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Paxillin and Steroid Signaling: From Frog to Human

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Abstract

Paxillin is a well-characterized cytoplasmic adaptor protein that is known to play important roles in cytoskeletal rearrangement, cell adhesion, and cell motility. In addition to its structural functions, paxillin has more recently been shown to function as a regulator of cell division—mediating steroid-triggered meiosis in oocytes as well as steroid- and growth factor-induced proliferation in prostate and breast cancer. Paxillin mediates these processes through a conserved pathway that involves both extranuclear (nongenomic) and nuclear (genomic) steroid signaling, as well as both cytoplasmic and nuclear kinase signaling. In fact, paxillin appears to serve as a critical liaison between extranuclear and nuclear signaling in response to multiple stimuli, making it a fascinating molecule to study when trying to determine how growth signals from the membrane lead to important proliferative changes in the nucleus. This chapter outlines recent advances in understanding how paxillin regulates both steroid and growth factor signaling, focusing on the conserved nature of its actions from a frog germ cell to a human cancer cell.

Keywords

Paxillin; Steroids; Growth factors; Prostate cancer; Breast cancer

1 Introduction

During normal and pathologic growth and development, cells constantly translate a myriad of extracellular stimuli into a complex array of intracellular signaling events. In order to coordinate these processes, cells utilize multi-domain scaffold proteins such as paxillin. Paxillin is primarily known for its actions in the cytoplasm and near the plasma membrane, where it interacts with structural proteins such as vinculin, actin, and integrins to regulate focal adhesion, cytoskeletal rearrangements, and cell motility [1, 2]. Paxillin is able to perform these functions due to the multiple protein-binding motifs interspersed throughout the protein, including several LD domains in the N-terminus (known to bind to multiple signaling molecules including kinases and G proteins) and several LIM domains in the carboxyl half of the protein (zinc-binding structures that are important for localization of paxillin to focal adhesions). By bringing together both structural and signaling molecules into one complex, paxillin helps to mediate efficient activation of multiple important signaling pathways.

One such pathway that paxillin regulates involves kinase signaling. The paxillin protein contains tyrosine residues that serve as phosphorylation targets for members of both the FAK and Src families of kinases [1, 3, 4]. Once phosphorylated on these tyrosine residues, paxillin becomes a critical and essential mediator of the mitogen-activated protein kinase

(MAPK) signaling cascade. For example, paxillin-deficient fibroblasts show reduced fibronectin-induced Erk activation [5]. In addition, paxillin has been shown to serve as a regulated MAPK scaffold that binds all three components of the MAPK cascade, Raf, Mek, and Erk, upon growth factor stimulation of epithelial cells [4, 6].

While most of paxillin's actions had initially been reported to involve kinase signaling within the cytoplasm, work in the early 2000s suggested that paxillin might also play a role mediating steroid hormone-induced transcription within the nucleus. For example, the paxillin-related protein Hic-5 was shown to associate with both glucocorticoid and androgen receptors [7], possibly playing a positive role in regulating glucocorticoid and androgen receptor-mediated transcription. Similarly, in some prostate cancer cell lines, early evidence suggested that paxillin might play a positive role in androgen receptor-mediated transcription [8]. With this background in place, a greater focus was then placed on understanding how paxillin interacts with androgen receptor signaling, revealing that, from frog oocytes to human prostate and breast cancer cells, paxillin regulates both genomic and nongenomic steroid receptor-mediated signaling via a conserved mechanistic pathway.

2 Paxillin and Meiosis

The first biological process where paxillin actions on steroid hormone signaling were examined in detail was in oocyte maturation, or meiotic progression. Female fertility relies on precise regulation of oocyte meiosis. In nearly all animals, immature oocytes are arrested in prophase I of meiosis until just prior to ovulation, when gonadotropin-induced signals within the ovary trigger them to reenter the meiotic cycle. Oocytes progress through meiosis until metaphase II, when meiotic progression is again arrested, and mature oocytes are ready for ovulation and fertilization [9, 10].

