G Protein βγ Subunits Inhibit Nongenomic Progesterone-induced Signaling and Maturation in Xenopus laevis Oocytes

EVIDENCE FOR A RELEASE OF INHIBITION MECHANISM FOR CELL CYCLE PROGRESSION*

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Progesterone-induced maturation of Xenopus oocytes is a well known example of nongenomic signaling by steroids; however, little is known about the early signaling events involved in this process. Previous work has suggested that G proteins and G protein-coupled receptors may be involved in progesterone-mediated oocyte maturation as well as in other nongenomic steroid-induced signaling events. To investigate the role of G proteins in nongenomic signaling by progesterone, the effects of modulating Ga and Gβγ levels in Xenopus oocytes on progesterone-induced signaling and maturation were examined. Our results demonstrate that Gβγ subunits, rather than Ga, are the principal mediators of progesterone action in this system. We show that over-expression of Gβγ inhibits both progesterone-induced maturation and activation of the MAPK pathway, whereas sequestration of endogenous Gβγ subunits enhances progesterone-mediated signaling and maturation. These data are consistent with a model whereby endogenous free Xenopus Gβγ subunits constitutively inhibit oocyte maturation. Progesterone may induce maturation by antagonizing this inhibition and therefore allowing cell cycle progression to occur. These studies offer new insight into the early signaling events mediated by progesterone and may be useful in characterizing and identifying the membrane progesterone receptor in oocytes.

Steroid hormones are traditionally known to mediate their signaling and subsequent biological activities via nuclear receptors (1). Interestingly, many steroid-induced signaling events appear to be triggered independently from the classic nuclear receptor pathways. In fact, these processes likely involve steroid signaling via membrane receptors (2). Examples of rapid, nongenomic signaling by steroids are myriad, including aldosterone-induced increases in intracellular calcium in vascular smooth muscle cells (9–8), estrogen-mediated induction of nitric-oxide synthase in vascular endothelial cells (9–12), vitamin D-induced increases in intracellular calcium in osteosarcoma cells (13–15), and progesterone-mediated maturation of amphibian and fish oocytes (16–18).

The phenomenon of progesterone-induced maturation of Xenopus oocytes serves as a useful experimental model for studying nongenomic steroid signaling (16, 19–21). The maturation of an oocyte refers to the meiotic stage at which an oocyte rests. “Immature” oocytes are arrested in prophase of meiosis I. Before ovulation, oocytes are induced to re-enter the cell cycle, finally resting in metaphase II. These “mature” oocytes are then competent for ovulation and subsequent fertilization, after which the final stages of meiosis are completed (16).

Evidence suggests that progesterone-induced maturation of Xenopus oocytes is mediated by cell surface rather than nuclear receptors. First, maturation is unaffected by the transcriptional inhibitor actinomycin D (21). Second, progesterone covalently attached to either polymers or bovine serum albumin and therefore unable to diffuse through the oocyte membrane still mediates maturation (16, 22). Third, progesterone injection directly into oocytes does not induce maturation (16, 21, 23). Finally, progesterone appears to bind specifically and with relatively high affinity to oocyte membranes (24, 25). At this point, however, no progesterone binding proteins have been identified in Xenopus oocyte membranes.

Progesterone signaling may be coupled to G proteins. Progesterone treatment of oocytes results in a rapid decrease in intracellular cAMP, perhaps through attenuation of adenyl cyclase activity (26–29). This suggests that Ga, and therefore a Ga-coupled receptor, may be involved in progesterone-mediated signaling. In addition, progesterone activates the mitogen-activated protein kinase (MAPK)1 cascade in oocytes (30–34), which could be mediated by Gβγ subunits. Finally, 1-methyladenine induces maturation of starfish oocytes in a pertussis toxin-sensitive fashion, suggesting that Ga mediates oocyte maturation in a comparable system (35–37). Interestingly, progesterone binds to the G protein-coupled oxytocin receptor, thereby partially blocking oxytocin binding and oxytocin-mediated signaling (38). One hypothesis is that the high amounts of progesterone present during pregnancy bind to the oxytocin receptor and prevent the induction of labor. When progesterone levels decrease at the end of pregnancy, this “release of inhibition” may allow oxytocin-mediated contractions, and therefore labor, to begin. Again, these data are consistent with progesterone modulation of G protein-coupled receptor signaling.

