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Review

Understanding extranuclear (nongenomic) androgen signaling: What a frog oocyte can tell us about human biology

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ABSTRACT

Steroids are key factors in a myriad of mammalian biological systems, including the brain, kidney, heart, bones, and gonads. While alternative potential steroid receptors have been described, the majority of biologically relevant steroid responses appear to be mediated by classical steroid receptors that are located in all parts of the cell, from the plasma membrane to the nucleus. Interestingly, these classical steroid receptors modulate different signals depending upon their location. For example, receptors in the plasma membrane interact with membrane signaling molecules, including G proteins and kinases. In contrast, receptors in the nucleus interact with nuclear signaling molecules, including transcriptional co-regulators. These extranuclear and intranuclear signals function together in an integrated fashion to regulate important biological functions. While most studies on extranuclear steroid signaling have focused on estrogens, recent work has demonstrated that nongenomic androgen signaling is equally important and that these two steroids modulate similar signaling pathways. In fact, by taking advantage of a simple model system whereby a physiologically relevant androgen-mediated process is regulated completely independent of transcription (*Xenopus laevis* oocyte maturation), many novel and conserved concepts in nongenomic steroid signaling have been uncovered and characterized.

Contents

1. Introduction

Androgens regulate a number of important biological processes, including male [1] and female reproduction [2–4], prostate growth and development [5,6], prostate cancer proliferation [7,8], and bone metabolism [9]. In all of these examples, androgens are believed to mediate their effects primarily through classical androgen receptors (ARs)[1]. While ARs are traditionally thought to mediate their effects primarily through the regulation of transcription in the nucleus, studies performed over the past 10 years have demonstrated that, in fact, ARs also mediate extranuclear (nongenomic) signaling pathways in response to androgens. These AR-mediated extranuclear pathways, that include G protein and kinase signaling, function synergistically with both AR-dependent and AR-independent transcriptional signals to regulate the afore-

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[1] AR, androgen receptor; Modulator of Nongenomic steroid Responses, MNAR; proline, glutamic acid, and leucine rich protein 1, PELP1; epidermal growth factor receptor, EGFR.

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mentioned physiologic processes. Here we provide an overview of extranuclear androgen actions, focusing on the remarkable conservation of nongenomic androgen-mediated signaling pathways from frog germ cell development to human prostate cancer proliferation.

2. Oocyte maturation: a biologically relevant model of nongenomic androgen signaling

Although great strides have been made in the identification and characterization of a number of extranuclear steroid signals, the physiological significance of many of these nongenomic actions remains elusive [10]. Since most steroid-induced processes involve both genomic and nongenomic effects, delineating the relative importance of nongenomic steroid actions in a biologically relevant process is a daunting task. To circumvent this problem, oocyte maturation in frogs has served as an ideal model system to study nongenomic steroid signaling in a physiologically important system [10–12]. Oocyte maturation refers to the resumption of meiosis. In all animals, oocytes are arrested in prophase I of meiosis (termed "immature" oocytes) until shortly before ovulation, when gonadotropins trigger meiotic re-entry, or maturation. Oocytes progress through meiosis to metaphase II, where they again arrest (termed "mature" oocytes). They remain in this stage until after ovulation and fertilization, when meiosis is finally completed [10,13–15]. Importantly, it is universally accepted that, not only do steroids induce this maturation process in oocytes from the frog *X. laevis*, but that they do so completely independent of transcription [16,17].

Historically, progesterone was considered the physiological mediator of *Xenopus* oocyte maturation [16–20]. However, gonadotropins stimulate more than 10 times more androgen than progesterone production at the time of ovulation, and androgens are equally or more potent promoters of oocyte maturation in vitro [12,21,22]. Furthermore, inhibition of androgen but not progesterone production in vivo almost completely blocks gonadotropin-induced oocyte maturation and ovulation [23]. Together, these observations prove that, in fact, androgens rather than progesterone are the physiologic regulators of oocyte maturation and release.

Androgen-induced *Xenopus* oocyte maturation is mediated by classical ARs, as both the androgen receptor antagonist flutamide [21] and AR knockdown by siRNA or antisense oligonucleotides [24] abrogates androgen-triggered maturation. Based on immunohistochemistry and biochemical studies, classical ARs are expressed throughout the cell, with approximately 5% found in the plasma membrane [24]. These membrane-localized ARs are presumed to be the regulators of androgen-mediated maturation, in part because testosterone coupled to BSA triggers oocyte maturation as well as free steroid; however, definitive proof of their importance has yet to be demonstrated.

