G\beta\gamma$ -coupled signaling by the M2R receptor [35]. These observations suggest that MNAR may enhance $G\beta\gamma$ signaling in *Xenopus* oocytes to maintain meiotic arrest. Activation of steroid receptors may then suppress $G\beta\gamma$ signaling through interactions with MNAR, ultimately leading to decreased cAMP and meiotic progression (Fig. 1).

5. Kinase Cascades and Oocyte Maturation

Steroid-mediated inhibition of G protein signaling leads to activation of a vast signaling network of downstream kinases [36,37]. A crucial step in this process is the accumulation of MOS protein, a germ cell specific Raf [38]. MOS protein is not detectable in immature oocytes; however, large amounts of *Mos* mRNA are present. Steroid stimulation leads to increased polyadenylation of *Mos* mRNA, followed by MOS protein translation. Once MOS is synthesized, it activates MEK1, followed by ERK and CDK1. Both ERK and CDK1 then enhance MOS protein accumulation, resulting in a powerful feedback loop that creates an irreversible signaling cascade to ensure an all-or-none response to steroid stimulation [39]. Recent studies have identified Paxillin as a key regulatory protein in this kinase cascade. Paxillin is a scaffolding protein that regulates MAPK signaling in other tissues [40-42]. Knockdown of Paxillin in *Xenopus* oocytes blocked MOS protein accumulation, while having no effect on *Mos* mRNA polyadenylation [43]. Furthermore, ERK-mediated phosphorylation of Paxillin was critical for this enhancement of MOS protein expression. Thus, Paxillin appears to regulate the potent all-or-none positive feedback loop that promotes *Xenopus* oocyte maturation (Fig. 1).

III. Oocyte Maturation in Mammals

1. Steroid-Triggered Mammalian Oocyte Maturation

While similar intracellular signaling pathways regulate meiotic arrest in frogs and mammals (e.g., GPR3, cAMP, and $G\alpha_s$) the role of steroids in triggering mammalian oocyte maturation has remained controversial. Some early studies suggested that steroids were not necessary for rodent oocyte maturation, as inhibitors of steroidogenesis appeared to have minimal effect on gonadotropin-mediated maturation [44-46]. In contrast, other studies found that inhibitors of steroidogenesis did indeed block gonadotropin-mediated maturation, and that, in fact, progesterone could promote oocyte maturation in a porcine model system [47,48]. These differences were likely due to variable culture conditions that can affect steroid-triggered maturation. For example, the use of oil overlay in cultures precludes examining steroid effects on maturation, as steroids will partition into the oil fraction [49,50]. In addition, variable carbohydrate content in the culture medium can have profound effects on the maturation process [51,52]. Finally, the fact that denuded mouse oocytes rapidly matured in the absence of any direct stimulation suggests that alternative strategies might be necessary to evaluate triggers of oocyte maturation in rodents.

Although one recent study could not demonstrate steroid-mediated mammalian oocyte maturation [53], studies from four different groups (in addition to the older porcine study mentioned above [48]) using different methods and model systems, have all indicated that steroids can indeed promote mammalian oocyte maturation. First, two groups showed that androgens trigger mouse or porcine oocyte maturation and activation of kinase cascades *in vitro* [54,55]. This process is transcription-independent and inhibited by AR antagonists. In fact, the pharmacology is almost identical to that of androgen-mediated oocyte maturation in *Xenopus* oocytes. In addition, two groups have shown that progesterone promotes mouse oocyte maturation *in vitro* [56,57]. This process is PR-dependent, as RU486 blocks progestin-induced maturation. Furthermore, oocyte maturation studies in PR null mice now demonstrate that, while oocytes from heterozygotic littermates respond to the progestin R5020, oocytes from PR null mice no longer mature when exposed to the progestin, but still mature in response to estradiol (Fig. 3). Together, these studies provide compelling evidence that steroids are capable of promoting mouse oocyte maturation *in vitro*, and that they are likely signaling through classical steroid receptors.

Finally, *in vivo* studies in primates have confirmed that progestins and androgens may be capable of promoting oocyte maturation. In primates primed with gonadotropins, injection of both the progestin R5020 and the androgen DHT promoted oocyte maturation in the absence of hCG [58].

Thus, steroid-triggered oocyte maturation may be conserved from frogs to primates, although the physiologic importance of steroid-mediated maturation in higher vertebrates has yet to be established.