The role of G proteins in progesterone-mediated Xenopus oocyte maturation was examined by systematically depleting or overexpressing G protein subunits in Xenopus oocytes. Our data suggest that, in fact, Ga is not important for this process. Rather, Gβγ subunits appear to be the principal mediators of progesterone action in Xenopus oocytes, acting as inhibitors of oocyte maturation. Progesterone may therefore induce matu-

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1 The abbreviations used are: MAPK, mitogen-activated protein kinase; GPCR, G protein-coupled receptor; MSBH, modified Barth’s solution; ERK, extracellular signal-regulated kinase; GTPγS, guanosine 5′-3-O-(thio)triphosphate.
by antagonizing constitutive Gβγ-mediated inhibition of cell cycle progression.

**EXPERIMENTAL PROCEDURES**

**Oocyte Preparation**—Oocytes were harvested from female *Xenopus laevis* (Nasco) and treated as described previously (39, 40). Briefly, follicular cells were removed by incubation of the oocytes for 3–4 h at room temperature with 1 mg/ml collagenase A (Roche Molecular Biochemicals) in modified Barth’s solution (MSBH) without Ca2+. Oocytes were then washed extensively and incubated overnight at 16 °C in MSBH with 1 mg/ml heat-inactivated bovine serum albumin, 1 mg/ml Ficoll, 100 units/ml penicillin, and 100 μg/ml streptomycin. Stage V–VI oocytes were selected, and maturation assays were performed on each preparation to determine its sensitivity to progesterone-induced maturation.

**Progesterone Maturation Assay**—Injected or un.injected stage V–VI oocytes were washed extensively with MSBH to remove the bovine serum albumin from the storage buffer. They were then incubated with various doses of progesterone (Sigma) for the times indicated in the figure legends. The ethanol concentration was always kept constant at 0.1% regardless of the progesterone concentration. Maturation was detected by visualizing germinal vesicle breakdown, which manifests itself as a white spot on the dark animal pole of the oocyte (16).

**Oocyte Membrane Preparation**—Oocyte membranes were prepared from Stage V–VI or Stage I–III oocytes as described (25). In summary, oocytes were homogenized with a Dounce homogenizer (Kontes, pestle A, 15 strokes) in membrane buffer (83 mM NaCl, 1 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 10 mM Hepes, pH 7.6) at 4 °C. The homogenate was centrifuged at 800 × g for 10 min, and the supernatant was removed and centrifuged again at 800 × g. After a third centrifugation at 800 × g, the supernatant was centrifuged at 20,000 × g for 25 min, and the membrane pellet was resuspended in membrane buffer, taking caution to avoid disturbing the black melanin component of the pellet. Membranes were centrifuged two more times at 20,000 × g and finally resuspended in MSBH at a protein concentration of 5 mg/ml. Samples were then frozen at −80 °C until needed.

**Binding Assay**—1,2,6,7-3H(N)-progesterone (PerkinElmer Life Sciences) was diluted in ethanol on ice to 100× the final concentrations indicated in the figure legends. 5 μl of each of the 100× solutions was added to individual 1.5-ml polypropylene microcentrifuge tubes with either 1 μl of unlabeled progesterone stock in ethanol (concentration indicated in the figure legends) or 1 μl of ethanol alone. Each condition (i.e. concentration of radiolabeled progesterone) was tested in triplicate for every experiment. Membranes were diluted to the concentrations indicated in the figure legends in MSBH at 4 °C, and 500 μl of this mixture was added to each tube containing radiolabeled steroid. The tubes were incubated at 4 °C for 1 h. The tubes were centrifuged at 4 °C for 10 min, 500 μl of the supernatant was collected, and the tube, diluted in scintillation fluid, and counted to determine the concentration of free progesterone at the end of the assay. Glass microfiber filters (Millipore) that had been preincubated in MSBH at 4 °C, and 500 μl of this mixture was added to each tube containing radiolabeled steroid. The tubes were incubated at 4 °C for 1 h. The tubes were centrifuged at 4 °C for 10 min, 500 μl of the supernatant was collected, and the tube, diluted in scintillation fluid, and counted to determine the concentration of free progesterone at the end of the assay. Glass microfiber filters (Millipore) that had been preincubated in MSBH at 4 °C, and 500 μl of each sample was applied to individual filters. The filters were washed with 20 μl of cold MSBH, removed, and placed in 5 ml of scintillation fluid (Budget-Solve, Research Products International Corp.) for counting on a Beckman LS1801 scintillation counter.