One of the best biological systems for studying meiosis is the *Xenopus laevis* oocyte. In fact many of the maturation signaling pathways discovered in *Xenopus laevis* oocytes now appear to be conserved in mammalian oocytes as well [11, 12]. Interestingly, the ovarian factors that promote *Xenopus laevis* oocyte maturation are steroids. Although many such steroids are capable of promoting *Xenopus laevis* oocyte maturation in vitro, androgens likely serve as the physiologic trigger in vivo [13]. Androgens promote *Xenopus laevis* oocyte maturation by signaling through classical androgen receptors (ARs) in a transcription-independent (nongenomic) fashion [13, 14]. In fact, transcription during meiosis is essentially nonexistent, making steroid-triggered oocyte maturation one of the few biologically relevant purely nongenomic steroid-mediated processes. Given the ease of collecting oocytes (thousands per frog), measuring the physiologic results (germinal vesicle breakdown, or GBVD, which manifests itself as a visible white spot on the animal pole of the *Xenopus* oocyte), and following intermediate signaling pathways (*see below*), steroid-triggered maturation of *Xenopus laevis* oocytes is an outstanding model to study nongenomic steroid signaling in a transcription-free setting, with the expectation that discoveries in this "simple" model system will then be applicable to more complex models where extranuclear and nuclear steroid signaling occurs at the same time.

With regard to the steroid-triggered signaling process during *Xenopus laevis* oocyte maturation, meiosis progresses in a “release of inhibition” fashion whereby oocytes are held in meiotic arrest by constitutive G protein signals that stimulate adenylyl cyclase to elevate intracellular cAMP [15–17] (Fig. 1). One receptor that may promote this cAMP production is GPR3, which is constitutively active on the oocyte membrane and promotes both G α s and G $\beta\gamma$ signaling, both of which appear to stimulate adenylyl cyclase and therefore cAMP production [18, 19]. During ovulation, gonadotropins stimulate ovarian androgen production in the *Xenopus laevis* ovary, leading to activation of extranuclear ARs, attenuation of the constitutive G α s and G $\beta\gamma$ signaling (and possibly activation of phosphodiesterases), and a drop in intracellular cAMP. Once intracellular cAMP is reduced, a powerful feed-forward kinase signaling loop is activated, ultimately leading to germinal vesicle breakdown (GVBD) [10, 20].

Interestingly, this potent kinase signaling pathway that regulates oocyte maturation is the mitogen-activated protein kinase (MAPK) pathway. At the top of the cascade is MOS, an oocyte-specific mitogen-activated protein kinase (Mek) kinase similar to Raf (Fig. 1). *Mos* mRNA is present in immature oocytes, but essentially none is translated into stable MOS protein. Steroid-triggered reductions in cAMP lead to increased polyadenylation of *Mos* mRNA in a process that involves interactions of regulatory proteins EG2 and cytoplasmic polyadenylation element-binding protein (CPEB) with the 3′ untranslated region of *Mos* mRNA [21–26]. Increased polyadenylation of *Mos* mRNA leads to increased MOS protein expression. Once present, MOS protein is constitutively active and activates Mek1 [27], which in turn activates extracellular signaling-regulated kinase 2 (Erk2, or p42), and finally cycle-dependent kinase 1 (CDK1). Activated Erk2 then feeds back to promote further increases in MOS protein expression and activity, thus markedly enhancing the signaling cascade in a feed-forward fashion [28–30].

While this unusual positive feedback loop was originally described in the 1960s and 1970s [10, 31–33], the description of regulatory factors that mediate this signaling pathway did not occur until nearly 30 years later, when paxillin was discovered to be an important component of steroid-triggered oocyte maturation. In retrospect, an association of paxillin with oocyte maturation makes sense, as, in somatic cells, paxillin is known to interact with and regulate the activity of several components of the MAPK signaling cascade, including Raf, MEK, and Erk [6, 34]. Since the same MOS/Mek/Erk pathway is crucial for steroid-triggered oocyte maturation, one might anticipate that paxillin would be involved. In fact, knockdown of paxillin expression in *Xenopus laevis* oocytes using either antisense oligonucleotides or siRNA almost completely abrogates androgen-induced maturation [35]. In addition, paxillin knockdown markedly attenuates androgen-triggered MOS protein expression and downstream Erk activation. In contrast, androgen-triggered polyadenylation of mRNA is unaffected by paxillin knockdown. Together, these results demonstrate that paxillin functions downstream of *Mos* mRNA modification and upstream of MOS translation to regulate nongenomic androgen-triggered oocyte maturation in *Xenopus laevis* oocytes (Fig. 1).

