The Classical Progesterone Receptor Associates with p42 MAPK and Is Involved in Phosphatidylinositol 3-Kinase Signaling in Xenopus Oocytes*

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The induction of Xenopus laevis oocyte maturation by progesterone is a striking example of a steroid hormone-mediated event that does not require transcription. Here we have investigated the role of the classical progesterone receptor in this nongenomic signaling. The Xenopus progesterone receptor (XPR) was predominantly cytoplasmic; however, a significant fraction (~5%) of one form of the receptor (p82 XPR) was associated with the plasma membrane-containing P-10,000 fraction, compatible with the observation that membrane-impermeant derivatives of progesterone can induce maturation. XPR co-precipitated with active phosphatidylinositol 3-kinase. The phosphatidylinositol 3-kinase (PI3-K) inhibitor wortmannin delayed progesterone-induced maturation and completely blocked the insulin-dependent maturation, indicating that the association of XPR with PI3-K could be functionally important. We also examined whether the nongenomic signaling properties of XPR can account for the ability of glucocorticoids and the progesterone antagonist RU486 to induce maturation. We found that none of these steroids cause XPR to become associated with active PI3-K; thus, association of XPR with active PI3-K is progesterone-specific. Finally, we showed that p42 mitogen-activated protein kinase (MAPK) associates with XPR after progesterone-induced germinal vesicle breakdown and that active recombinant MAPK is able to phosphorylate p110 XPR in vitro. These findings demonstrate that the classical progesterone receptor is involved in progesterone-induced nongenomic signaling in Xenopus oocytes and provide evidence that p42 MAPK and PI3-K activity are directly associated with the classical progesterone receptor.

Many effects of steroid hormones result from changes in transcription; the hormone binds to a classical steroid hormone receptor in the cytoplasm or nucleus and changes the receptor's transcriptional regulatory properties (1–3). However, steroids can also exert fast, nontranscriptional effects (4–7). One physiologically important process that clearly depends upon nontranscriptional effects of steroid hormones is the maturation of fish and amphibian oocytes. Maturation is the series of events through which a fully grown oocyte becomes ready for ovulation and fertilization (8–10). Immature oocytes are arrested in a G2-like state with an intact nuclear envelope or germinal vesicle. The steroid hormones progesterone (in frogs) or 17α,20β-dihydroxy-4-pregnen-3-one (in fish) cause the oocyte to undergo germinal vesicle breakdown (GVBD),1 condense its chromosomes and organize a meiotic spindle, complete the first meiotic division, enter meiosis II, and then arrest in metaphase of meiosis II. Steroids may be important in the regulation of mammalian oocyte maturation as well (11–13).

Many of the biochemical and cell biological events of progesterone-induced frog oocyte maturation can occur in the absence of transcription. Progesterone can induce germinal vesicle breakdown in the presence of the transcriptional inhibitor actinomycin D (14) and can trigger Cdc2 activation in oocytes whose nuclei have been microsurgically removed (15, 16). Cdc2 activation is weaker and less sustained in enucleate Xenopus oocytes than it is in intact oocytes (17); nevertheless, it does occur. These findings demonstrate that nontranscriptional effects are of central importance in progesterone-induced oocyte maturation.

The nongenomic effects of progesterone on oocytes could be mediated either by a classical steroid hormone receptor or by some novel type of steroid hormone receptor. Until recently, it was generally believed that the latter was the case, based on several observations. First, progesterone is more effective in inducing maturation when applied to the outside of an oocyte than it is when microinjected into the cytoplasm or nucleus, consistent with a plasma membrane localization for the maturation-inducing receptor (15, 18). In addition, steroids immobilized on agarose beads (19) or covalently coupled to a synthetic polymer (20) can still induce maturation. Since classical steroid hormone receptors are generally found in the cytoplasm or nucleus, the receptor responsible for oocyte maturation was thought not to be a classical steroid hormone receptor. In addition, progesterone can cause rapid, GTP-dependent inhibition of adenyl cyclase in washed oocyte membranes (21–23). This supports the idea that the progesterone receptor may be localized to the membrane and suggests that the plasma membrane progesterone receptor is a seven-pass, G-protein-coupled receptor.

