

Activators of Protein Kinase C Trigger Cortical Granule Exocytosis, Cortical Contraction, and Cleavage Furrow Formation in *Xenopus laevis* Oocytes and Eggs

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Abstract. Prophase I oocytes, free of follicle cells, and metaphase II eggs of the amphibian *Xenopus laevis* were subjected to transient treatments with the protein kinase C activators, phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-didecanoate, and 1-oleoyl-2-acetyl-*sn*-glycerol. In both oocytes and eggs, these treatments triggered early events of amphibian development: cortical granule exocytosis, cortical contraction, and cleavage furrow formation. Surprisingly, activation of oocytes occurred in the absence of meiotic resumption, resulting in cells with an oo-

cytelike nucleus and interior cytoplasm, but with a zygotelike cortex. PMA-induced activation of oocytes and eggs did not require external calcium, a prerequisite for normal activation of eggs. PMA-induced activation of eggs was inhibited by retinoic acid, a known inhibitor of protein kinase C. In addition, pretreatment of eggs with retinoic acid prevented activation by mechanical stimulation and inhibited activation by calcium ionophore A23187. The results suggest that protein kinase C activation is an integral component of the *Xenopus* fertilization pathway.

THE program of fertilization in frog eggs is similar to that of sea urchin eggs (20, 49), fish eggs (24, 25), and mammalian eggs (17, 40) in that it involves a wave-like increase in intracellular free calcium (7, 36), which precedes a wave of cortical granule exocytosis (26, 36). Cortical granule exocytosis in the frog *Xenopus laevis* is followed by contraction of the cortex and later, the formation of the first cleavage furrow (for a table of postfertilization events, see reference 55). Cortical granule exocytosis, cortical contraction, and cleavage furrow formation are the morphological hallmarks of frog egg activation, and provide convenient markers for use in the dissection of the activation process (21, 28).

The discovery that inositol-1,4,5-trisphosphate (IP₃),¹ an intermediate of the polyphosphoinositide pathway, triggered the intracellular wave of calcium and activated *Xenopus* eggs led to the suggestion that polyphosphoinositide turnover mediates the activation of *Xenopus* eggs (8, 44). Added support for this hypothesis is derived from studies of fertilization in sea urchins, mammals, and fish which indicate that IP₃ formation triggers the wave of increase in intracellular free calcium (16, 39, 43, 52, 54). More recently, this hypothesis has been further strengthened by the finding that stimulation of receptors that trigger IP₃ formation induces activation of *Xenopus* eggs (35).

IP₃ is one of two intermediates produced by the activity of phospholipase C in the polyphosphoinositide pathway. This bifurcating signal pathway results in the formation of IP₃ with the stoichiometric production of diacylglycerol (for reviews, see reference 4 and 42) and the latter activates protein kinase C (PKC), a calcium and phospholipid-dependent serine kinase (32, 34). PKC is thought to mediate a variety of cellular events in both somatic cells (18, 19, 45) and fertilized eggs (15, 22, 51). In the case of sea urchin eggs, PKC is believed to mediate an increase in intracellular pH after sea urchin fertilization via phosphorylation of the sodium/hydrogen pump in the plasma membrane (13, 48, 51). At present, however, it is not known what role, if any, PKC has in the fertilization of amphibian eggs.

We have used three activators of PKC, phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-didecanoate (PD), and 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) (for review, see reference 6) to investigate the role of PKC in the activation of the *Xenopus* egg. We show that these agents not only activate *Xenopus* eggs but follicle-free oocytes as well. Our findings indicate that PKC functions in cortical granule exocytosis as well as cortical contraction and cleavage furrow formation.