Surprisingly, in addition to this important upstream role in regulating activation of the MOS/Mek/Erk pathway, paxillin is also regulating important maturation-related processes

downstream of the MAPK pathway. As mentioned earlier, paxillin contains serine residues that are targets for Erk-dependent phosphorylation. Interestingly, while re-expression of wild-type paxillin rescues steroid-triggered maturation in paxillin-knockdown oocytes, rescue with a paxillin protein containing alanine substitutions on serine residues 107 and 111 is ineffective at rescuing maturation. MOS and Erk activation are still intact in the presence of this mutated paxillin, though at significantly lower levels [35]. These data, plus others, suggest that paxillin subserves two separate functions doing androgen-triggered oocyte maturation. First, it regulates activation of the MAPK pathway, most likely by upregulating MOS protein expression and then possibly serving as a scaffold for Mek and Erk signaling. Second, after Erk-dependent phosphorylation on serine residues, phosphoserine-paxillin regulates downstream processes that are still not yet known to regulate oocyte maturation. In fact, since MOS expression and Erk activation are slightly reduced in the absence of phosphoserine-paxillin, it is possible that one of the functions of phosphoserine-paxillin is to enhance the aforementioned positive feedback loop in MAPK signaling that is critical for normal oocyte maturation (Fig. 1). Further studies will help elucidate how paxillin functions to both enhance MOS protein expression and promote downstream signals necessary for oocyte maturation; however, as seen below, the concept that paxillin and phosphoserine-paxillin play different but equally important roles in MAPK-mediated processes is a theme that is conserved from frog oocytes to human somatic cells.

3 Paxillin and Prostate Cancer

As mentioned, one of the advantages of studying nongenomic steroid signaling in oocyte maturation is that, during meiosis, no significant transcription is occurring. Therefore, the investigator is not clouded by trying to differentiate between the relative importance of genomic and nongenomic signaling during the observed physiologic process. However, once nongenomic signaling pathways have been characterized in this “simpler” model of oocyte maturation, these concepts can then be applied to more complex systems where both genomic and nongenomic steroid signaling are intertwined. Specifically, the role of paxillin in regulating prostate cancer cell proliferation has been studied, with evidence suggesting that, similar to its role in frog oocytes, paxillin regulates extranuclear androgen signaling in prostate cancer cells, and that this paxillin-dependent nongenomic signaling is required for androgen-triggered proliferation [36].

What is the androgen-triggered nongenomic pathway that regulates prostate cancer cell growth? Similar to androgen-induced oocyte maturation, Erk signaling appears to be of major importance [37]. However, whereas androgens trigger Erk activation by up-regulating *Mos* mRNA polyadenylation and therefore MOS protein expression in frog oocytes, in prostate cancer cells, androgens bind to extranuclear ARs at or near the plasma membrane, leading to activation of matrix metalloproteinases (MMPs) that then cleave membrane-bound epidermal growth factor receptor (EGFR) ligands (Fig. 2a). These ligands then *trans*-activate the EGFR, leading to Akt and Erk signaling [37–39]. Notably this concept of growth factor receptor *trans*-activation is not unique, as the estrogen receptor is known to function similarly in many cell types, including breast cancer and endothelial cells [40, 41]. Thus, whether referring to extranuclear androgen signaling prostate cancer cells or extranuclear estrogen signaling in breast cancer cells, an important concept to be taken from both

examples is that steroid-triggered kinase signaling is essentially the same as EGF-triggered kinase signaling, only steroids indirectly activate the EGFR through released EGFR ligands, while EGF directly activates the EGFR. In other words, outside the nucleus steroids signal just like a growth factor [37, 42].