Two recent papers have prompted reexamination of the idea that the oocyte receptor is something other than a classical steroid hormone receptor (24, 25). Both papers reported the

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1 The abbreviations used are: ERK, extracellular signal-regulated kinase; GVBD, germinal vesicle breakdown; MAPK, mitogen-activated protein kinase; PI3-K, phosphatidylinositol 3-kinase; PR, progesterone receptor; PVDF, polyvinylidene difluoride; XPR, Xenopus progesterone receptor; PMSF, phenylmethylsulfonyl fluoride.
cloning and characterization of cDNAs for Xenopus homologs of the classical progesterone receptor, one of which appears to represent a complete cDNA, designated XPR-1 (25). The XPR-1 sequence shows high similarity to the mammalian progesterone receptor proteins in the C-terminal hormone binding domain and central DNA binding domain and more limited similarity in the N-terminal region that is present in mammalian PR-B proteins and absent from the smaller PR-A proteins (26). Both groups presented evidence that this classical progesterone receptor plays a role in progesterone-induced maturation. Injection of a truncated XPR mRNA was found to accelerate progesterone-induced Mos synthesis, p42 MAPK activation, and oocyte maturation, showing that a classical steroid hormone receptor can promote maturation (24, 25). The transcription inhibitor actinomycin D did not affect the ability of XPR to promote maturation (24, 25). In addition, XPR-1 antisense oligonucleotides were found to inhibit progesterone-induced maturation, and co-injection of sense XPR or human PR-B mRNAs restored progesterone-induced maturation (25). These findings support the hypothesis that oocyte maturation is mediated by nontranscriptional effects of a classical progesterone receptor.

However, there are a number of aspects of steroid-induced oocyte maturation that are difficult to reconcile with the hypothesis that it is mediated by XPR-1 (27). The first is the apparent localization of the maturation-inducing progesterone receptor to the plasma membrane. Although some classical steroid hormone receptors have been found to associate with the plasma membrane when overexpressed (28), Bayaa et al. reported that endogenous XPR-1 is exclusively cytosolic (24). If so, it would seem unlikely that XPR-1 mediates the progesterone-induced inhibition of adenyl cyclase in washed membrane preparations or the induction of maturation by immobilized steroids. In addition, a diverse group of nonprogesterone-like steroids, including the glucocorticoids hydrocortisone and deoxycorticosterone, are potent inducers of oocyte maturation (29). Unless XPR-1 differs markedly from other progesterone receptors in its ability to be activated by nonprogestins, it seems unlikely that it could mediate the effects of these steroids.

Here we have examined whether the location of XPR is consistent with its hypothesized role as a mediator of maturation and whether the steroid specificity of XPR-associated effects is consistent with the steroid specificity of maturation. In addition, we looked for a mechanistic connection between XPR and the signal transduction machinery of maturation. We found that mammalian progesterone receptor antibodies recognize two main protein bands in blots and immunoprecipitates of Xenopus oocyte lysates, a prominent 82-kDa band and a less prominent 110-kDa band. We found that about 5% of the oocyte’s 82-kDa XPR protein can be recovered from washed oocyte membranes, indicating that XPR could initiate signals at the plasma membrane. Moreover, progesterone caused XPR to become associated with phosphatidylinositol 3-kinase (PI3-K) activity. This association is detectable within 30 min of progesterone treatment and continues to increase until the time of germinal vesicle breakdown. The physical association of XPR with PI3-K provides one possible functional link between XPR and maturation. We also found that XPR becomes associated with p42 MAPK after germinal vesicle breakdown and that p42 MAPK is able to phosphorylate p110 XPR in vitro. This raises the possibility that XPR is regulated by the p42 MAPK pathway. Finally, we showed that RU486, hydrocortisone, and deoxycorticosterone, three steroids that can induce maturation but are not agonists at classical progesterone receptors, do not cause XPR to become associated with PI3-K activity. This finding suggests that although XPR may contribute to progesterone-induced maturation, it probably does not account for the maturation-inducing effects of nonprogestins.

**Experimental Procedures**

**Antibodies, Reagents, and Recombinant Proteins—** Several antibodies were obtained from commercial sources. A polyclonal anti-progesterone receptor antibody (SC-539) and its corresponding blocking peptide (SC-539P) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), mouse monoclonal anti-C2D9 was from Immunotest, mouse monoclonal anti-ERK2 (D2 SC1647) was from Santa Cruz Biotechnology, and mouse monoclonal anti-phospho-ERK from New England Biolabs. The DC3 rabbit polyclonal anti-p42 MAPK antiseraum was raised against a C-terminal peptide (30). The mouse monoclonal anti-N1 antibody b2-2B10 was a generous gift from C. Dreyer (31). The PI3-K inhibitor wortmannin was purchased from Calbiochem, phosphatidylinositol 3-kinase (PI3-K) was obtained from Avanti, and recombinant, active p42 MAPK (ERK2) was obtained from New England Biolabs. All other biochemicals, including hydrocortisone, deoxycorticosterone, and (RU486) mifepristone, were obtained from Sigma.