Materials and Methods

Oocyte and Egg Procurement

Ovaries were surgically removed from female *Xenopus laevis* (Nasco Biologicals, Fort Atkinson, WI) anesthetized by hypothermia. For complete removal of follicle cells, clumps of 20 oocytes were treated for 1 h with 1%

1. *Abbreviations used in this paper:* IP₃, inositol-1,4,5-trisphosphate; PD, phorbol 12,13-didecanoate; PDD, 4 α -phorbol 12,13-didecanoate; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; OAG, 1-oleoyl-2-acetyl-*sn*-glycerol.

collagenase (unless otherwise stated, all reagents were purchased from Sigma Chemical Co., St. Louis, MO) in $1\times$ O-R2 (57; 82.5 mM NaCl, 2.5 mM KCl, 1 mM Na_2HPO_4 , 3.8 mM NaOH, 1 mM MgCl_2 , 1 mM CaCl_2 , pH 7.4). After collagenase treatment, full grown oocytes (1.1–1.4 mm) were isolated, washed extensively, and incubated in plastic petri plates for 4–6 h in $1\times$ O-R2. After 4–6 h, oocytes were transferred to plastic petri plates containing fresh O-R2. The follicle cells adhered to the petri plates and were removed as a complete sheath by simply pushing the oocytes across the petri plate with forceps. In separate experiments, follicle cells were removed by incubation of manually isolated oocytes in calcium-free O-R2. Follicle-free oocytes obtained in this manner gave similar results in all experiments; however, since incubation in calcium-free O-R2 has been shown to result in ultrastructural transformations (3), the collagenase treatment was used preferentially. It was essential to remove follicle cells completely for this study because follicle cells markedly altered the effects of PMA, and resulted in the breakdown of cortical granules within the oocyte and a general disruption of the cytoplasm (see Discussion).

Meiotically mature eggs were obtained by incubation of follicle-free oocytes in O-R2 containing progesterone (2 $\mu\text{g}/\text{ml}$) until a white spot on the animal hemisphere became visible, which signified the release of the first polar body. These eggs were then incubated 3 h more, since activability is known to arise several hours after white spot appearance (10, 14, 28).

Experimental Media

PMA and PD were dissolved in DMSO at 1 mM, stored at -20° , and used in $1\times$ O-R2 at the final concentration indicated in the Results section. OAG was stored at -20° under nitrogen. Before use, it was thawed, added to a final concentration of 50 $\mu\text{g}/\text{ml}$ in $1\times$ O-R2, and subjected to sonication. Medium containing OAG was used immediately to minimize OAG oxidation. The inactive PMA analogue, 4 α -phorbol 12,13-didecanoate (PDD), was dissolved in DMSO, stored in a manner identical to PMA, and used at the final concentrations indicated in the Results section. Retinoic acid was dissolved at 10 mM in 100% ethanol, stored at -70° , and diluted in $1\times$ O-R2 to a final concentration of 100 μM . $1\times$ O-R2 made without CaCl_2 and with 10 mM EGTA was employed as calcium-free medium. Eggs were activated by incubation in O-R2 containing the calcium ionophore A23187 (0.25 μM) or by puncturing the plasma membrane of the animal hemisphere with a fine glass needle. In initial experiments, A23187 was used at 2 μM . We found that while this concentration was no more successful in activating eggs than 0.25 μM , it tended to result in the nonspecific release of cytoplasm that could be detected spectrophotometrically.

Electron Microscopy

Oocytes and eggs were fixed as described previously (3) in *s*-collidine buffer (70 mM KCl, 15 mM NaCl, 1 mM CaCl_2 , 34 mM *s*-collidine, pH 7.2; reference 5) containing 2.5% glutaraldehyde. Samples were postfixated for 3 h in 0.1 M sodium cacodylate buffer (pH 7.9) with 1% osmium tetroxide. Samples were dehydrated in a graded ethanol series, the ethanol was replaced with acetone, and the samples were embedded in Spurr embedding medium. Ultrathin sections were cut on glass knives, poststained with uranyl acetate and lead citrate, and viewed on an electron microscope (model 201; Philips Electronic Instruments, Inc., Mahwah, NJ).

Spectrophotometry

10 oocytes or eggs were gently transferred with a wide bore pipette into a 1-ml quartz cuvette containing 0.4 ml of the experimental media described in the Results section. Absorbance readings were taken at 280 nm on a spectrophotometer (Du-G4; Beckman Instruments, Inc., Palo Alto, CA) every 2 min after gentle agitation of the cuvette. Care during the agitation was required to prevent cell lysis in the oocytes and eggs and to prevent mechanical activation of the eggs. A standard curve was generated using BSA. Since retinoic acid absorbs strongly in the ultraviolet range, a BCA Protein Assay Kit (Pierce Chemical Company, Rockford, IL) was employed to measure protein release in oocytes and eggs treated with retinoic acid. Absorbance was measured at 562 nm, where the retinoic acid absorbed only weakly.