**Oligonucleotide Design, Plasmid Construction, cRNA Synthesis, and cRNA Injection**—Sense and antisense oligonucleotides directed against sequences near the start codons of *Xenopus* (UTSW). All of these cDNAs were cloned into the expression vector pGEM HE (a gift from L. Jan (University of California at San Francisco)) and were sequenced using the dyeoxy method. The bovine Gβ3 cDNA in pGEM HE and bovine Gγ2 cDNA in pFROG were gifts from L. Jan (University of California at San Francisco). The bovine transducin Go cDNA in pFROG was a gift from S. Coughlin (University of California at San Francisco), and the GRK-minigene in pGEM HE was a gift from E. Reuveny (Tel Aviv). All constructs were linearized and transcribed in vitro with either T7 or SP6 (Promega transcription kit). Stage V–VI oocytes were injected with 50.6 nl of cRNA at a concentration of approximately 200 ng/μl using a Drummond automatic injector, and injected oocytes were then incubated 30–48 h in MSBH II before the maturation or MAPK assays were performed.

**MAPK Assay**—Injected oocytes were washed with MSBH and incubated with 50 nM progesterone as described in the experimental procedures under "Progesterone Maturation Assay." Oocytes were lysed at the indicated times with lysis buffer (150 mM NaCl, 2 mM EDTA, 0.5 mM sodium orthovanadate, 2 mM NaF, 0.5 mM Tris, pH 7.4) at 4 °C. 12,6,7-3H(N)-progesterone (PerkinElmer Life Sciences, Boston, MA) or 1% Tween-20, 50 mM Tris, pH 7.4 for 1 h, incubated overnight at 4 °C with a rabbit anti-phospho-p44/42 MAPK antibody (New England Biolabs), washed four times with TBST, incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:4000, Bio-Rad), and washed another four times with TBST. Blots were then treated with ECL-Plus (Amersham Pharmacia Biotech) to visualize the proteins. Next, blots were stripped in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris, pH 6.7 at 55 °C for 30 min, washed four times with TBST, and reblotted as above using a rabbit anti-p44/42 MAPK polyclonal antibody (New England Biolabs) that would bind all p44/p42 regardless of its phosphorylation status.

**RESULTS**

The EC50 of Progesterone-induced Maturation Correlated with Specific High Affinity Binding of Progesterone to Maturation-competent Oocyte Membranes—Maturation experiments using increasing concentrations of progesterone revealed an EC50 for maturation in the range of 75–150 nM, depending upon the oocyte preparation (Fig. 1A). The sensitivity of the in vitro maturation response to low concentrations of progesterone suggests that it could be relevant in vivo, where physiologic concentrations of progesterone in the ovary could easily be in the 100 nM range (38). The maturation response was also relatively specific to progesterone, because much higher concentrations of progrenenolone (EC50 = −1 μM), 17-OH progesterone, corticosterone, and aldosterone (all with EC50 values of ~0.5–1 μM) were needed to induce maturation (data not shown).

Progesterone binding studies were performed on *Xenopus* oocyte membranes to compare the affinity of progesterone binding to the EC50 for progesterone-induced maturation. Scatchard analysis revealed two different sets of progesterone binding sites on the oocyte membranes: a set of lower affinity binding sites (Stage V–VI) and a set of higher affinity binding sites (Stage I–III). The density of high affinity binding sites in this in vitro model could easily be in the 100 nM range (38). The high affinity binding sites were also relatively specific to progesterone, because much higher concentrations of progrenenolone (EC50 = −1 μM), 17-OH progesterone, corticosterone, and aldosterone (all with EC50 values of ~0.5–1 μM) were needed to induce maturation (data not shown).