Once the EGFR has been activated by androgens in prostate cancer cells, Erk activation occurs via the well-established MAPK pathway that involves Raf-Mek-Erk. Notably, this is the same MAPK pathway described above in *Xenopus* oocytes, only, in germ cells, MOS replaces Raf at the top of the pathway (Fig. 2a). Thus, although different means are used in oocytes versus prostate cancer cells, the end result of extranuclear androgen-triggered signaling is ultimately the same: activation of the Raf/MOS-Mek-Erk pathway. As such, the expectation might be that paxillin regulates MAPK signaling in prostate cancer cells similarly to how it does so in *Xenopus* oocytes. In fact, paxillin indeed functions at the same locations in the MAPK signaling cascade in oocytes and somatic cells [37, 42]. First, paxillin is required for Raf activation. Activated EGFR stimulates Src, which then phosphorylates paxillin on tyrosine residue 31 and 118—a process necessary for Raf activation and subsequent Erk phosphorylation. In support of this pathway, siRNA-mediated paxillin knockdown in prostate cancer cells eliminates androgen-triggered Mek and Erk signaling, but not activation of the EGFR or the Src. Furthermore, Erk activation can be rescued by over-expression of constitutively activated Mek or Raf. Together, these results place paxillin actions just downstream of Src and upstream of Raf.

Second, once Erk is activated, it regulates phosphorylation of paxillin at serine residues 83, 126, and 130, the latter two being homologous to serines 107 and 111 on frog paxillin, which are targeted during androgen-triggered oocyte maturation [35, 43]. This process of Erk-dependent phosphorylation of paxillin is essential for downstream proliferation effects of androgens, as paxillin protein lacking these serine residues can still mediate androgen-triggered Erk activation but can no longer promote androgen-triggered physiologic responses (*see below*) [37]. Thus, as observed in oocytes, paxillin serves as both an effector and an effector of androgen-triggered Erk signaling in prostate cancer cells (Fig. 2a).

How is phosphoserine-paxillin regulating proliferation in prostate cancer cells? As mentioned, in oocytes, phosphoserine-paxillin is required for meiotic progression; however, mechanistically how paxillin performs this function is not understood. In prostate cancer cells, phosphoserine-paxillin is essential for DHT-mediated proliferation. In contrast to its actions in oocytes, however, more is known about how phosphoserine-paxillin regulates proliferation. For example, immunofluorescence studies examining the subcellular localization of paxillin demonstrate that paxillin is localized primarily in the cytoplasm in resting prostate cancer cells [42]; however, upon stimulation with androgens, some paxillin relocates to the nucleus, and the majority of phosphoserine-paxillin is nuclear. These observations suggest that phosphoserine-paxillin might regulate nuclear processes. Interestingly, studies [42] in androgen-responsive prostate cancer cells show that androgen-induced expression of *PSA* mRNA or activation of the *PSA* promoter is dependent on both EGFR and Mek signaling, as inhibitors of either kinase block both processes. Similar results are seen with other AR targets such as FKBP5 and NKX3-1. In addition, knockdown of paxillin in various prostate cancer cells prevents androgen-induced induction of these

androgen-sensitive genes. Expression of wild-type paxillin in these knockdown cells restores the androgen-induced transcription, while the paxillin mutant lacking the Erk-targeted serine residues, which still allows Erk activation, does not. Together, these studies suggest that extranuclear androgen-triggered kinase signaling is required for maximal intranuclear transcription, and that phosphoserine-paxillin is the “liaison” that mediates this cross talk between genomic and nongenomic androgen signaling.

