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Xenopus $G_q\alpha$ subunit activates the phosphatidylinositol pathway in *Xenopus* oocytes but does not consistently induce oocyte maturation

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ABSTRACT **We cloned the *Xenopus laevis* form of $G_q\alpha$ subunit to study its effects on oocyte maturation. Injection of *Xenopus* $G_q\alpha$ mRNA into stage 6 oocytes activated the phospholipase C/phosphatidylinositol pathway. The oocyte membrane became permeable to calcium ions and was able to generate transient inward currents (T_{in}), due to the opening of Ca^{2+}-dependent Cl^- channels. The T_{in} amplitude developed over several hours and disappeared by 24 hr. Diacylglycerol levels were found to parallel the appearance and disappearance of the T_{in}. The concurrent decline of T_{in} values and diacylglycerol was not due to a failure in the synthesis of $G_q\alpha$ protein, which was produced continuously for >24 hr. After *Xenopus* $G_q\alpha$ mRNA injection, germinal vesicle breakdown (GVBD) was variable (0–100%) in stage 6 oocytes, whereas none of the stage 4 oocytes underwent GVBD. In contrast, stage 6 oocytes injected with mRNA encoding the $G_o\alpha$ G protein consistently underwent GVBD but did not acquire T_{in}. Our results show that activation of phospholipase C is not an absolute requisite for the induction of maturation, although in oocytes of some frogs phospholipase C activation can trigger a pathway to GVBD.**

The induction of oocyte maturation by progesterone leads to a series of events, involving both inhibition and activation of second-messenger systems, that culminates in germinal vesicle breakdown (GVBD) and progression to metaphase of meiosis II (1). Changes in second-messenger concentrations are usually the result of agonist–receptor association and activation of second-messenger effectors, either directly or through receptor-associated G proteins. However, it is not yet clear whether the only progesterone receptor described so far in *Xenopus* oocytes (2) is directly responsible for maturation.

Immediately after exposure to progesterone the diacylglycerol (DAG) level in the oocyte falls ≈30%, and after 2 min it begins to rise, reaching control levels by 15 min and rising further until GVBD (3, 4). A similar increase in DAG, but without the initial drop, has been reported (5), and a decrease in inositol 1,4,5-triphosphate ($InsP_3$) immediately after progesterone exposure has also been reported (3). Changes in both DAG and $InsP_3$ suggest an involvement of phospholipase C (PLC) in the process of oocyte maturation because PLC converts phosphatidylinositol 4,5-bisphosphate into DAG and $InsP_3$.

The α subunits of the G_q family of G proteins (α_q, α_{11}, α_{14}, α_{15}, α_{16}) are known to activate directly the β isoforms of PLC (PLC_β) (6–9). To study further the possible role of the phosphatidylinositol (PtdIns) signaling cascade in *Xenopus* oocyte maturation, we investigated the effect of expressing additional G protein α subunits in the oocyte. The work was done throughout 1991–1992 and has been presented in preliminary form (10).

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METHODS

Oocyte Collection. *Xenopus laevis* frogs were laboratory raised, and segments of ovaries were surgically removed during hypothermic anesthesia. Oocytes were manually defolliculated, staged according to size (11), and maintained in OR2 medium (12) supplemented with gentamicin at 0.1 mg/ml (GIBCO) or incubated at 17°C with either cholera toxin at 2 μg/ml (Sigma) or pertussis toxin at 4 μg/ml (Sigma) 24 hr before and after mRNA injection. To induce maturation, oocytes were continuously exposed to progesterone at 1 μg/ml. Maturation was monitored by the appearance of a white spot on the animal hemisphere, indicative of GVBD, and confirmed by manual dissection (1).

Cloning of *Xenopus* G_q α Subunit ($xG_q\alpha$). A mouse $G_q\alpha$ cDNA clone (13) was used to screen a *X. laevis* whole-ovary cDNA library (14). Two partial cDNA clones were ligated to make the full-length $xG_q\alpha$ homolog. Sequence data are available from GenBank under accession no. L05540.

mRNA Transcription and Injection. To ensure addition of a poly(A) tail, the coding regions of $xG_q\alpha$ and $xG_o\alpha$ were transferred to the SP64T (15) transcription vector, and the mouse $G_q\alpha$ coding region was transferred to the SP65A (16) transcription vector. Capped mRNAs were synthesized by using Ambion reagents. Oocytes were injected with 0.4–40 ng of mRNA (40 nl) and then incubated at room temperature (20–23°C).