The EC50 for progesterone-induced maturation correlated with the equilibrium constants calculated for both sets of binding sites, suggesting that progesterone binding to either set of receptors could be mediating the maturation response. To confirm that the high affinity sites were mainly responsible for progesterone-mediated effects, membranes from maturation-competent (Stage V–VI) and incompetent (Stage I–III) oocytes were tested for progesterone binding. A radiolabeled progesterone concentration of 10 nM was used to favor detection of the high affinity binding sites. Although stage V–VI oocytes contained specific high affinity binding sites for [3H]progesterone, the earlier stage oocytes (Stages I–III) did not (Fig. 1C). The presence of the high affinity progesterone binding sites almost
exclusively on maturation-competent oocytes suggests that the specific binding of progesterone to the high affinity sites may indeed be linked to the maturation process. Competition studies with other steroids revealed that progesterone binding to the stage V–VI oocyte membranes using a wide range of radiolabeled progesterone concentrations was performed. 300 μg of membranes were used for each sample. Data represent the means of three samples at each concentration of progesterone and are expressed as a Scatchard plot. This experiment was performed three times with nearly identical results.

**FIG. 1.** Progesterone-mediated oocyte maturation and progesterone binding to oocyte membranes. A, the EC\textsubscript{50} for progesterone-mediated maturation was determined by treating oocytes with the indicated concentrations of progesterone (x-axis) for 18 h. Maturation, which was detected by looking for germinal vesicle breakdown, is displayed on the y-axis as a percentage of the total number of oocytes/concentration of progesterone (n = 20). This experiment was performed over 10 times with a range of EC\textsubscript{50} from 50 to 150 nM. B, binding studies on stage V–VI oocyte membranes using a wide range of radiolabeled progesterone concentrations was performed. 300 μg of membranes were used for each sample. Data represent the means of three samples at each concentration of progesterone and are expressed as a Scatchard plot. This experiment was performed three times with three different membrane preparations with nearly identical results. C, binding assays were performed on membranes from stage I–III and stage V–VI oocytes. 50 μg of membranes were incubated with 10 nM radiolabeled progesterone ± 100 nM unlabeled progesterone for each sample. Data represent the means ± S.D. (n = 3) of the total counts after subtracting background, with background defined as the number of counts in samples incubated with labeled plus excess unlabeled progesterone. The total counts bound in Stage V–VI membranes was approximately 2-fold above background. D, binding studies on stage V–VI oocyte membranes (300 μg/point) were done using the indicated radiolabeled progesterone concentrations. Experiments were performed as described in the experimental procedures only the MSBH contained 5 mM MgCl\textsubscript{2}, and half of the membranes were preincubated with 200 μM GTP\textsubscript{S} in MSBH for 30 min at 4 °C (open squares) before being added to the radiolabeled progesterone. Data represent the means of three samples at each concentration. This experiment was performed three times with nearly identical results.

**Progesterone Binding to Its Membrane Receptor Was Unaffected by the State of Gα Activation**—The affinity of a GPCR agonist for its receptor is generally decreased when associated Gα subunits are in the GTP-bound, activated state (42). For example, agonist affinity for the β-adrenergic receptor is markedly decreased in the presence of GTP (43). If progesterone is acting as a GPCR agonist to mediate oocyte maturation, then, accordingly, its affinity for this receptor might be expected to be...
lower when Go subunits are in the activated state. Xenopus oocyte membranes were incubated both with and without the nonhydrolyzable GTP analogue GTPγS. As demonstrated in Fig. 1D, incubation with GTPγS had no effect on progesterone binding to the oocyte membranes, suggesting that progesterone is not binding to its receptor in a fashion typical for a GPCR agonist.