**Oocyte Isolation—** Xenopus ovari an tissue was surgically removed, and oocytes were defolliculated for 1–1.5 h at room temperature with 2 mg/ml collagenase and 0.5 mg/ml polyvinylpyrrolidone in Ca<sup>2+</sup>-free modified Barth’s solution (88 mM NaCl, 1 mM KCl, 0.82 mM MgSO<sub>4</sub>, 2.4 mM NaHCO<sub>3</sub>, 10 mM HEPES, pH 7.5). The oocytes were then washed four times with modified Barth’s solution. Stage VI oocytes were sorted manually and incubated at 16 °C in OR2 solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM Na<sub>2</sub>HPO<sub>4</sub>, 5 mM HEPES, pH 7.5) supplemented with 1 mg/ml bovine serum albumin and 50 μg/ml gentamicin for at least 10 h to allow the oocytes to recover from the collagenase treatment. Oocytes were then treated with progesterone and scored for GVBD by the appearance of a white dot at the animal pole.

**Oocyte Lysis—** Frozen oocytes were thawed rapidly and lysed by pipetting up and down in 60 μl of ice-cold extraction buffer (0.25 M sucrose, 0.1 mM NaCl, 2.5 mM MgCl<sub>2</sub>, 20 mM HEPES, pH 7.2) containing 10 mM EDTA, protease inhibitors (10 μg/ml leupeptin, 10 μg/ml pepstatin, 1 μg/ml aprotinin, 1 μg/ml phenylmethylsulfonyl fluoride) and phosphatase inhibitors (50 μM 2-glycerophosphate, 1 mM sodium orthovanadate, 2 μM microcystin). Samples were clarified by centrifugation for 2.5 min in a Beckman E microcentrifuge with a right angle rotor. Crude cytoplasm was collected and processed for immunoblotting as described (30).

**Subcellular Fractionation—** Xenopus oocytes were isolated as above and homogenized in 10 volumes of membrane preparation buffer (83 mM NaCl, 1 mM MgCl<sub>2</sub>, 10 mM HEPES, pH 7.9) in a Dounce homogenizer (20 strokes, pestle A). The pellet obtained at 100 × g (1100 rpm; Eppendorf tabletop centrifuge) for 10 min (P-100) contained mainly yolk platelets. Centrifugation of the supernatant at 1000 × g (3500 rpm; Eppendorf tabletop) for 10 min gave a melanosome-enriched pellet (P-1000). The plasma membrane-containing fraction P-10,000 was obtained by centrifugation of the supernatant at 10,000 × g (11,100 rpm; Eppendorf tabletop) (32). The supernatant, which contained cytosol, microsomes, and small vesicles, was saved. The pellet was then overlaid once with membrane preparation buffer and centrifuged for 10 min at 10,000 × g to wash away any residual cytosol. Nuclei were isolated by mechanical dissection.

**Immunoprecipitation—** Pools of 10–100 oocytes were lysed by pipetting. Lysates were precleared with protein A-Sepharose beads at 4 °C for 1 h. Afterward, lysates were immunoprecipitated with anti-PR antibody (1.4 μg) and fresh protein A-Sepharose beads at 4 °C for 3 h. The immunoprecipitates were washed once with lysis buffer, twice with buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 10 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, pH 7.4, and 0.15 mM NaCl. In some immunoprecipitations, the PR antibody was preincubated with a 10-fold excess of blocking peptide for 30 min at 4 °C prior to binding to the protein A-Sepharose beads.