Electrophoresis

Groups of 10 oocytes were treated with 3 μM PMA in $1\times$ O-R2 and exocytosis was monitored spectrophotometrically as described above. When the exocytotic reaction reached a plateau, 200 μl of oocyte exudate was removed from the cuvette and precipitated in a fourfold excess of ice-cold 100% etha-

nol. The precipitate was dissolved into SDS sample buffer, electrophoresed on a 10% SDS polyacrylamide gel, and then stained with Coomassie Blue and silver stain (11).

Results

Transient PMA Treatment Activates *Xenopus* Oocytes without Inducing Meiotic Maturation

Oocytes treated with PMA were examined by transmission EM. Analysis of their cortical regions demonstrated that the cortical granules had undergone exocytosis (Fig. 1 *A*). For comparison, meiotically mature eggs, manually activated by pricking, were examined and these cortices were identical with those of PMA-activated oocytes (Fig. 1 *B*). Both had flat plasma membranes, remnants of a cortical endoplasmic reticulum, and were devoid of cortical granules. Oocytes treated with the inactive PMA analogue, PDD (Fig. 1 *C*), were not activated as can be seen from the fully intact cortical granules, the highly invaginated plasma membrane, and the surface studded with microvilli, all characteristics of the untreated, full-grown oocyte cortex (3, 10, 14).

In contrast to the cortex of the oocyte, PMA-induced activation did not affect the oocyte interior. The germinal vesicle envelope and the nuclear pores remained intact (Fig. 2 *A*), prominent nucleoli were present (Fig. 2 *B*), and annulate lamellae were present. Fig. 2 *C* shows a low magnification view of a PMA-activated oocyte, demonstrating that the cortex resembles that of an activated egg, while an annulate lamellae is present in the cell interior, a characteristic of the oocyte cytoplasm (3).

PMA-activated oocytes and eggs developed cleavage furrows ~ 90 min after activation. The cleavage furrow typically formed in the center of the animal hemisphere, which is normal location for the first cleavage furrow in fertilized eggs (55). In sections of PMA-activated oocytes, the cleavage furrow could be seen above the germinal vesicle (Fig. 2 *D*). The cleavage furrows eventually regressed, as is typical for eggs activated in the absence of sperm (28, 55).

In all of the above cases, the transient PMA application was followed by extensive washing in PMA-free O-R2. Continuous PMA treatment (beyond 25 min with micromolar concentrations and beyond 1 h with nanomolar concentrations) resulted in numerous pigmentation abnormalities in treated oocytes and eventual cell lysis and death. Acute treatments followed by extensive washing, however, resulted in activated oocytes that could be cultured at 20°C for as long as 1 wk.

Nanomolar Concentrations of PMA Trigger Cortical Granule Exocytosis in Oocytes and Eggs

For rapid, quantitative analysis of cortical granule exocytosis in oocytes and eggs, the spectrophotometric technique of Wolf (58) was used. Protein absorbance (280 nm) of the medium surrounding oocytes and eggs provided a direct measure of the degree of cortical granule exocytosis. The application of nanomolar concentrations of PMA resulted in the linear release of protein over time, whereas 3 μM PMA triggered a rapid protein release which reached a plateau after 18 min (Fig. 3). Oocytes treated with the inactive PMA analogue, PDD (3 μM), did not release protein.