**Progesterone-induced Maturation of Xenopus Oocytes Was Affected by Changes in Go Expression**—The role of Go in progesterone-induced maturation was examined by modulating its expression in oocytes. Depletion of endogenous Go was attained by injection of antisense oligonucleotides directed against mRNAs encoding three known Xenopus Go proteins into oocytes. Antisense experiments work well in oocytes because the oligonucleotides can be injected directly into the cell, thus avoiding the usual problems of degradation and inconsistent uptake seen with cultured cells (44). Note that progesterone concentrations used in this and subsequent experiments were just below the EC50 calculated from Fig. 1A. This allowed more subtle changes in progesterone sensitivity to be detected. Given the slight variability in EC50 between oocyte preparations, however, all experiments were performed multiple times to confirm the results. Injection of antisense oligonucleotides against mRNA encoding each individual Go subunit (Go1, Go2, and Go3) had minimal effect on progesterone-induced maturation when compared with injection of matching sense oligonucleotides (Fig. 2A). In contrast, simultaneous injection of antisense oligonucleotides against mRNAs encoding all three Go subunits significantly attenuated progesterone-mediated maturation when compared with oocytes injected with matching sense oligonucleotides (Fig. 2A).

The results from these antisense experiments are difficult to interpret, because antibodies against Xenopus Go proteins are not available to confirm that the antisense oligonucleotides are indeed reducing Go expression. To further examine the effects of Go on maturation, cRNA encoding the Xenopus Go2 protein was injected into oocytes. Injection of this cRNA resulted in marked spontaneous maturation when compared with oocytes injected with 10 mM Hepes (mock) or nonspecific cRNA (PAR1) (Fig. 2B). Interestingly, this spontaneous maturation was inhibited by nearly 80% with co-injection of the antisense oligonucleotide directed against Go2 (Fig. 2C, Go2-WT). In contrast, co-injection of the Go2 antisense oligonucleotide with a modified cRNA encoding the wild-type Go2 protein but containing 21 of 33 mismatches in the region corresponding to antisense oligonucleotide binding had less of an inhibitory effect on maturation (Fig. 2C, Go2-Mod). This suggests that at least the Go2 antisense oligonucleotide can inhibit Go activity via binding to its complementary RNA sequence. Together, these results indicate that the amount of Go expressed in oocytes directly correlates with the maturation response to progesterone.

**Gβγ Inhibited Progesterone-induced Maturation of Xenopus Oocytes**—Two interpretations can explain the observation that decreased Go, attenuated, whereas excess Go enhanced, progesterone-induced maturation. First, Go itself may be signaling to induce maturation. Second, Gβγ that is released by activation of Go may be affecting the maturation response. To differentiate between these two possibilities, oocytes were injected with cRNAs encoding bovine Gβγ or Gγγ subunits. These cRNAs encode functional proteins, because they have been injected together into Xenopus oocytes and markedly enhanced Gβγ-dependent G protein inward rectifying potassium channel activity (45). Individual expression of either Gβγ or Gγγ had no effect on progesterone-induced maturation when a progesterone concentration just below the EC50 was used (50 nM). In contrast, simultaneous expression of both subunits in oocytes significantly attenuated progesterone-induced maturation (Fig. 3A). Incubation with higher concentrations of progesterone overcame this inhibition by Gβγ (Fig. 3B), indicating that the processes of Gβγ-mediated inhibition and progesterone-mediated induction of maturation can compete with each other. In addition, these data demonstrate that injected Gβγ subunits are not simply damaging their host oocytes in a nonspecific fashion.

The effect of lowering endogenous Xenopus Gβγ was examined by injecting oocytes with cRNA encoding the bovine retinal transducin Go subunit. Transducin Go itself has no apparent activity in oocytes but has been used as a Gβγ “sink” to inhibit Gβγ-mediated signaling (46). Oocytes injected with transducin Go exhibited enhancement of progesterone-induced maturation (Figs. 3C and 2B). In addition, injection of a GRK minigene encoding the carboxyl portion of the GRK1 protein, which has been shown to inhibit Gβγ function in a very specific fashion (47, 48), markedly enhanced progesterone-mediated maturation at a concentration of progesterone just below the EC50 (50 nM) (Fig. 3D). The observation that sequestration of endogenous Gβγ enhances, while overexpression of exogenous Gβγ attenuates, progesterone-induced maturation suggests that increased Go activity is not activating maturation; instead, endogenous Gβγ is inhibiting maturation.