**In Vitro Phosphorylation with Recombinant Active p42 MAPK—** Immunoprecipitates were washed with 0.25 M sucrose, 1 mM NaCl, 2.5 mM MgCl<sub>2</sub>, 20 mM HEPES, pH 7.2 containing 10 mM EDTA, protease inhibitors (10 μg/ml leupeptin, 10 μg/ml pepstatin, 1 μg/ml aprotinin, 1 μM phenylmethylsulfonyl fluoride), and phosphatase inhibitors (50 μM 2-glycerophosphate, 1 mM sodium orthovanadate, 2 μM microcystin) and twice with the same buffer without EDTA. Another wash was performed in the kinase reaction buffer (50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 2 mM dithiothreitol, 0.01% Brij 35). The kinase accounted in the page dimensions.
reaction was performed in 25 μl of kinase reaction buffer containing 5 μCi of [γ-32P]ATP, 50 μM unlabeled ATP, and 100 units of active MAPK (New England Biolabs) for 30 min at 30 °C.

**PI3-K Assay**—Pools of 100 oocytes were subjected to immunoprecipitation with PR antibody and washed twice with phosphate-buffered saline containing 1% Nonidet P-40 and 1 mM dithiothreitol; twice with 100 mM Tris, pH 7.4, 0.5 μL of [γ-32P]ATP. The reaction was stopped by adding 15 μl of 1 x xHCl, and the lipid layer was extracted with 120 μl of chloroform/methanol (1:1, v/v). The lipids were separated by thin layer chromatography on silica gel 60 plates (200-300 mesh; Alltech) in chloroform/methanol/H2O/NH4OH (43:38:7:5, v/v/v/v).

**RESULTS**

**XPR Is Present in the Cytosolic and Membrane Fractions of Xenopus Oocytes**—We looked for a progesterone receptor-related protein in *Xenopus* oocyte lysates by immunoblotting with a polyclonal antibody (SC-539) that recognizes both the short ~80-kDa PR-A and longer ~100-kDa PR-B isoforms of the mammalian progesterone receptor. In agreement with a previous report (24), we found that oocytes possess a prominent 82-kDa PR-related protein, here designated p82 XPR (Fig. 1A). Its molecular mass is compatible with its being either a full-length B-isoform of XPR-1 (predicted molecular mass 82 kDa) or an A-isoform (predicted molecular mass 72 kDa) (25). There was also a less prominent 110-kDa band that could represent a B-isoform; the mammalian B-isoforms generally migrate ~20 kDa above their predicted molecular masses. Alternatively, p110 XPR could represent an XPR-2 protein (25) or some other related protein. Both p82 XPR and p110 XPR were immunoprecipitated by SC-539 (Fig. 1A, right panel). The peptide against which the PR antibody was raised blocked immunoprecipitation of both p82 XPR and p110 XPR (Fig. 1A, right panel). Neither the p82 nor the p110 band changed in intensity during maturation (Fig. 1, A and B); thus, progesterone treatment appeared not to alter either the abundance or immunoprecipitability of either protein.

Next we examined the localization of p82 XPR and p110 XPR by subcellular fractionation. Because Bayaa et al. observed no detectable p82 XPR in immunoblots of one oocyte’s worth of washed membranes (24), we chose to analyze larger amounts of material, 50 oocytes’ worth of washed membranes/lane and five oocytes’ worth of cytoplasmic membrane (Fig. 1B). The p82 XPR protein was detected in both the membrane and supernatant fractions of progesterone-treated and -untreated oocytes, with roughly 5% of the p82 XPR present in the membrane fraction (Fig. 1B). Progesterone treatment did not detectably alter the partitioning of p82 XPR between membrane and cytoplasm. As controls, we examined the fractionation of a plasma membrane protein, CD29, a cytoplasmic protein, α-tubulin, and a nuclear and cytoplasmic protein, N1. The washed membrane fraction contained all of the detectable CD29, no detectable N1, and less than 0.4% of the detectable α-tubulin (Fig. 1B). Thus, the membrane-associated p82 XPR cannot be accounted for by cytoplasmic or nuclear contamination. The p110 XPR protein was detected only in the cytoplasm (Fig. 1B).

**Association of PI3-K Activity with XPR**—Phosphatidylinositol 3-kinase is a critical intermediary in growth factor-induced mitogenesis in somatic cells. PI3-K also becomes activated in oocytes treated with progesterone or insulin (33). Recent work has shown that the steroid hormone estradiol causes PI3-K to become activated and to associate with ERα estrogen receptor (34). PI3-K activity was also found to be associated with glucocorticoid and thyroid hormone receptors in a hormone-dependent fashion (34). Although the same study reported that PI3-K activity did not become associated with progesterone receptors in response to progestins (34), we nevertheless addressed the possibility that PI3-K might be a mechanistic link between the oocyte’s classical progesterone receptors and the signal transduction of maturation.