How do androgens trigger *Xenopus* oocyte maturation? most studies implicate a "release of inhibition" mechanism whereby oocytes are held in meiotic arrest by constitutive inhibitory $G\alpha_s$ [25] and $G\beta\gamma$ signaling [24,26–28] (Fig. 1A) (Table 1). These inhibitory G protein signaling are mediated at least in part via the constitutively activated G protein-coupled receptor called GPR3 [29–31]. Combined $G\alpha_s$ and $G\beta\gamma$ signaling stimulate adenylyl cyclase to elevate intracellular cAMP levels [25,32], which then prevents meiotic progression through mechanisms that are not well understood [33,34] but may involve the scaffold protein named Modulator of Nongenomic steroid Responses (MNAR), or proline, glutamic acid, and leucine rich protein 1 (PELP1) [35]. In somatic cells, MNAR/PELP1 acts as a scaffold that links steroid receptors to Src and other signaling molecules [36]. In *Xenopus* oocytes, MNAR/PELP1 directly interacts with Gβ and AR to enhance $G\beta\gamma$-mediated stimulation of adenylyl cyclase [10,15,35]. Following gonadotropin stimulation, testosterone binding to ARs might cause a conformational change in the AR–PELP1–$G\beta\gamma$ complex that suppresses G-protein mediated signaling, leading to decreased intracellular cAMP levels and subsequent oocyte maturation [15,35].

Once cAMP levels drop, downstream kinases are activated, starting with the germ cell specific Raf homolog called MOS. In immature *Xenopus* oocytes, though there is sufficient *Mos* mRNA, little is translated into MOS protein [37–41]. When cAMP levels drop, *Mos* mRNA becomes polyadenylated, resulting in a small increase in MOS protein expression. MOS in turn activates the MEK–Erk pathway [42]. Androgen-induced expression of MOS and subsequent Erk activation requires the scaffolding protein called paxillin [43]. Paxillin is a 68 kDa focal adhesion protein that, in somatic cells, acts as a multi-domain adaptor and/or scaffold molecule to integrate many signals from integrins, cell surface receptors and growth factors [44]. Interestingly, in oocytes, after paxillin assists in androgen-triggered MOS and Erk activation, Erk phosphorylates paxillin on serine residues, which in turn leads to increased MOS protein expression and more Erk activation. Thus, paxillin functions both upstream and downstream of Erk, and this positive feedback loop ultimately leads to activation of cyclin dependent kinase CDK1 and subsequent meiotic resumption [43,45–48].

Androgens are also capable of promoting mammalian oocyte maturation. Studies using mouse [10,15,49] and porcine [50,51] oocytes show that testosterone induces oocyte maturation in a transcription independent manner that involves activation of MAPK and CDK1 signaling. In fact, androgen-induced maturation of mouse oocytes is blocked by the AR antagonist flutamide [49] and no longer occurs in oocytes lacking androgen receptors [4], providing pharmacologic and genetic evidence that, as in frogs, androgen-triggered oocyte maturation requires the classical AR. However, unlike in frog oocytes, the physiologic role of androgens (and progestins) in regulating mammalian oocyte maturation is still uncertain.

3. Extranuclear androgen signaling regulates transcription in the testes

While the physiologic role of androgens in murine oocyte development remains obscure, the role of testosterone and ARs in spermatogenesis is well established [52,53]. Surprisingly, although ARs are expressed in Sertoli cells [54] and testosterone is known to promote germ cell development [55,56], microarray studies of Sertoli cells in AR knockout mice reveal few transcriptional targets for testosterone. Furthermore, relatively few genes expressed in Sertoli cells are known to have AR binding elements (AREs) [57–60], although evidence in other tissues suggests that AREs might be located further from promoters than initially assumed [61]. Nonetheless, together, these observations suggest that extranuclear, or nongenomic, androgen signaling might be important for spermatogenesis [62].

In Sertoli cells, androgen-induced extranuclear signaling involves trans-activation of the epidermal growth factor receptor (EGFR) [56] (Fig. 1B) (Table 1). Testosterone binds to classical ARs [56,62–64] located at or near the plasma membrane, leading to activation of Src [65–67]. Src in turn promotes phosphorylation of the EGFR either directly or through factors yet to be identified, resulting in activation of the MAPK pathway (Raf-MEK-Erk) [67]. Once Erk is phosphorylated, it activates the kinase $p90^{RSK}$, which promotes phosphorylation of the cAMP Response Element Binding (CREB) protein. CREB then acts as a transcriptional co-activator upon binding to cAMP Response Elements (CREs) [62,65,67,68]. This pathway