**GTPγS Inhibited Progesterone-induced Maturation**—One way to formally prove that Gβγ, and not Go, is important for progesterone-induced maturation would be to examine the effect of injected GTPγS on progesterone-induced maturation. GTPγS would be expected to bind irreversibly to Go subunits, thus activating all GTP binding proteins within the oocytes. In addition, the activated Go subunits would no longer interact with Gβγ subunits; thus, free Gβγ activity would be expected to increase as well. Indeed, injection of GTPγS has been shown to enhance signaling by Gβγ-activated G protein inward rectifying potassium channels (49, 50). Injection of GTPγS significantly blocked oocyte maturation in response to all concentrations of progesterone tested when compared with Mock-injected cells (Fig. 4A), providing further evidence that intrinsic Go activity is not stimulating oocyte maturation; rather, Gβγ subunits are inhibiting maturation.

**Constitutively Activated Go Had Minimal Effect on Progesterone-induced Oocyte Maturation**—Injection of GTPγS inhibited maturation in the presence of relatively high concentrations of progesterone (1 μM; Fig. 4A). GTPγS may therefore be inhibiting maturation simply by disabling the oocyte in a nonspecific fashion. A more elegant way to delineate the role of Go and Gβγ subunits in oocyte maturation would be to compare the effects of a constitutively active rat Go protein to its matched wild-type isoform. If Gβγ suppression is indeed the more important signaling event, then wild-type rat Go should behave similarly to the Xenopus Go, whereas the constitutively active Go protein, which cannot bind to and sequestrer Gβγ, should have little effect on progesterone-induced maturation. As expected, wild-type rat Go markedly enhanced progesterone-induced maturation at progesterone concentrations just below the EC50 (50 nM). In contrast, constitutively active rat Go, containing a mutation that decreased the intrinsic GTPase activity of the protein (Q204L) had minimal effect on maturation (Fig. 4B). The lack of any substantial change in progesterone sensitivity in oocytes overexpressing the activated Go clearly implies that Go activity is not important in the progesterone-mediated maturation response. Note that expression of these two Go proteins was equal as measured

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2 S. R. Hammes, unpublished results.
by Western blot analysis of extracts from injected oocytes (see Fig. 7C).

GI Effects Are Taking Place Upstream of Activation of Extracellular Signal-regulated Kinase Phosphorylation—One of the early signaling events triggered by progesterone is activation of the MAPK cascade. This process can be measured by immunoblotting for phosphorylated MAPK p42 (ERK1). Of note, Xenopus oocytes contain only the p42 isoform of ERK (51, 52). Oocytes were injected with cRNAs encoding the aforementioned G proteins to determine whether their effects were occurring proximal to this point. Overexpression of Gβγ resulted in decreased phosphorylation of ERK1 in response to suboptimal concentration of progesterone (50 nM) over the course of 4 h (Fig. 5A), which correlated with the decrease in maturation observed over an 18-h time period (Fig. 3). In contrast, injection of the GRK minigene markedly enhanced both the rate and amount of progesterone-induced ERK phosphorylation, consistent with the GRK protein’s ability to enhance progesterone-mediated maturation (Fig. 6A). Overexpression of transducin had a similar effect on ERK phosphorylation in response to progesterone (data not shown). Finally, injection of wild-type rat Gαi enhanced both the rate and amount of pro-
gesterone-induced ERK phosphorylation, whereas expression of the constitutively active GaQ (Q204L) had minimal overall effect on MAPK activation (Fig. 7A). Although phosphorylated ERK was detected at the 2-h time point in GaQ-Q204L-injected oocytes, the amount of phosphorylation at 4 h remained similar to mock injected cells. This contrasted with the markedly increased phosphorylation of ERK1 after 4 h detected in GaQ-WT expressing cells. Of note, Western blot analysis of these samples using an antibody against rat GaQ protein revealed that both GaQ subunits were equally expressed in the injected oocytes (Fig. 7C). In addition, equal amounts of sample were present in each lane, as confirmed by blotting for total ERK (Figs. 5B, 6B, and 7B). Together, these data indicate that Gβγ effects are occurring early in the progesterone-mediated signaling pathway, upstream of MAPK activation.

