Molecular and Cellular Endocrinology 448 (2017) 87–97

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Paxillin and embryonic PolyAdenylation Binding Protein (ePABP) engage to regulate androgen-dependent *Xenopus laevis* oocyte maturation - A model of kinase-dependent regulation of protein expression

Susanne U. Miedlich, MD [a, *, 1], Manisha Taya [a, 1], Melissa Rasar Young, MD PhD [b], Stephen R. Hammes, MD PhD [a]

[a] Division of Endocrinology and Metabolism, Department of Medicine, University of Rochester School of Medicine and Dentistry, Box 693, 601 Elmwood Ave, Rochester, NY 14642, USA
[b] Department of Therapeutic Radiology, Smilow Cancer Hospital at Yale, Yale School of Medicine, 35 Park Street, New Haven, CT 06511, USA

ARTICLE INFO

Article history:
Received 15 October 2016
Received in revised form
25 March 2017
Accepted 25 March 2017
Available online 28 March 2017

Keywords:
ePABP
Paxillin
Androgen
Mos
Erk
Oocyte
Maturation

ABSTRACT

Steroid-triggered *Xenopus laevis* oocyte maturation is an elegant physiologic model of nongenomic steroid signaling, as it proceeds completely independent of transcription. We previously demonstrated that androgens are the main physiologic stimulator of oocyte maturation in *Xenopus* oocytes, and that the adaptor protein paxillin plays a crucial role in mediating this process through a positive feedback loop in which paxillin first enhances Mos protein translation, ensued by Erk2 activation and Erk-dependent phosphorylation of paxillin on serine residues. Phosphoserine-paxillin then further augments Mos protein translation and downstream Erk2 activation, resulting in meiotic progression. We hypothesized that paxillin enhances Mos translation by interacting with embryonic PolyAdenylation Binding Protein (ePABP) on polyadenylated *Mos* mRNA. Knockdown of ePABP phenocopied paxillin knockdown, with reduced Mos protein expression, Erk2 and Cdk1 activation, as well as oocyte maturation. In both *Xenopus* oocytes and mammalian cells (HEK-293), paxillin and ePABP constitutively interacted. Testosterone (*Xenopus*) or EGF (HEK-293) augmented ePABP-paxillin binding, as well as ePABP binding to *Mos* mRNA (*Xenopus*), in an Erk-dependent fashion. Thus, ePABP and paxillin work together in an Erk-dependent fashion to enhance Mos protein translation and promote oocyte maturation.

1. Introduction

Over the past several decades, research has revealed that steroids regulate both nuclear as well as extranuclear signals through the same classical steroid hormone receptors located either inside or outside of the nucleus, respectively. Extranuclear, or nongenomic, steroid responses involve modulation of G protein-coupled as well as kinase signaling at or near the plasma membrane, and appear to be just as important as the genomic actions of their nuclear counterparts (reviewed in (Hammes and Davis, 2015)). The dissection of whether a specific biologic outcome is controlled by nongenomic as opposed to genomic steroid signaling can be difficult, especially since both pathways are intricately linked (Hammes and Davis, 2015). Interestingly, *Xenopus laevis* oocyte maturation (meiotic progression) is triggered by many steroids *in-vitro* but primarily by androgens *in-vivo*, and is entirely controlled on a post-transcriptional level (Lutz et al., 2001, 2003; White et al., 2005). *Xenopus laevis* oocyte maturation therefore serves as an elegant physiologic model to exclusively study nongenomic androgen receptor signaling.

Normal oocyte maturation relies on the precise regulation and timing of a multitude of intracellular signaling events (Whitaker, 1996). After duplication of the DNA early in embryogenesis, *Xenopus laevis* oocytes are held in the first meiotic arrest (prophase I) by constitutive Gβγ- and $G\alpha_s$-mediated stimulation of cAMP production (Gallo et al., 1995; Guzman et al., 2005; Lutz et al., 2000; Sheng et al., 2005), which is at least in part mediated through G protein-coupled receptor-3 (GPR3) signaling (Deng et al., 2008;

* Corresponding author.
E-mail address: susanne_miedlich@urmc.rochester.edu (S.U. Miedlich).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.mce.2017.03.028
0303-7207/

Mehlmann et al., 2004; Rios-Cardona et al., 2008). The powerful surge of gonadotropin release and subsequent sex steroid production then suppresses G protein signaling and reduces intracellular cAMP levels, leading to increased polyadenylation of *Mos* mRNA and translation of Mos protein (the germ cell equivalent of Raf). Mos then promotes activation of MEK and then Erk2, which feeds back in a positive fashion to promote more Mos protein production and Erk signaling, ultimately leading to activation of cyclin-dependent kinase 1 (Cdk1) and meiotic resumption. Meiosis is halted again at the second meiotic arrest (metaphase II) until fertilization.

We have previously shown that the versatile adaptor protein paxillin is a critical regulator of sex-steroid triggered meiotic progression in *Xenopus laevis* oocytes by functioning as a potent enhancer of the aforementioned positive feedback loop. We have shown that paxillin first acts downstream of androgen-triggered *Mos* mRNA polyadenylation to promote Mos protein expression and subsequent Erk2 activation (Rasar et al., 2006). Erk2 then regulates phosphorylation of *Xenopus* paxillin at serine residues 107 and 111, after which phosphoserine-paxillin further enhances Mos protein production and Erk2 activation, ultimately resulting in meiotic progression. In fact, replacement of endogenous paxillin with a mutated paxillin protein containing alanine mutations at serines 107 and 111 no longer supported oocyte maturation, confirming the importance of these residues (Rasar et al., 2006).