The concentration range that successfully triggers exocyto-

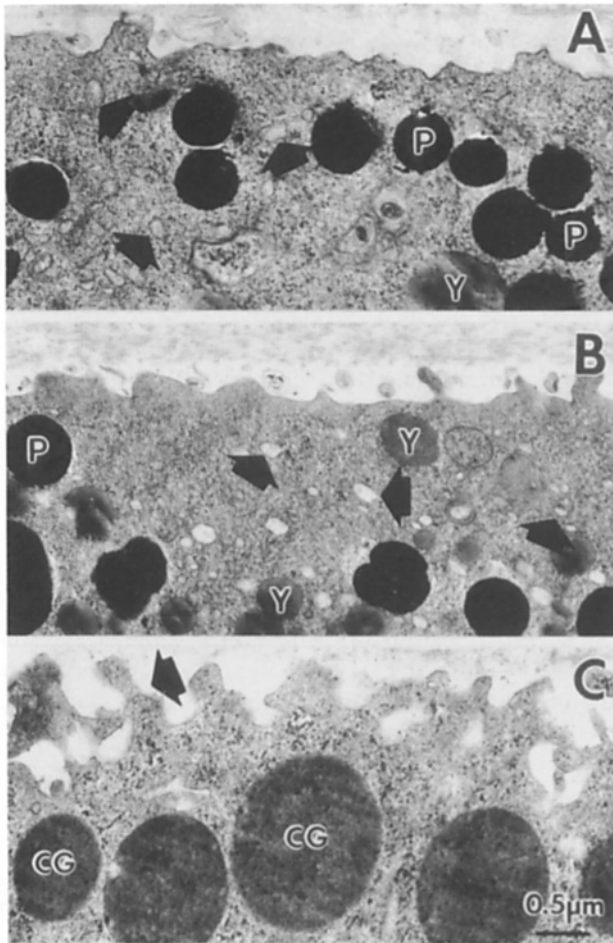


Figure 1. PMA treatment triggers oocyte activation. Electron micrographs of cortices from an oocyte treated with PMA (A), an egg activated by mechanical stimulus (B), and an oocyte treated with an inactive PMA analogue (C). (A) PMA treatment (300 nM) resulted in oocyte activation: the cortical granules are absent, the plasma membrane is flattened, and remnants of a cortical endoplasmic reticulum are visible (arrows). Pigment granules (P) and yolk platelets (Y) are also present. (B) A prick-activated egg also lacks cortical granules, and has a smooth plasma membrane and remnants of a cortical endoplasmic reticulum (arrows). (C) An oocyte treated with the inactive PMA analogue, PDD (300 nM), did not activate: the cortical granules are intact (CG), and the plasma membrane is highly invaginated and studded with numerous microvilli (arrow).

sis in oocytes (Fig. 3) is also sufficient to trigger exocytosis in eggs (Fig. 4). The reaction proceeded faster in eggs than oocytes, and the final amount of protein released was generally greater in eggs (compare Figs. 3 and 4). To obtain a control for the amount of protein released during exocytosis, meiotically mature eggs were activated by calcium ionophore A23187, and the absorbance of the extracellular medium was measured as described in the Materials and Methods section for eggs. Protein release triggered by A23187 averaged $1.83 \mu\text{g}/\text{egg} \pm 0.35$ ($n = 3$ experiments) while eggs activated by PMA averaged $1.93 \mu\text{g}/\text{egg} \pm 0.24$ ($n = 3$ experiments). Oocytes activated with PMA released $1.53 \mu\text{g}/\text{oocyte} \pm 0.1$ ($n = 3$ experiments). There is no comparable control for oocytes, however, since neither ionophore application nor mechanical stimulation cause cortical granule exocytosis in oocytes. Wolf (58) reported similar values for the amount of

protein released after activation of meiotically mature eggs.

To verify that the spectrophotometric assay reflected cortical granule exocytosis rather than nonspecific protein release, samples of medium from activated oocytes were concentrated and subjected to gel electrophoresis (Fig. 5). The lane that contained the exudate exhibited four bands of molecular masses similar to those reported for *Xenopus* cortical granule contents (47, 59); another band was also present at the top of the lane that may correspond to the 500-kD lectin found in cortical granules (47). The lane containing total oocyte protein has numerous prominent bands not observed in the exudate lane, indicating that protein release by PMA-treated oocytes reflects specific release of cortical granule contents.

PD and OAG Trigger Cortical Granule Exocytosis in Full-grown Oocytes

Oocytes were also treated with two other activators of PKC, PD and OAG to determine if oocyte activation was a PMA-specific phenomenon or whether it was associated with activators of PKC in general. Incubation of oocytes in O-R2 containing $3 \mu\text{M}$ PD induced cortical granule exocytosis at a level similar to that induced by $3 \mu\text{M}$ PMA (Fig. 6). Furthermore, incubation of oocytes in O-R2 containing OAG at $50 \mu\text{g}/\text{ml}$ (32) also triggered exocytosis (Fig. 6). The time course of exocytosis induced by both PD and OAG is comparable to that of oocytes incubated in O-R2 containing PMA (Fig. 3). In contrast, oocytes incubated in O-R2 containing DMSO at the same concentration used for the PD-treated samples released virtually no protein within 20 min (Fig. 6).