As shown in Fig. 2, PI3-K activity was found to be associated with progesterone receptor immunoprecipitates. The XPR-associated PI3-K activity began to increase within 30 min of progesterone treatment and was maximal at about the time of GVBD (Fig. 2). This is similar to the time course of p85-associated PI3-K activity in progesterone-treated oocytes (33). The PI3-K inhibitor wortmannin inhibited the XPR-associated...
PI3-K activity, as expected if the activity were due to a bona fide PI3-K (Fig. 2). These findings show that the classical progesterone receptor is functional in Xenopus oocytes and that it rapidly engages the PI3-K pathway upon progesterone treatment.

Next we examined whether other steroids that are effective at inducing GVBD also cause PI3-K activity to become associated with XPR. We treated oocytes with the glucocorticoids hydrocortisone and deoxycorticosterone, which are potent inducers of maturation (29). We found that 100% of the oocytes treated with either glucocorticoid matured within 3 h of hormone treatment, but neither glucocorticoid caused PI3-K activity to become associated with XPR (Fig. 3A). We also treated oocytes with mifepristone (RU486), a progesterone receptor antagonist that acts as a weak inducer of oocyte maturation (22). We found that only about 10% of the mifepristone-treated oocytes matured after an overnight incubation, but even among those oocytes that did mature, there was no detectable increase in the amount of XPR-associated PI3-K activity (Fig. 3B). Thus, the signaling properties of the classical progesterone receptor do not account for the broad steroid specificity of oocyte maturation. Mifepristone neither blocked nor augmented the progesterone-stimulated PI3-K activity in XPR immunoprecipitates and did not appreciably affect progesterone-induced maturation (Fig. 3B and data not shown).

Inhibition of PI3-K Activity Delays Progesterone-induced Maturation—There is conflicting evidence on whether PI3-K is important for progesterone-induced maturation. As mentioned above, PI3-K is activated in response to both progesterone and insulin (33, 35, 36). Moreover, a putative dominant negative form of PI3-K inhibits progesterone-induced oocyte maturation (33), suggesting that PI3-K is an important mediator of progesterone-induced maturation. However, the phosphatase SIP/SHIP inhibits the induction of oocyte maturation by PI3-K overexpression and by insulin but does not inhibit progesterone-induced maturation, arguing against an important role for PI3-K in progesterone-induced maturation (36). In addition, the PI3-K inhibitor wortmannin has been reported to block insulin-induced maturation but not progesterone-induced maturation (35). These findings argue that PI3-K is at most a redundant, inessential mediator of progesterone-induced maturation.

In light of the association of XPR with PI3-K activity, we reassessed the role of PI3-K in progesterone-stimulated oocyte maturation. We used relatively low (more physiological) concentrations of progesterone (0.6 μM) to increase the likelihood that any redundant progesterone-induced pathways would not overwhelm any contribution made by the PI3-K pathway and...
used various concentrations of the irreversible PI3-K inhibitor wortmannin. As shown in Fig. 4A, as little as 10 nM wortmannin was effective in inhibiting the co-precipitation of active PI3-K with XPR. Wortmannin also caused a significant delay in progesterone-induced maturation as assessed by GVBD. The extent of the delay depended on the dosage of wortmannin used (Fig. 4B). Low doses (10 nM) of wortmannin delayed maturation by about 2 h, whereas high doses (1 μM) blocked maturation. In contrast to the relatively modest effects of wortmannin on progesterone-stimulated maturation, insulin-induced maturation was already completely blocked by the lowest dose of wortmannin used (10 nM) (Fig. 4C). Similar results were also obtained with the inhibitor LY294002, which caused a delay in progesterone-induced oocyte maturation and complete inhibition of insulin-stimulated oocyte maturation (data not shown). Thus PI3-K activity may contribute to progesterone-induced oocyte maturation, but it is more important for insulin-induced maturation.

XPR Associates with Active p42 MAPK and Can Be Phosphorylated by p42 MAPK in Vitro—The phosphorylation of human PR-B at serine 294 by p42 MAPK signals its degradation by the 26 S proteasome in human breast cancer cells (37). In Xenopus oocytes, p42 MAPK (ERK2) becomes quantitatively activated just prior to GVBD and remains active in mature oocytes and eggs. p42 MAPK activation promotes Cdc2 activation, suppresses DNA replication between meiosis I and meiosis II, and helps establish the metaphase arrest of mature Xenopus oocytes and eggs (10, 38, 39). We therefore investigated whether p42 MAPK might associate with and phosphorylate XPR in oocytes.