Labeling Newly Synthesized Protein. mRNA-injected oocytes were cultured in OR2 medium and then injected with [^{35}S]methionine/[^{35}S]cysteine (Tran^{35}S-label; ICN) as described (17). After 1 hr, the oocytes were homogenized in 100 μl of 50 mM NaCl/0.5 mM phenylmethylsulfonyl fluoride and centrifuged; the supernatant was then precipitated with 4 vol of acetone. The protein precipitate was resuspended and subjected to SDS/PAGE.

Measurement of Diacylglyceride Mass. At specified times, five oocytes were homogenized in 3 ml of chloroform/methanol, 1:2, 1 ml of chloroform and 1.8 ml of 1 M NaCl were added, and the organic phase was dried under N_2 as described (3). The DAG mass was quantitated using a DAG kinase assay (18). The resulting ^{32}P-labeled phospholipids were spotted on TLC plates. The plates were developed in chloroform/methanol/acetic acid, 130:30:10, and exposed to film. Spots corresponding to phosphatidic acid (DAG plus PO_4) were scraped, and radioactivity was counted in a scintillation counter.

Abbreviations: DAG, diacylglycerol; GVBD, germinal vesicle breakdown; PtdIns, phosphatidylinositol; PLC, phospholipase C; T_{in}, transient inward current(s); $InsP_3$, inositol 1,4,5-triphosphate; PKC, protein kinase C.

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Electrophysiological Recording. A two-electrode voltage clamp was used to record oocyte membrane currents while the membrane potential was clamped at −20 mV. Pulses to −60 and −100 mV were used to test for the presence of transient inward currents (T_{in}). The oocytes were superfused with normal frog Ringer's solution at 20–23°C (19, 20).

RESULTS

Cloning $xG_q\alpha$. The predicted amino acid sequence of the *Xenopus* $G_q\alpha$ shares 86% identity to mouse, 77% to *Drosophila*, and 78% to squid (13, 21) and retains the characteristics that distinguish $G_q\alpha$ from other GTP-binding proteins. These characteristics include an amino-terminal site for palmitoylation (22) but not for myristoylation, a change in the conserved GTP-binding region from GAGE to GTGE, and the absence of a pertussis toxin-modification site in the carboxyl terminus. In addition, $xG_q\alpha$ contains six extra amino acids beginning with methionine (MTLESI) N-terminal to the methionine of *Drosophila* and squid.

Expression of $xG_q\alpha$ and T_{in}. The *Xenopus* oocyte membrane contains many Cl^- channels that are activated by intracellular Ca^{2+} (20, 23). These Cl^- channels are frequently used to monitor Ca^{2+} influx across the oocyte membrane, as well as the intracellular Ca^2 released from internal stores after activation of various types of neurotransmitter receptors (24). For example, activation of brain serotonin receptors expressed in *Xenopus* oocytes activates the PLC/PtdIns pathway and causes increases in intracellular Ca^{2+} that trigger characteristic oscillatory Cl^- currents. In addition, receptor activation leads to a prolonged increase in Ca^{2+} permeability, and if the membrane is hyperpolarized, calcium ions enter the oocyte and cause a T_{in}, due to opening of the Ca^{2+}-dependent Cl^- channels (25). A similar T_{in} is seen after intracellular injection of $InsP_3$ (26).

Just a few hours after injection of $xG_q\alpha$ some oocytes had "spontaneous" oscillatory Cl^- currents. The oocytes then became able to generate T_{in}, which were rapidly blocked by Mn^{2+} (Fig. 1), La^{3+}, and other ions that block Ca^{2+} channels. The block by Mn^{2+} was rapidly reversible, whereas that produced by La^{3+} recovered only slowly. Later after injection many oocytes failed to generate T_{in}, suggesting that their appearance was a transient phenomenon. To see whether that was so we followed individual oocytes and also tested fresh oocytes for several days after mRNA injection. In both types of experiments, injection of $xG_q\alpha$ clearly led to a transient appearance of T_{in}. Furthermore, when less mRNA was injected, the T_{in} appeared later and persisted longer (Fig. 2). This ON–OFF pattern was consistently seen after each injection, although the amplitude of the T_{in} and the time of their onset and disappearance varied appreciably among oocytes from different donors. Similar results were obtained after injection of the mouse $G_q\alpha$ mRNA (see Fig. 4).