PMA-Activation Is Inhibited by Retinoic Acid and Insensitive to Lack of External Calcium

Retinoic acid and other retinoids are known to antagonize PMA (56) and have been shown to inhibit PKC (29, 53). To determine what inhibitory effect, if any, retinoic acid had on oocyte and egg activation, eggs were incubated in O-R2 containing $100 \mu\text{M}$ retinoic acid (29) and then challenged with PMA, calcium ionophore A23187, or pricking. Exocytosis was monitored spectrophotometrically as described in the Materials and Methods and cortical contraction was monitored with a dissecting microscope. A preincubation of 2 h in retinoic acid inhibited PMA and ionophore-induced cortical granule exocytosis (Table I). Retinoic acid also delayed the onset of cortical contraction in eggs treated with PMA or ionophore, and prevented activation of eggs stimulated by pricking.

Xenopus egg activation by means of mechanical stimulation and fertilization are known to require external calcium (60). To determine the dependence of PMA-induced activation on external calcium, oocytes and eggs were incubated in calcium-free O-R2 containing PMA. Activation was monitored as above and confirmed with EM analysis. PMA-induced protein release in oocytes and eggs showed no dependence on external calcium (Fig. 7).

PMA and Calcium Ionophore A23187 Synergistically Trigger Cortical Granule Exocytosis in Oocytes

It has been noted in several systems that the threshold amounts of either PMA or calcium ionophore A23187 required to elicit the desired cellular response can be greatly