Interestingly, the ability of paxillin to modulate extranuclear androgen steroid signaling through Erk is not restricted to frog oocytes. In human prostate cancer cells, paxillin regulates Erk signaling that is initiated at the plasma membrane through activation of the EGF receptor either directly by EGF or indirectly through androgen-triggered release of EGF receptor ligands. Specifically, as seen in frog oocytes, paxillin is required for Raf-mediated activation of Erk1/2 in prostate cancer cells, after which Erk1/2 mediates phosphorylation of human paxillin on serine residues. Phosphoserine-paxillin then travels to the nucleus and acts as a co-activator of both androgen- and growth factor-mediated gene transcription, ultimately promoting cell proliferation (Sen et al., 2010, 2012).

Additionally, in primary mouse granulosa cells, we have shown that paxillin mediates androgen-dependent FSH receptor protein expression exclusively on a post-transcriptional, translational level (Sen et al., 2014), reminiscent of its effects on Mos protein expression in *Xenopus laevis* oocyte maturation (Rasar et al., 2006).

How might paxillin regulate protein expression in *Xenopus laevis* oocytes and mammalian granulosa cells? Interestingly, paxillin can interact with the RNA-binding protein PABP1 (Poly-Adenylation Binding Protein 1) in NIH3T3 fibroblasts (Woods et al., 2002). Abolishing this interaction resulted in nuclear trapping of PABP1, as well as impaired cell spreading and migration (Woods et al., 2005). It was suggested by the authors that paxillin may act as a carrier of RNA-binding proteins such as PABP1, and thus by inference as a carrier of mRNAs, possibly targeting mRNA for translation (Woods et al., 2002). Details regarding which signals regulate these interactions and how they might regulate biologically important processes are still not known.

We proposed that paxillin might interact with RNA-binding proteins such as the PABP family in *Xenopus laevis* oocytes, thus enhancing translation of polyadenylated *Mos* mRNA to promote Mos protein expression and subsequent oocyte maturation. We further proposed that Erk-dependent phosphorylation of paxillin might be an important regulator of its interaction with PABP. In fact, knockdown of embryonic PABP (ePABP) in *Xenopus laevis* oocytes phenocopied the effects of paxillin knockdown, with normal steroid-triggered *Mos* mRNA polyadenylation but reduced Mos protein expression, Erk2 and Cdk1 activation, as well as germinal vesicle breakdown. Furthermore, paxillin-ePABP interactions were dependent on Erk-mediated phosphorylation of serines 107 and 111 on paxillin, suggesting that Erk signaling is a critical regulator of ePABP-paxillin-mediated translation.

2. Materials and methods

2.1. Antibodies

Anti-Mos (sc-86) was obtained from Santa Cruz Biotechnology, mouse anti-HA (#901501) from BioLegend, mouse anti-FLAG (F3165) from Sigma-Aldrich, rabbit anti-PABP1 (#4992), rabbit anti-phospho-p44/42 MAPK (#9101) and anti-total p44/42 MAPK (#9102) from Cell Signaling Technologies. For initial experiments determining ePABP and PABP1 expression, a polyclonal antibody directed against the C-terminal tail of ePABP was kindly provided by Joan Steitz (Voeltz et al., 2001). Rabbit polyclonal antibodies directed to *Xenopus* ePABP amino acids 449-462, were used for all other experiments (generated by Biosynthesis Inc., Lewisville, TX). The phospho-Cdk1 antibody was from Cell Signaling Technologies (#9111) and the total-Cdk1 antibody from Thermofisher Scientific (#33-1800).

2.2. Plasmid construction

cDNAs encoding *Xenopus* paxillin, *Xenopus* Mos, and the 3′UTR of *Mos* mRNA were cloned into pcDNA3.1(+) (Invitrogen) for eukaryotic expression and pGEM-HE for *Xenopus laevis* oocyte expression (Rasar et al., 2006). The primers used to clone *Xenopus* paxillin and *Xenopus* Mos included a sequence to incorporate an amino-terminal HA tag. Serine mutated versions of paxillin were cloned using site-directed mutagenesis to convert serine residues to alanine.

cDNAs encoding *Xenopus* ePABP (Mammalian Gene Collection ID: BC080020) and PABP1 (Mammalian Gene Collection ID: BC052100) were cloned into pGEM-HE for *Xenopus laevis* oocyte expression or pcDNA3.1(+) for eukaryotic expression. The primers included a sequence to incorporate an amino-terminal FLAG tag.

2.3. Oocyte preparation

Oocytes were harvested from female *Xenopus laevis* (NASCO) and treated as previously described (Lutz et al., 2000). Briefly, follicular cells were removed by incubation of the ovaries in 0.8 mg/ml collagenase A (Roche) in modified Barth's solution (MBSH) without Ca^{2+} for 3 h. Oocytes were then washed and incubated overnight at 16 °C in MBSH containing 1 mg/ml Ficoll, 1 mg/ml bovine serum albumin, 100U/ml penicillin and 0.1 mg/ml streptomycin. Testosterone-induced maturation assays (10–1000 nM) were performed on stage V/VI oocytes from each preparation to determine sensitivity to the steroid. Maturation was scored as germinal vesicle breakdown, which was visualized as a white spot on the animal pole of the oocyte. Twenty oocytes were used for each data point in all experiments.

2.4. Cell culture/transfection

HEK-293 cells (CRL1573, ATCC) were cultured in 1× high glucose Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific), supplemented with 10% FBS (Seradigm), 100 units/ml penicillin and streptomycin (Thermo Fisher Scientific) at 37C, 5% CO_2. For transient transfections, cDNA of *Xenopus laevis* paxillin and ePABP were premixed at a 1:3 ratio (total cDNA 2 μg per well of 6-well plate) with Xtreme Gene 9 (Roche) and medium (DMEM) according to manufacturer's instructions, added to 50% confluent