No p42 MAPK was detected in XPR immunoprecipitates from G2 phase oocytes, which possess inactive p42 MAPK, or from oocytes treated with progesterone for 120 min, which possess active p42 MAPK but have not yet undergone GVBD (Fig. 5B). However, p42 MAPK was detected in XPR immunoprecipitates from GVBD-stage oocytes (Fig. 5B). The XPR-associated p42 MAPK was detectable with p42 MAPK antibodies and with antibodies to active phospho-MAPK (Fig. 5B). Only trace levels of p42 MAPK were detectable in control immunoprecipitates (Fig. 5B). Thus, XPR associates with p42 MAPK, but only after GVBD.

Since active p42 MAPK was associated with XPR after GVBD, we examined whether p42 MAPK can phosphorylate XPR in vitro. We incubated control and XPR immunoprecipitates with active p42 MAPK in the presence of [γ-32P]ATP. As shown in Fig. 5C, active p42 MAPK brought about the phosphorylation of a 110-kDa protein in the XPR immunoprecipitates but not in the control immunoprecipitates. The phospho-
protein band co-migrated with p110 XPR. There was no detectable phosphorylation of the p82 XPR protein (Fig. 5C).

**DISCUSSION**

The recent reports that overexpression of XPR accelerates progesterone-induced maturation (24) and antisense XPR oligonucleotides inhibit progesterone-induced maturation (25) support the idea that a classical progesterone receptor mediates progesterone-induced oocyte maturation, a physiological process long thought to be mediated by some other type of receptor. Here we have examined three questions that emerge out of these studies: (i) Can the localization of XPR account for the ability of bead-linked steroids to induce maturation? (ii) Is XPR able to directly engage signal transduction pathways that are involved in maturation? (iii) Can the steroid specificity of XPR-mediated signaling responses account for the steroid specificity of maturation?

**Can the Localization of XPR Account for the Ability of Bead-linked Steroids to Induce Maturation?**—Baya et al. (24) found no evidence for association of XPR with the plasma membrane. They proposed instead that the evidence that the maturation-inducing receptor was located in the plasma membrane needed to be reexamined. They pointed out that the induction of maturation by bead-linked steroids could result from the leaching of steroids off the beads and into the cytoplasm. They also noted that not all laboratories agree that microinjected progesterone is ineffective at inducing maturation (24). Even so, it would be difficult to reconcile the observation that washed plasma membranes respond to progesterone with the hypothesis that the progesterone receptor is exclusively cytoplasmic.

The present studies provide a resolution to this problem. We found that about 5% of the oocytes’ p82 XPR is present in washed membranes. This amount of membrane-associated p82 XPR cannot be accounted for by cytoplasmic or nuclear contamination. Thus, p82 XPR could be responsible for the maturation-inducing effects of bead-linked steroids and the effects of progesterone on adenylyl cyclase activity in washed plasma membrane preparations (15, 18, 19–23).

**Is XPR Able to Directly Engage Signal Transduction Pathways That Are Involved in Maturation?**—We found two links between XPR and the biochemistry of oocyte maturation. First, we found that progesterone causes XPR to become associated with PI3-K activity. No significant amount of PI3-K activity was found in control immunoprecipitates, indicating that the association is specific to XPR, and no XPR-associated PI3-K activity was found in oocytes induced to mature with RU486, hydrocortisone, or deoxycorticosterone. Thus, some receptor other than XPR is probably responsible for the maturation-inducing effects of these hormones. One attractive hypothesis is that other classical steroid hormone receptors, such as the glucocorticoid receptor, mediate these effects, although the potent glucocorticoid dexamethasone, which would be expected to interact with a classical glucocorticoid receptor, fails to induce maturation (29). Recently, Morrison et al. (40) have shown that progesterone can bring about the activation of a tyrosine-specific protein kinase in oocyte membrane/cortex preparations. It will be of interest to see whether this tyrosine kinase is also stimulated by RU486, hydrocortisone, and deoxycorticosterone.

In summary, our studies provide new evidence for the involvement of a classical progesterone receptor in meiotic maturation. The identity of the receptor responsible for maturation is becoming less elusive, although perhaps not yet conclusive.

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**Footnotes**

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