2. The Role of G Protein Signaling in the Regulation of Mouse Oocyte Maturation

While $G\beta\gamma$ and $G\alpha_s$ signal together to stimulate adenylyl cyclase and maintain meiotic arrest in *Xenopus laevis* oocytes, $G\alpha_s$ is the dominant regulator of cAMP production in mouse oocytes [59,60]. In contrast to *Xenopus* oocytes, where sequestration of $G\beta\gamma$ enhances steroid-triggered oocyte maturation, sequestration of $G\beta\gamma$ in mouse oocytes had no effect on spontaneous oocyte maturation [61]. Similarly, while increased $G\beta\gamma$ signaling elevated intracellular cAMP and blocked *Xenopus* oocyte maturation, increased $G\beta\gamma$ signaling in mouse oocytes lowered cAMP and enhanced oocyte maturation. This fundamental difference is likely due to the specific expression of $G\beta\gamma$ -sensitive adenylyl cyclase VII in *Xenopus* oocytes [29,62]. In fact, the enhanced stimulation of cAMP production by $G\beta\gamma$ in frog oocyte may partially explain why they remain in meiotic arrest even after removal from the ovary.

As mentioned, a novel family of G protein-coupled receptors that includes GPRs 3 and 12 contribute to maintaining meiotic arrest in mammalian oocytes [31,63–66]. Consistent with the G protein studies described above, GPR3 and 12 promoted cAMP production primarily through $G\alpha_s$ in rodent oocytes. Interestingly, these receptors appear to have differential effects in mice versus rats, with GPR3 playing a dominant role in mouse oocytes, while GPR12 being more important in rat oocytes [64].

Recent work utilizing a GPR3 knockout mouse showed the presence of spontaneous oocyte maturation in *Gpr3*^{-/-} antral follicles [31,67]. In addition, female *Gpr3*^{-/-} null mice had smaller than normal litter sizes, with premature ovarian failure. However, these mice were still fertile [67], and anywhere from 10–30% of the antral oocytes in *Gpr3*^{-/-} null mice still remain in meiotic arrest [31,67]. Furthermore, similar numbers of oocytes in wild-type mice remained in meiotic arrest after being injected with short interfering RNAs directed against GPR3 mRNA [32]. Taken together, these observations confirm that although GPR3 is important, it is not essential nor is it the only player in the complex signaling processes that regulate oocyte maturation.

3. Paracrine Signaling and Oocyte Maturation in the mouse ovary

Although several labs have now demonstrated that steroids can promote mammalian oocyte maturation both *in vitro* and *in vivo*, the physiologic importance of steroids in this process remains uncertain. However, evidence suggests that steroid production may at least play partial role in regulating LH-induced oocyte maturation in mice. This concept is based on seminal findings describing the role of paracrine signaling in LH-induced oocyte maturation [68–72]. A longstanding mystery had been how LH activation of its receptor, which is located on mural granulosa and theca cells in ovarian follicles, leads to expansion of inner cumulus granulosa cells and meiotic resumption in oocytes, both of which lack LH receptors. In fact, stimulation of LH receptors in the mural granulosa and theca cells activates metalloproteinases, which then cleave membrane-bound EGF receptor ligands (e.g., amphiregulin and epiregulin). Once released, these ectodomains bind to EGF receptors on cumulus granulosa cells, leading to cumulus cell expansion and eventual oocyte maturation. Evidence supporting this model includes studies in genetic mouse models where EGF receptor or amphiregulin levels are

reduced [68], as well as inhibition studies whereby blockade of metalloproteinase activity or EGF receptor function abrogates LH-induced maturation [69,72].

Notably, EGF receptor ligands cannot directly promote maturation of denuded oocytes, suggesting that stimulation of EGF receptors in cumulus granulosa cells activates other pathways, and possibly releases paracrine signaling molecules that then directly promote meiotic progression of oocytes. These secondary signaling mechanisms are likely to be complex, involving changes in cell-cell communication between somatic cells and oocytes, alterations of G protein signaling within oocytes, changes in phosphodiesterase activity in oocytes, or release of maturation-promoting factors from cumulus cells. However, one intriguing factor that is produced by cumulus granulosa cells in response to LH and that also regulates oocyte maturation is steroid. In fact, the same LH/EGF receptor network that regulates oocyte maturation also mediates LH-induced steroidogenesis in the ovary [56]. Like oocyte maturation, EGF receptor activation is sufficient to promote rapid steroid production in oocyte-granulosa cell complexes (OGCs) and cumulus-oocyte complexes (COCs). Also similar to oocyte maturation, LH-mediated steroidogenesis is blocked by inhibitors of metalloproteinases and EGF receptor signaling, indicating that cleavage of membrane-bound EGF receptor ligands and downstream activation of the EGFR signaling in cumulus cells are critical processes regulating steroidogenesis.

Although several steroids promote mammalian oocyte maturation *in vitro*, progesterone levels are the highest in most mammals at the time of the LH surge [56,73-75]. In addition, progesterone actions via the progesterone receptor are known to be essential for ovulation, as mice lacking progesterone receptors do not ovulate in response to gonadotropin [73]. These observations suggest that LH-induced progesterone release might play at least a partial role in regulating oocyte maturation and subsequent ovulation.