Since nuclear phosphoserine-paxillin appears to be required for AR-mediated transcription, the next question to be addressed would be how phosphoserine-paxillin is regulating nuclear AR effects. Interestingly, paxillin knockdown and rescue experiments demonstrate that phosphoserine-paxillin is required for androgen-triggered nuclear localization of the AR. Studies suggest that the AR might normally cycle between the cytoplasm and nucleus. Upon addition of androgen, rapid AR-mediated Erk signaling leads to phosphorylation of paxillin and nuclear localization of phosphoserine-paxillin. Once in the nucleus, co-immunoprecipitation studies demonstrate that phosphoserine-paxillin binds to AR, holding in the nucleus and allowing AR-mediated transcription to progress. Interestingly, chromatin immunoprecipitation assays demonstrate that, upon androgen stimulation of prostate cancer cells, paxillin can co-localize with the AR at the androgen-responsive elements of several AR-dependent genes [42]. Together, these data suggest that, just as paxillin serves as a scaffold for signaling molecules in the cytoplasm, phosphoserine-paxillin serves as a scaffold for transcription factors in the nucleus (Fig. 2a).

Notably, an important distinction between paxillin actions in prostate cancer cells versus oocytes is that paxillin serves a broader purpose beyond steroid signaling in the somatic cancer cells. In frog oocytes, only one major biological trigger activates Erk signaling: androgens [43]. In contrast, prostate cells use multiple triggers to activate Erk, including androgens and growth factors. In fact, based on its location in the signaling pathway (just downstream of the EGFR and Src), as well as paxillin knockdown studies in prostate cancer cells and experiments in mouse embryonic fibroblasts from paxillin null mice, paxillin appears to be a broad regulator of Erk-mediated processes in response to a myriad of inputs that signal through receptor tyrosine kinases and/or Erk [37]. Specifically, phosphoserine-paxillin seems to play an important role in regulating transcription of some Erk-dependent genes such as *c-Fos* and *cyclin D1* (Fig. 2b). Evidence demonstrates that, in PC3 prostate cancer cells [42], Erk-dependent serine phosphorylation of paxillin is necessary for EGF-mediated upregulation of *c-Fos* and *cyclin D1* mRNA expression. Similar to its interactions with the AR, phosphoserine-paxillin coprecipitates with activated Erk in nuclear extracts. In contrast to androgen signaling, however, phosphoserine-paxillin is not required for EGF-induced nuclear localization of Erk, and does not appear to bind to Erk-dependent promoters [42]. Instead, phosphoserine-paxillin is required for Erk-mediated phosphorylation of Elk1, which then enhances *c-Fos* gene expression. Thus, although phosphoserine-paxillin is not required for Erk nuclear localization and is not a co-activator of *c-Fos* promoter, phosphoserine-paxillin is still required for Erk-mediated actions by acting as a nuclear scaffold, facilitating in the formation of Erk-Elk1 complex (Fig. 2b).

Since paxillin is a critical regulator of both androgen- and growth factor-mediated signaling, it stands to reason that paxillin expression or activity might be upregulated in prostate

cancer. Studies in tissue microarrays of human prostate biopsies containing malignant, normal, and benign prostate hyperplasia samples show significant elevated levels of AR, paxillin, cyclin D2, and ki67 in malignant patient samples relative to normal and/or benign prostate hyperplasia samples. Furthermore, phosphoserine-paxillin expression, which was almost entirely nuclear, trended toward increased expression in prostate cancer samples [42]. These results confirm that the paxillin may play an important proliferative role in prostate cancer and perhaps other tumors as well, and may have value as a diagnostic or a therapeutic target.

4 Paxillin and Breast Cancer

Similar to prostate cancer, sex steroid and growth factor signaling pathways have been recognized as major driving forces for breast cancer growth [44, 45]. In breast cancers expressing estrogen receptor alpha (ER α), which represent the majority of breast tumors [46], estradiol is an extremely potent stimulus for breast cancer cell proliferation in vitro and in vivo [44]. In vitro, estradiol also triggers breast cancer cell migration [47] implying a role of estrogen for breast cancer invasion and metastasis. Accordingly, estrogen deprivation and antagonizing receptor signaling strategies are well established and successful breast cancer therapies [48]. However, just as prostate cancer eventually becomes resistant to hormone deprivation, over time persisting breast tumor cells adapt to the new growth conditions and cancer proliferation resumes.

