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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.

1. 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 maturation of *X. 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 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 *X. 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.

2. Oocyte maturation in *X. laevis*

2.1. Androgens are the physiologic mediators of Xenopus oocyte maturation

As mentioned in the introduction, *X. 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 *X. 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

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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 *X. 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 10-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 their 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.

2.2. 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), while others stimulate only genomic AR-mediated signaling (transcription) [13,17]. Interestingly, approximately 5% of AR was expressed on the oocyte cell 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.

2.3. 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 mPRβ, and injection of an antibody against this mPR into Xenopus oocytes inhibited progesterone-mediated oocyte maturation [21].

To further address the role of mPRβ in regulating *Xenopus* oocyte maturation, we examined the effect of the anti-mPR antibody on testosterone-induced oocyte maturation. First, we confirmed

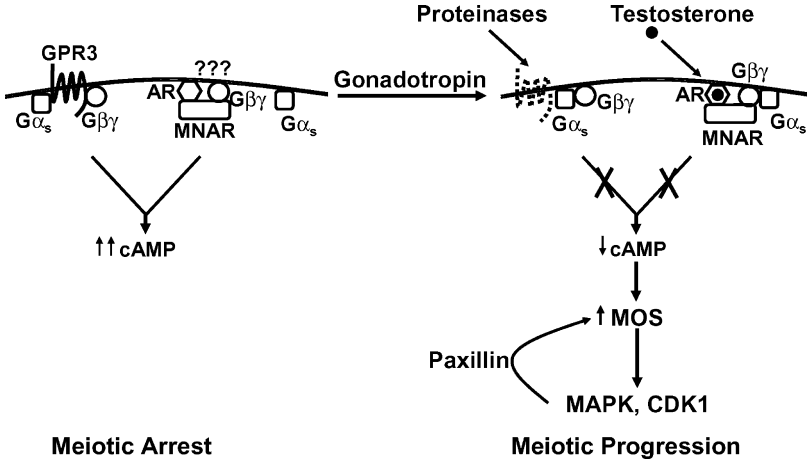

Fig. 1. Model for gonadotropin-mediated oocyte maturation in *Xenopus laevis*. Prior to ovulation, oocytes are held in meiotic arrest in prophase I by constitutive $G\alpha_s$ and $G\beta\gamma$ signaling that stimulates adenylyl cyclase to elevate intracellular cAMP. Part of this inhibitory G protein 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 Gβ [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 CDK1, 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.