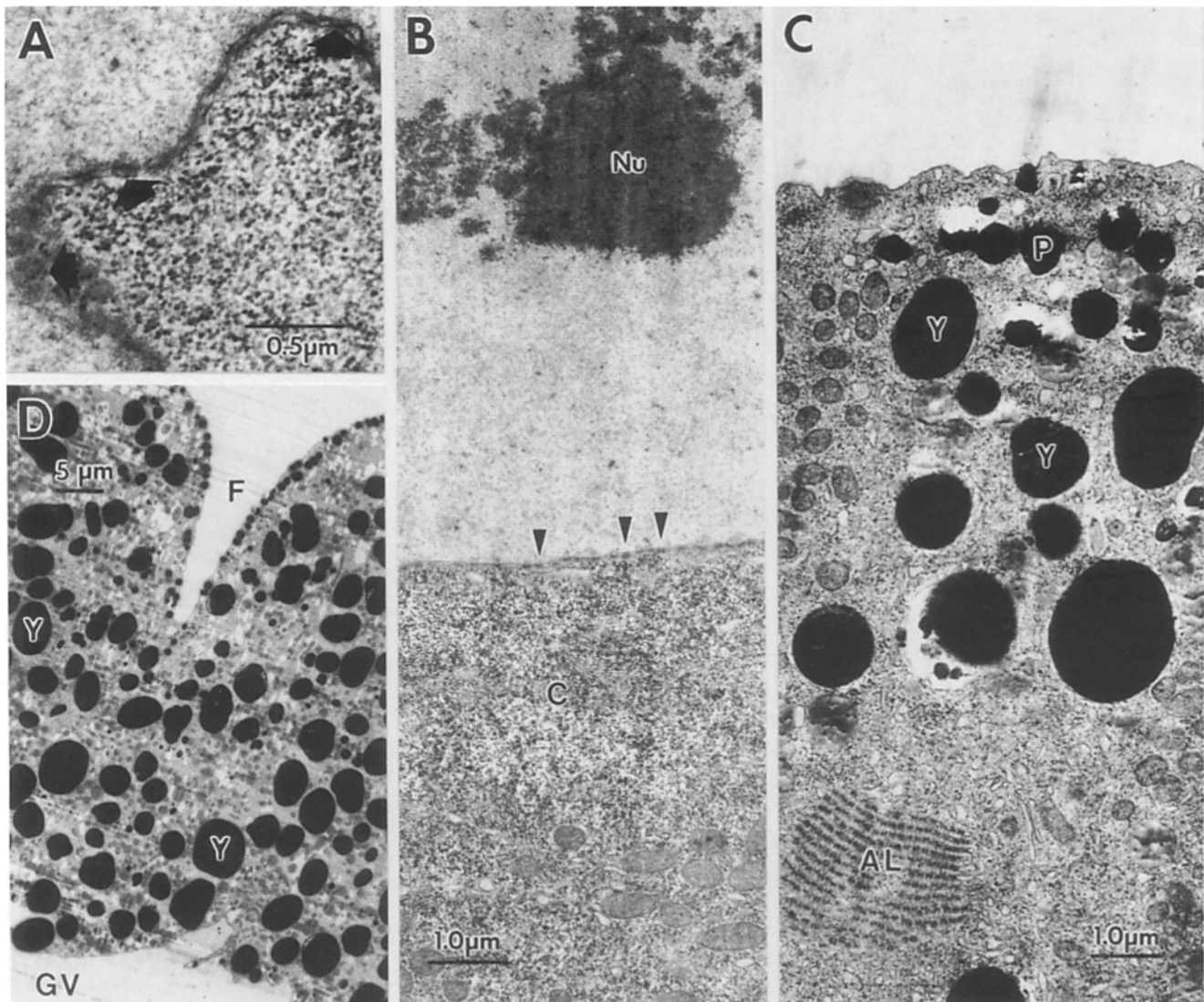


Figure 2. PMA-induced activation occurs in the absence of germinal vesicle breakdown. Electron micrographs of oocytes activated with PMA (300 nM). (A) High magnification view of a germinal vesicle in an oocyte activated with PMA demonstrating the nuclear envelope with intact nuclear pores (arrows). (B) Germinal vesicle of an oocyte activated with PMA showing a nucleolus (Nu) in the germinal vesicle interior. The arrowheads indicate the nuclear envelope. C denotes the cytoplasm. (C) Cortex and cell interior of an oocyte activated with PMA. In the cortex, no cortical granules are present, the plasma membrane is flat, and remnants of the cortical endoplasmic reticulum are visible; in the cytoplasm, an annulate lamellae is present (AL). Yolk platelets (Y) and pigment granules (P) are also present. (D) Cross-section of an oocyte activated with PMA showing a cleavage furrow (F) forming above the germinal vesicle (GV). Pigment granules line the furrow.

reduced if both agents are applied simultaneously (38, 61, 62). Potential synergy between PMA and A23187 was tested by incubating oocytes in 6 nM PMA, 0.25 μ M A23187, or both. 6 nM PMA or 0.25 μ M A23187 alone triggered virtually no protein release (Fig. 8). In contrast, the combination of these agents resulted in exocytosis near the level induced by 600 nM PMA (Fig. 3), demonstrating that calcium ionophore and PMA interact synergistically.

Discussion

The principal finding of this study is that activators of PKC are capable of triggering exocytosis, cortical contraction,

and cleavage furrow formation in oocytes and eggs of the amphibian, *Xenopus laevis*. Phorbol esters and OAG activate PKC in many systems (1, 12, 32, 33, 46), including *Xenopus* oocytes and eggs (37). Furthermore, the PMA concentrations effective in triggering activation in this study are within the range required to activate *Xenopus* PKC in vitro (37). Consequently, a simple interpretation of this report is that PKC stimulation is a normal component of the activation reaction in *Xenopus*. PKC-mediated phosphorylation events are thought to be important in sea urchin activation (13, 48), which supports this idea. However, in contrast to sea urchin egg activation, where PKC activation did not induce cortical granule exocytosis (15), our findings suggest that PKC acti-