As is true for androgens, in addition to classic genomic signaling, estradiol also signals in an extranuclear (nongenomic) fashion through membrane-located receptors by activating MMPs, thus releasing ligands which then *trans*-activate the EGFR and related receptor tyrosine kinases, in turn leading to activation of the MAPK signaling cascade [49]. Furthermore, as in androgen signaling in prostate cancer cells, estradiol-induced activation of MAPK is also an important regulator of both breast cancer growth and migration [47, 50, 51]. Given these similarities between breast and prostate tumor biology, it therefore would make sense that paxillin would play a similar role in breast cancer pathophysiology as it does in prostate cancer. In fact, several lines of evidence suggest a role of paxillin in regulating estrogen-sensitive breast cancer. First, estradiol-mediated cell migration of estrogen-sensitive breast cancer cells is abrogated by paxillin knockdown [47]. Second, as seen in androgen-dependent prostate cancer, estrogen-dependent breast cancer cell migration is dependent on kinases that, as mentioned, are known to mediate paxillin phosphorylation, including Src and Erk. In fact, inhibition of either of these kinases abolishes both estrogen-triggered paxillin phosphorylation on tyrosine residues and subsequent estradiol-mediated cell migration, in the estrogen-dependent breast cancer T47D cell line [47]. Since tyrosine and serine phosphorylation of paxillin by Src and Erk, respectively, facilitate androgen-dependent migration and proliferation in prostate cancer cell lines, one might speculate that estrogen-dependent breast cancer cell migration and proliferation may similarly depend on consecutive Src and Erk phosphorylation of paxillin. Accordingly, Erk-dependent serine phosphorylation of paxillin, followed by nuclear localization of phosphoserine-paxillin, has been described in primary granulosa cells [42], and therefore likely occurs in breast cancer cells. However, downstream nuclear effects of phosphoserine-paxillin have not yet been studied in breast cancer.

As mentioned, paxillin expression as well as other components of the MAPK proliferative signaling cascade appear to be upregulated in human prostate cancer tissues [42], suggesting that paxillin-mediated signaling may be an important regulator of prostate cancer growth. Interestingly, evidence suggests that paxillin expression is also upregulated in breast cancer, and may be related to receptor tyrosine kinase activity and tumor aggressiveness. First, in 20–30 % of breast cancers, HER2 (human epidermal growth factor receptor 2—a member of the receptor tyrosine kinase family) gene amplification is noted. This upregulated HER2 expression confers a more aggressive phenotype and is associated with poor disease outcome [52]. Importantly, HER2 over-expression is correlated with paxillin expression in a study of 314 primary invasive breast cancer samples by immunohistochemistry [53]. Second, in vitro studies using the breast cancer cell line MCF7 demonstrate that paxillin expression is upregulated by heregulin, and paxillin expression appears to correlate with HER2 expression in breast cancer cell lines and in grade III tumors [54]. These data suggest that paxillin may act downstream of HER2 as described for EGF signaling in prostate cancer [42]. Finally, paxillin knockdown in MDA-MB-231 cells, ER-negative but EGFR-positive breast cancer cells, causes a significant inhibition of cell migration in vitro and, more importantly, a dramatic reduction of lung metastasis formation in vivo when paxillin-depleted MDA-MB-231 cells were injected into SCID mice [55]. In a similar breast cancer metastasis model, MLK3-dependent phosphorylation of paxillin on serine 178 appears to upregulate mouse lung metastases [56]. Taken together, these data suggest that, as seen in prostate cancer, paxillin-mediated signaling may be an important regulator of breast cancer proliferation, and may serve as a potential therapeutic or diagnostic target in the future. Accordingly, in a study of 105 breast cancer tumors, increased paxillin expression was positively correlated with EZH2, Ki-67, and progesterone receptor expression—all markers of breast cancer aggressiveness [57].