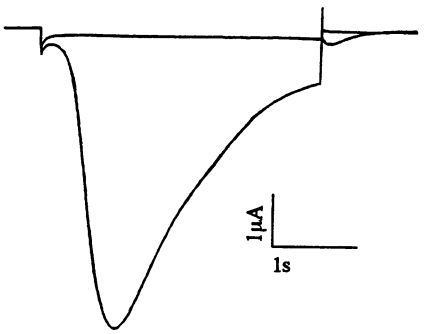

FIG. 1. T_{in} in an oocyte 3 hr after injection of 40 ng of $xG_q\alpha$ mRNA. The T_{in} was rapidly blocked by 5 mM Mn^{2+}. For Figs. 1–7 T_{in} was generated by stepping the membrane potential from −20 to −100 mV.

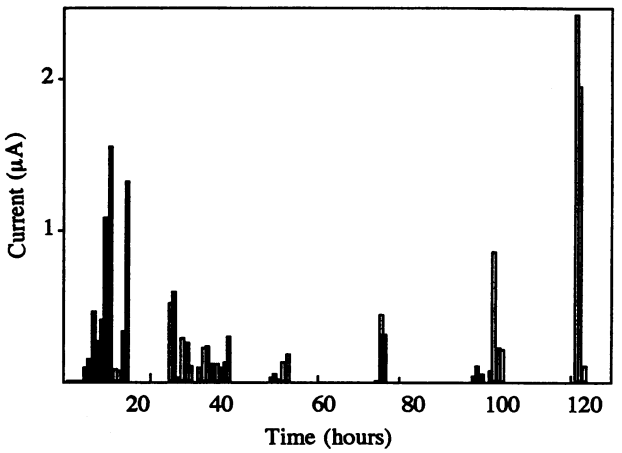

FIG. 2. Amplitudes of T_{in} in stage 6 oocytes after injection of 40 ng (solid bars) or 0.4 ng (open bars) of $xG_q\alpha$ mRNA.

All these data suggested strongly that the ability to generate T_{in} was from accumulation of newly synthesized $xG_q\alpha$. But why did the T_{in} disappear? One possibility was that this disappearance was from a cessation in $G_q\alpha$ production. To examine this hypothesis we monitored the synthesis of $G_q\alpha$. In general, oocytes translate injected mRNA very well, and large quantities of specific proteins are produced; $G_q\alpha$ is no exception. After injection of $xG_q\alpha$ mRNA oocytes were homogenized, and the protein was run on an SDS/polyacrylamide gel. One highly abundant band was visible, which corresponded to $xG_q\alpha$. Fig. 3 shows that $G_q\alpha$ was continuously produced for >48 hr, at which time the T_{in} had already disappeared in oocytes that were similarly injected. Thus, it seems more likely that some component of the PtdIns receptor–channel coupling pathway had been inhibited or exhausted. This hypothesis is supported by the fact that when the T_{in} disappeared, the characteristic oscillatory Cl^- current response to a serum factor, which activates the PLC/PtdIns system (27, 28), was also greatly reduced or abolished.

Effects of Pertussis and Cholera Toxins. To see whether the T_{in} were induced directly by the exogenous $G_q\alpha$ and not through endogenous G proteins, we tested the effects of pertussis toxin and cholera toxin on oocytes injected with $G_q\alpha$ mRNA. Both toxins covalently ADP-ribosylate certain α subunits; pertussis toxin inhibits activation of $G_o\alpha$ and $G_i\alpha$, whereas cholera toxin irreversibly activates $G_s\alpha$ (29). The $G_q\alpha$ family is not susceptible to either toxin.

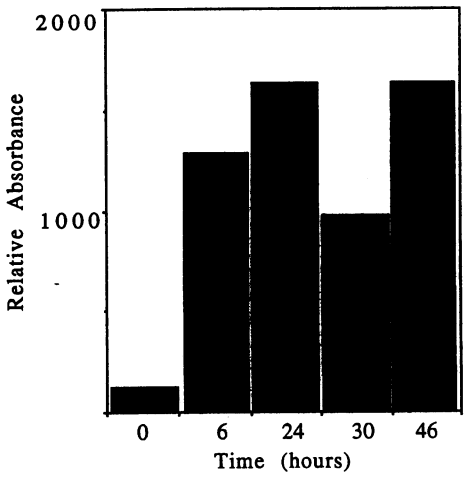

FIG. 3. Newly synthesized protein in stage 6 oocytes injected with $xG_q\alpha$ (40 ng). Levels of protein (three oocytes per lane) quantitated by laser scanning.