**DISCUSSION**

Progesterone induces maturation of Xenopus oocytes via a nongenomic pathway. Very little is currently understood about the early progesterone-induced signaling events, including the identity of the putative membrane progesterone binding protein. Previous work reported the presence of specific high affinity progesterone binding sites on Xenopus membranes (24, 25). Our results confirm these reports, demonstrating high affinity progesterone binding sites (Kd = 3 nM) on Stage V–VI oocyte membranes but not on Stage I–III oocyte membranes.
The presence of high affinity sites almost exclusively on maturation-competent oocytes, combined with the similarities between the $K_d$ for high affinity progesterone binding and the $EC_{50}$ for progesterone-induced maturation (~100 nM; Fig. 1A), suggest that the specific binding of progesterone to these sites is linked to the maturation process. An active role for the lower affinity sites cannot be completely ruled out, however, given that their equilibrium constant (140 nM) is also close to the $EC_{50}$ value.

Earlier work has implicated Ga$_i$ as a possible mediator of progesterone signaling in *Xenopus* oocytes (16, 26–29). The inability of constitutively active rat Ga$_i$ to significantly alter progesterone-mediated maturation and MAPK signaling, whereas wild-type rat Ga$_i$ enhanced both maturation and MAPK activation (Figs. 4B and 7A), argues against this hypothesis. Instead, these data suggest that intrinsic Ga$_i$ activity (for example, its ability to inhibit adenylyl cyclase) is unimportant for progesterone-induced maturation. We propose that the changes in progesterone sensitivity observed by modulating Ga$_i$ levels in oocytes are in fact due to changes in free Gbg levels. If so, Gbg must be acting as an inhibitor of maturation: the more free Gbg, the less sensitivity to progesterone-induced maturation. Accordingly, reduction of endogenous Ga$_i$, which would increase intracellular free Gbg, inhibited progesterone-induced maturation (Fig. 2A). Overexpression of Ga$_i$, which would deplete free Gbg, enhanced progesterone-induced maturation as well as MAPK activation (Figs. 2B, 4B, and 7A). Finally, constitutively active Ga$_s$, which cannot bind to Gbg and therefore would not alter endogenous Gbg levels, minimally affected both progesterone-mediated signaling and maturation (Figs. 4B and 7A).

In further support of the inhibitory role of Gbg in maturation, overexpression of bovine Gbg in oocytes markedly reduced the sensitivity of the maturation response to progesterone.
maturation (35, 37), suggesting that activation of Ga induced maturation. To date, in addition to the Ga, their respective constitutively active forms, on progesterone-inhibited Xenopus against its importance for their respective constitutively active forms, on progesterone-induced maturation of starfish oocytes. Injection of Ga overexpressed in oocytes (data not shown).

Importantly, sequestration of endogenous Gβγ by overexpression of either transducin or the more specific Gβγ-binding mini-GRK protein enhanced responses to progesterone. This suggests that endogenous Gβγ plays a real role in attenuating Xenopus oocyte maturation in response to progesterone and perhaps in resting oocytes as well.

It is still possible that progesterone-induced maturation is mediated by a Ga subunit other than Ga. An argument against this possibility is that injection of GTPγS, which activates all Ga subunits, still inhibited maturation (Fig. 5A). In addition, progesterone-mediated activation of Ga would be unlikely given the observed decrease in cAMP after progesterone treatment (26–29). Formal exclusion of this possibility is difficult, however, and will involve examining the effects of overexpression of all of the known classes of Ga subunits, and perhaps of their respective constitutively active forms, on progesterone-induced maturation. To date, in addition to the Ga subunits presented in these studies, Ga2, as well as multiple mammalian and Xenopus Ga subunits, enhance maturation when overexpressed in oocytes (data not shown).

Our results are strikingly different than earlier data reported using starfish oocytes. Injection of Ga subunits into starfish oocytes inhibits, whereas injection of Gβγ enhances, maturation (35, 37), suggesting that activation of Ga and release of Gβγ are critical for starfish oocyte activation. These contrasting results are not surprising, however. First, 1-methyladenine-induced maturation of starfish oocytes is inhibited by pertussis toxin (53–55), whereas progesterone-induced maturation of Xenopus oocytes is not (26, 56–58). This supports the involvement of Ga in starfish oocyte maturation and argues against its importance for Xenopus oocyte maturation. Second,