5 Conclusion

In summary, accumulating studies suggest that paxillin has two important functions in cells. First, paxillin serves as a remodeling molecule in the cytoplasm to regulate important structural processes such as cytoskeletal rearrangements, focal adhesions, and motility. In modulating these processes, paxillin functions as a scaffold to regulate cytoplasmic kinase signaling. Second, paxillin serves as a proliferative molecule, functioning as a scaffold protein in the nucleus to regulate kinase- and steroid receptor-mediated transcription during cell cycle progression (either meiosis or mitosis). In fact, paxillin appears to be a liaison that mediates cross talk between these cytoplasmic and nuclear processes, as Erk-dependent serine phosphorylation of paxillin in the cytoplasm allows phosphoserine-paxillin to move to the nucleus, where it can then regulate transcription. Given this clear separation of localization and function, is it possible that suppressing the proliferative actions of paxillin by targeting the serine phosphorylation sites or the nuclear localization process might serve as a general means of suppressing cancer cell proliferation? Specifically, paxillin expression seems to be upregulated in both prostate and breast cancer, and tumor progression appears to utilize this nuclear paxillin-mediated pathway for both steroid-dependent and steroid-independent proliferation. Further studies will determine whether paxillin can serve as a *bona fide* diagnostic or therapeutic target; however, the remarkable conservation of function

from a frog germ cell to human cancer cell, combined with the early embryonic lethality seen in paxillin knockout mice [5], suggests that paxillin is a critical molecule in nearly every cell in the body.

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Fig. 1.

Paxillin mediates androgen-triggered *Xenopus laevis* oocyte maturation. Oocytes are held in meiotic arrest through constitutive $G\alpha_s$ - and $G\beta\gamma$ -mediated cAMP production, perhaps in part due to constitutive GPR3 activation at the membrane. Upon exposure to androgens, the androgen receptor (AR) complexes with G proteins to attenuate their signaling, thus reducing cAMP production. Decreased cAMP then leads to increased polyadenylation of existing *Mos* mRNA, followed by increased translation of MOS protein. MOS is a constitutively activated homologue of Raf (a MAPK kinase kinase) that then promotes Mek activation, followed by Erk2 activation. Erk2 then mediates phosphorylation of paxillin on serine residues, which is required for subsequent maturation. In addition, Erk2-dependent serine phosphorylation of paxillin appears to be necessary for maximal MOS protein production, suggesting that paxillin might be playing a role in the feed-forward MAPK kinase loop that is essential for normal meiotic progression

Fig. 2.

Paxillin serves as a liaison between extracellular and nuclear signaling. **(a)** Paxillin regulates AR-mediated extracellular kinase signaling and intranuclear transcription. Androgens bind to androgen receptors at or near the membrane, leading to activation of matrix metalloproteinases (MMPs) and release of membrane-bound EGF receptor (EGFR) ligands. These ligands *trans*-activate the EGFR, leading to Src-mediated phosphorylation of paxillin on tyrosine residues. Once tyrosine phosphorylated, paxillin then mediates activation of Raf, which then promotes Mek activation, followed by Erk1/2 activation. Erk1/2 next mediates phosphorylation of paxillin on serine residues, after which phosphoserine-paxillin enters the nucleus and interacts with androgen-bound AR to hold it in the nucleus. The AR can then mediate transcription of AR target genes. Notably, paxillin is associated with androgen-responsive elements of some, but not all, AR target genes. **(b)** Paxillin regulates Erk-mediated transcription. Activation of the EGFR leads to Src-mediated phosphorylation of paxillin on tyrosine residues, followed by MAPK activation and serine phosphorylation of paxillin as described above. Once in the nucleus, phosphoserine-paxillin binds to Erk1/2 and regulates Erk-dependent phosphorylation or Elk1. After phosphorylation, Erk1/2 and Elk1 can mediate transcription of important proliferative genes, including *c-Fos*. Notably, in contrast to phosphoserine-paxillin interactions with androgen-responsive elements, phosphoserine-paxillin has not yet been shown to associate Erk1/2- or Elk1-sensitive promoters