Pertussis toxin did not alter significantly the amplitude of T_{in} values in oocytes injected with $xG_q\alpha$ mRNA (Fig. 4). In contrast, the T_{in} amplitude was reduced in oocytes exposed to cholera toxin (Fig. 5). To ascertain that this was not simply due to an earlier onset of the decay phase of the T_{in} (Fig. 2), we tested oocytes at different times after injection. Fig. 5 shows that the T_{in} in oocytes exposed to cholera toxin was consistently smaller than that of control oocytes. In fact, in all three experiments, the oocytes exposed to cholera toxin seemed to acquire the T_{in} slightly earlier.

$G_o\alpha$ protein has been reported to activate Ca^{2+}-dependent Cl^- channels in *Xenopus* oocytes and subsequently induce oocyte maturation (30, 31). Therefore, we decided to inject *Xenopus* $G_o\alpha$ mRNA to see whether this would lead to activation of the PLC/PtdIns system and appearance of T_{in}, as well as to oocyte maturation. Although oocytes injected with mouse or *Xenopus* $G_q\alpha$ acquired T_{in} currents, oocytes from the same donor injected with $G_o\alpha$ mRNA did not develop T_{in} (Fig. 4). However, and in contrast to $G_q\alpha$, which frequently failed to induce maturation, $G_o\alpha$ consistently induced maturation in 80–100% of the oocytes. Furthermore, GVBD occurred 13–15 hr after mRNA injection, which was ≈8 hr slower than after progesterone. This difference is similar to that previously reported for oocytes injected with activated $G_o\alpha$ protein (31), but the lack of T_{in} in the oocytes injected with $G_o\alpha$ mRNA suggests that $G_o\alpha$ may not act via PLC to induce maturation.

DAG Levels After $G_q\alpha$ mRNA Injection. We assume that the appearance of T_{in} after injection of $G_q\alpha$ mRNA is due to $G_q\alpha$ activation of PLCβ and the production of InsP_3 and DAG. To further test this assumption, we measured DAG levels at various times after injection of $xG_q\alpha$ mRNA (Fig. 6). One hour after injection the level of DAG had already increased; it continued to rise up to 10–13 hr and then returned to control levels at 24 hr. This rise and fall pattern resembles that of the T_{in} amplitude. In three experiments of this type, the largest DAG increase was 160% of the control, a small but significant difference. One explanation for the low magnitude of the increase may be that *Xenopus* oocytes already contain a large pool of DAG in their membranes (168 pmol per oocyte, ref. 3), which may buffer any localized change.

Effects of $xG_q\alpha$ Injection on Maturation. During oocyte maturation DAG levels fall and then rise (3). The process leading to these changes is unknown, but one hypothesis is that it involves activation of the PLC/PtdIns pathway. We showed above that $xG_q\alpha$ mRNA induced increases in Ca^{2+} membrane permeability and DAG, indicative of PLC activation. To see whether the PLC activation was sufficient to induce maturation, we injected 40 ng of $xG_q\alpha$ mRNA into stage 6 oocytes and monitored maturation. The proportion of injected oocytes exhibiting GVBD varied greatly (0–100%) in different frogs, and GVBD occurred at 8–24 hr (data not shown). The timing of GVBD in these oocytes ranged from nearly that of progesterone-induced GVBD (6 hr) to much slower. Injection of mouse $G_q\alpha$ mRNA gave similar results.

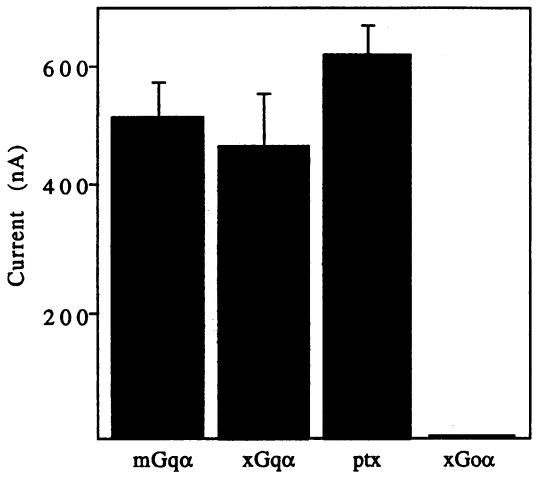

FIG. 4. T_{in} in oocytes injected with (40 ng) mouse or *Xenopus* $G_q\alpha$ or $G_o\alpha$ and $xG_q\alpha$ plus incubation with pertussis toxin (ptx) for 24 hr before and after injection. Columns represent means + SEMs of six to nine oocytes. No T_{in} was evident in $xG_o\alpha$ oocytes.