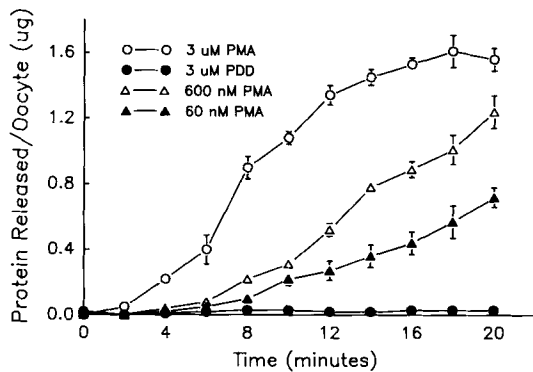


Figure 3. PMA triggers cortical granule exocytosis in oocytes. The amount of protein released from oocytes incubated in O-R2 containing the indicated concentration of PMA or the inactive PMA analogue, PDD, was determined spectrophotometrically. Data expressed as mean \pm SEM from three experiments, each using oocytes from three different frogs. When standard error bars are absent it indicates a SEM of <0.02 .

vation is an important prerequisite for cortical granule exocytosis in *Xenopus* eggs.

It is not likely that PMA, PD, and OAG indirectly activate eggs by triggering an influx of calcium since these agents trigger activation in the oocyte, which does not undergo activation when treated with calcium ionophore. Further, the effectiveness of PMA is undiminished by the absence of extracellular calcium, indicating the PMA does not act by introducing calcium into the oocyte or egg. Additional evidence for a direct role in activation comes from the observation that nanomolar concentrations of PMA result in the continuous release of cortical granule contents from oocytes and eggs. If PMA acted by triggering an intracellular free calcium wave, one would expect a rapid release of cortical granule contents followed by a plateau as the cortical granule population is depleted; however, this does not occur with nanomolar amounts of PMA. While micromolar PMA concentrations induce rapid release followed by a plateau,

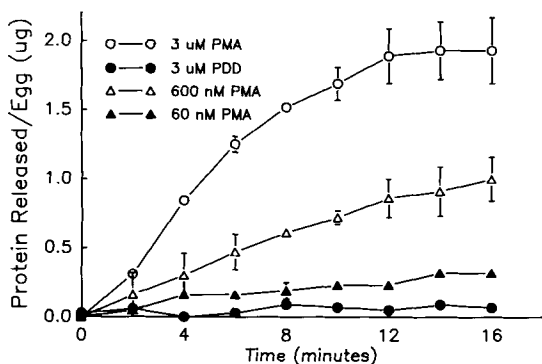


Figure 4. PMA triggers cortical granule exocytosis in eggs. The amount of protein released from eggs incubated in O-R2 containing the indicated concentration of PMA or the inactive PMA analogue, PDD, was determined spectrophotometrically. Data were analyzed as described in the legend to Fig. 3, except that eggs were used instead of oocytes.

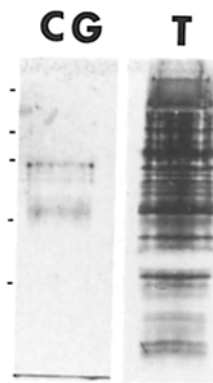


Figure 5. SDS polyacrylamide gel showing comparison of cortical granule exudate material with total oocyte proteins indicates that cytoplasmic leakage did not result from PMA treatment. The gel was subjected to Coomassie Blue and then silver staining. The cortical granule exudate lane (CG) was reacted 10 times longer in silver stain relative to the lane of total proteins (T) to ensure that any cytoplasmic protein would be detected. The sample for the CG lane was collected 18 min after treatment in an experiment identical to the 3- μ M treatment with PMA in Fig. 3, except that

samples were pooled from the exudate of 80 oocytes. Molecular mass markers are indicated by the bars on the left of the figure (from top to bottom, 200, 97.4, 68, 43, 25.7 kD).

this presumably reflects a rapid direct release of cortical granule contents resulting from a high degree of PKC activation.

The potential for direct PKC involvement in *Xenopus* egg activation is strengthened by the observation that retinoic acid inhibits activation induced by both pricking and calcium ionophore. Retinoids are known inhibitors of PKC (29, 53) and retinoic acid has been shown in this report to delay PMA-induced egg activation. However, since it was not directly shown that PKC is responsible for phosphorylation events during activation, it could be argued that PMA-, PD-, and OAG-induced activation of *Xenopus* oocytes and eggs is the result of nonspecific phosphorylation events. Such an argument would require the invocation of another phosphorylation system functioning at activation, also sensitive to retinoic acid inhibition. While this is a possibility, the fact that nanomolar concentrations of PMA rapidly induce the gamut of events characteristic of *Xenopus* egg activation renders the possibility of nonspecific effects unlikely.