Interestingly, recent work has shown that LH/EGF receptor crosstalk is also critical for LH-induced steroidogenesis in Leydig cells of the testes [76,77]. MMP-mediated release of EGF receptor ligands also occurs in these Leydig cells; however, unlike in the ovary, MMP inhibition does not significantly reduce LH-induced steroidogenesis, suggesting that it is not necessary for steroidogenesis in the testes. Finally, in Leydig cells, transactivation of the EGFR is regulated by LH-induced upregulation of cAMP and PKA, both of which also regulate EGFR-mediated oocyte maturation in follicles. Thus, LH/EGF receptor cross talk is critical for normal gonadal function and fertility in both males and females.

IV. Conclusions

In summary, G protein signaling plays a critical role in maintaining meiotic arrest in vertebrate oocytes, mainly by stimulating adenylyl cyclase and increasing intracellular cAMP. However, the nature of this signaling can vary between animals, depending upon the expression of G protein-coupled receptors, G proteins, and adenylyl cyclases within the oocytes. Androgens appear to be the primary physiologic mediators of oocyte maturation in *Xenopus laevis*, signaling through classical receptors in a transcription-independent fashion to decrease G protein-mediated stimulation of adenylyl cyclase. While steroids are capable of promoting mammalian oocyte maturation through similar mechanisms, the physiologic significance of this process has yet to be determined and will require further studies both *in vitro* and in whole animals.

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Figure 1.

Model for gonadotropin-mediated oocyte maturation in *Xenopus Laevis*. Prior to ovulation, oocytes are held in meiotic arrest in prophase I by constitutive G α_s and G $\beta\gamma$ signaling that stimulates adenylyl cyclase to elevate intracellular cAMP. Part of this inhibitory G protein signaling comes from GPR3; however, alternative mechanisms of G protein activation are likely present as well [25]. MNAR also contributes to the constitutive G $\beta\gamma$ signaling via direct interactions with G β [35]. Upon gonadotropin stimulation, proteinases might degrade and inactivate GPR3, thus enhancing but not initiating oocyte maturation. The most important signal that triggers oocyte maturation is gonadotropin-induced testosterone production, which acts via classical ARs and possibly MNAR to attenuate G $\beta\gamma$ signaling. These events lead to decreased intracellular cAMP, resulting in increased MOS protein translation. MOS then activates the MAPK cascade as well as CKD1, which in turn promote increased MOS production in a powerful feedback loop that is regulated at least in part by Paxillin. This all-or-none response ultimately leads to meiotic progression.

Figure 2.

Xenopus mPR regulates oocyte maturation independent of steroid binding A) COS cells were transfected with pcDNA3.1, an expression vector encoding Myc-tagged *Xenopus* mPR β (a generous gift from James Maller) [21], or a cDNA encoding Myc-tagged classical *Xenopus* AR. Binding assays with increasing amounts of radiolabeled testosterone were performed as described [20]. Note that, while significant testosterone binding to the classical AR was observed, testosterone binding to cells expressing mPR β was no higher than binding to mock-transfected cells. Expression levels were confirmed by Western blot using an anti-Myc antibody. B) Oocytes were injected with 50.6 nl of either 10 mM Hepes, nonspecific rabbit IgG (400 ng/ul), or anti-mPR immunoglobulin (1000 ng/ul). Oocytes were then treated with 300 nM testosterone or 300 nM progesterone. Oocyte maturation was quantified by percent GVBD (white spot on the animal pole) after 12 hours. Note that injection of the anti-mPR antibody significantly lowered both progesterone and testosterone-induced oocyte maturation, despite the lack of testosterone binding (A). Injection of the anti-mPR antibody also reduced MAPK and CDK1 activity (data not shown). Twenty oocytes were used for each point, and similar experiments were performed three times with nearly identical results.

Figure 3.

Oocytes from PR null mice do not mature in response to progestin. Follicles were isolated as described [56] from the ovaries of 4-week old PR null mice or heterozygotic littermates (generous gifts from O. Conneely, Baylor College of Medicine). 10-12 follicles were used for each condition, and were treated with 0.1% ethanol (ETOH), the progestin R5020 (250 nM), or estradiol (250 nM). After 8 hours, follicles were quickly dissected using 30 gauge needles, and oocytes scored for percent GVBD (visual loss of nuclear membrane). Note that oocytes from the heterozygotic mice matured in response to both estradiol and R5020, while oocytes from the PR null mice only responded to estradiol. This suggests that classical PRs are regulating progestin-induced oocyte maturation in mice. The graph represents the average of

two different experiments performed by two independent investigators. Each bar represents the average $\pm$ SD ($p=0.03$ when comparing responses to R5020 by students t-test).