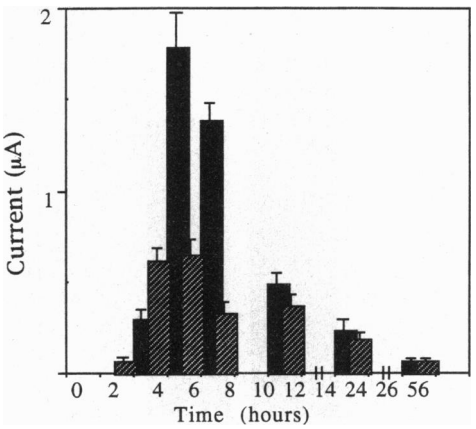

FIG. 5. T_{in} at different times after injection of $xG_q\alpha$ mRNA in control stage 6 oocytes (solid bars) and oocytes incubated with cholera toxin at 2 μg/ml (hatched bars). Columns are means + SEMs of four to seven oocytes.

Stage 4 oocytes are not matured by progesterone, due perhaps to a block or deficiency in the signaling pathway. This block can be overcome after injection of cyclin or maturation-promoting factor (MPF-1, ref. 32). These substances presumably induce GVBD because they act far enough downstream of progesterone for stage 4 oocytes to respond. Therefore, we injected $G_q\alpha$ mRNA to see whether that would also bypass the block. The stage 4 oocytes did not undergo GVBD, even 48 hr after injection.

To see whether $xG_q\alpha$ would allow maturation by progesterone in stage 4 oocytes or potentiate its effect on stage 6 oocytes, we treated the oocytes with progesterone 1 hr before $xG_q\alpha$ mRNA injection. In all cases the effect of progesterone was not potentiated, and the injected oocytes responded as the controls—that is, stage 4 oocytes never underwent GVBD, and stage 6 oocytes matured at the same time in the presence of progesterone, with or without $xG_q\alpha$. In addition, stage 6 oocytes injected with guanosine [γ-thio]triphosphate, a universal G protein activator, 4–20 hr after $xG_q\alpha$ mRNA injection and then treated or not treated with progesterone responded as the

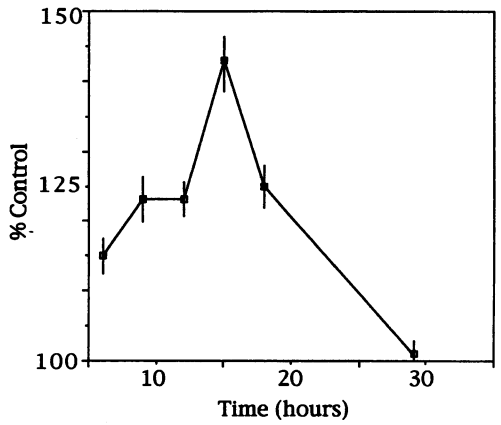

FIG. 6. DAG levels of stage 6 oocytes after injection (40 ng) of $G_q\alpha$ mRNA. Points represent the averages (± SEMs) as a percentage of control values, in three donor frogs.

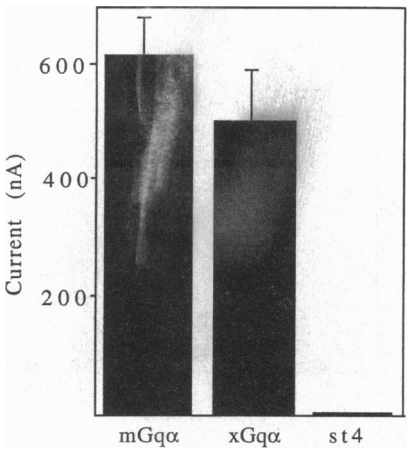

FIG. 7. T_{in} values in stage 6 and stage 4 oocytes injected with $G_q\alpha$ mRNA (40 ng). Values represent means + SEMs of four to five stage 6 or 10 stage 4 oocytes. Stage 4 (st4) oocytes failed to generate T_{in}.

controls. Altogether our results indicate that $G_q\alpha$ is probably not strictly involved in progesterone-induced maturation.

Although stage 4 oocytes did not undergo GVBD after $xG_q\alpha$ mRNA injection, it was still possible that PLC had been activated. To see whether the failure of stage 4 oocytes to mature was due to an inability to activate PLC or to lack of a downstream event, stage 4 oocytes were injected with $xG_q\alpha$ and tested for the presence of T_{in}. Fig. 7 compares the maximum T_{in} induced in stage 6 oocytes injected with mouse or *Xenopus* $G_q\alpha$ with that of stage 4 oocytes injected with $xG_q\alpha$. Mouse and *Xenopus* $G_q\alpha$ induced approximately equal T_{in} values in stage 6 oocytes, but T_{in} were not seen in stage 4 oocytes.

DISCUSSION

Effects of $G_q\alpha$. One of our main findings is that injection of $G_q\alpha$ mRNA into *Xenopus* oocytes mobilizes the PtdIns pathway by activating PLC and leads to a rise in DAG and the appearance of Ca^{2+}-dependent Cl^- currents that last only ≈1 day. The decline is not due to a failure in $G_q\alpha$ synthesis because protein continues to be produced >24 hr after mRNA injection. Instead, the decline appears to be from a failure in the PtdIns pathway because the response to serum factor, which is mediated by that pathway, also declines after $G_q\alpha$ mRNA injection. The decline in activity of this pathway may be due to exhaustion of a component in the PtdIns pathway or to negative-feedback control. One possible negative feedback may be via protein kinase C (PKC), which is presumably activated by the increased levels of Ca^{2+} and DAG that follow $G_q\alpha$ mRNA injection. It is known that PKC activation inhibits receptor-coupled PLC activity, although the PKC substrate is unknown (33). Potential PKC substrates are G-coupled receptors, which does not apply in our case, and $G_q\alpha$ or PLCβ itself. It has been proposed that PKC phosphorylates PLCβ, which then hinders proper interaction between PLCβ and $G_q\alpha$ (34). Our study suggests that PLC is down-regulated because both the Ca^{2+}-dependent T_{in} current and DAG drop to control levels.

Although $G_q\alpha$ stimulates PLC in stage 6 oocytes, it does not consistently induce oocyte maturation. Moreover, when oocytes are treated with $G_q\alpha$ mRNA and progesterone or guanosine [γ-thio]triphosphate, no cooperativity is found. These data indicate that $G_q\alpha$ is not necessarily involved in the *in vivo* induction of maturation and that PLC activation alone is not always sufficient to induce maturation. On the other hand, stage 4 oocytes do not respond to $G_q\alpha$ mRNA injection by generating T_{in}. This result is somewhat surprising because after $xG_q\alpha$ mRNA injection, stage 4 oocytes produced $G_q\alpha$ protein as well as stage 6 oocytes. Thus, the protein is synthesized, but it seems that PLC is not activated or there is a deficiency in the Ca^{2+}-dependent Cl^- channels.

Effects of $G_o\alpha$. In stage 6 oocytes $G_o\alpha$ mRNA was more efficient in inducing maturation than $G_q\alpha$ mRNA. Surprisingly, however, $G_o\alpha$ mRNA did not induce the appearance of T_{in}, indicating that $G_o\alpha$ probably acts through an effector other than PLC to stimulate maturation. This result agrees with work on transfected COS cells in which $G_q\alpha$, but not $G_o\alpha$, activated PLCβ1 (35) and with recent work implicating $\beta\gamma$ subunits, and not α subunits, in pertussis toxin-sensitive PLC activation (36–38). However, our data conflict with the reported activation of the PtdIns pathway in oocytes injected with $G_o\alpha$ protein (30). Perhaps the different results are due to injection of $G_o\alpha$ mRNA, as in our case, versus injection of $G_o\alpha$ protein. Furthermore, because $G_o\alpha$ mRNA efficiently induces maturation but does not induce T_{in}, this procedure serves as a control for $G_q\alpha$ mRNA injections, showing that neither the process of injection alone nor the induction of maturation induces the T_{in}.

Altogether our results indicate that activation of PLC is not an indispensable requirement for maturation. Although the natural pathway for progesterone-induced maturation may not necessarily involve $G_q\alpha$, our observations also imply that PLC activation by $G_q\alpha$ and the production of DAG and InsP_3 can sometimes lead to GVBD. The delayed appearance of GVBD, compared with that of progesterone, may simply reflect the time required to activate the pathway.

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