A number of treatments capable of artificially activating amphibian eggs have been identified, including mechanical

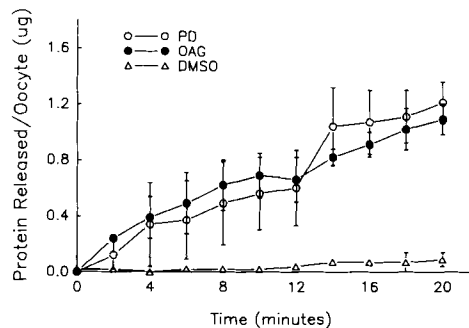


Figure 6. PD and OAG trigger cortical granule exocytosis in oocytes. The amount of protein released from eggs incubated in O-R2 containing 3 μ M PD, 50 μ g/ml OAG, or 0.3% DMSO was determined spectrophotometrically. Data were analyzed as described in the legend to Fig. 3.

Table 1. Effects of Retinoic Acid on Ionophore, PMA, and Mechanically Induced Activation

| Egg treatment* | Protein release/egg | Onset of cortical contraction |
|----------------|-----------------------------|-------------------------------|
| A23187 | 1.85 $\mu\text{g} \pm 0.14$ | 11 min ± 2 |
| RA + A23187 | 0.93 $\mu\text{g} \pm 0.20$ | 23 min ± 4 |
| PMA | 0.94 $\mu\text{g} \pm 0.18$ | 13 min ± 4 |
| RA + PMA | 0.50 $\mu\text{g} \pm 0.09$ | 19 min ± 1 |
| Pricking | — | 12 min ± 5 |
| RA + Pricked | — | No contraction |

* Concentrations were as follows: 0.25 μM calcium ionophore A23187 (A23187); 100 μM retinoic acid (RA); 600 nM PMA. 10 eggs were used in each experiment; the values shown represent the mean \pm standard error ($n = 3$).

stimulus (58), calcium ionophore (14), calcium microinjection (30), and microinjection of inositol trisphosphate (8, 44). These agents trigger activation only if meiotic maturation has progressed to the point of germinal vesicle breakdown, the achievement of metaphase II, or several hours beyond that (14, 28, 30, 44). It has been suggested that activability requires the development of intracellular calcium stores in the egg cortex (9, 10, 14, 23). The finding that PMA, PD, and OAG trigger activation in oocytes suggests the limit on activation competence may also involve PKC. Indeed, the fact that exposure of oocytes to calcium ionophore does not induce activation (14) indicates that calcium alone is not the limiting factor in the acquisition of activability.

Stith and Maller (50) have previously reported that *Xenopus* oocytes treated with PMA undergo germinal vesicle breakdown. There are two significant differences between the experiments of Stith and Maller (50) and those reported here. First, we applied PMA for 20 min and then washed the oocytes extensively. Stith and Maller subjected oocytes to prolonged treatments (up to 5 h) with PMA. We have found that prolonged treatments with PMA result in extensive reorganization of the cytoplasm and eventual deterioration of the oocyte. Second, we found that PMA-induced activation required complete removal of follicle cells before treatment. Stith and Maller (50) isolated oocytes manually, a procedure that leaves an intact follicle cell sheath detectable by ultrastructural examination. We have found that oocytes treated with PMA in the presence of an intact follicle cell sheath undergo cortical granule disruption beneath the plasma membrane (3). It is likely that both the collagenase treatment and

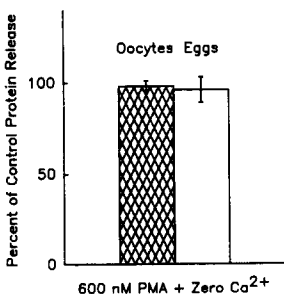


Figure 7. Calcium-free medium does not inhibit PMA-induced exocytosis. Protein release from oocytes and eggs exposed to 600 nM PMA in calcium-free O-R2 was determined spectrophotometrically and expressed as the percent of the appropriate control. Data were collected 18 min after treatment and expressed as mean \pm SEM from three experiments each using oocytes (or eggs) from three different frogs.

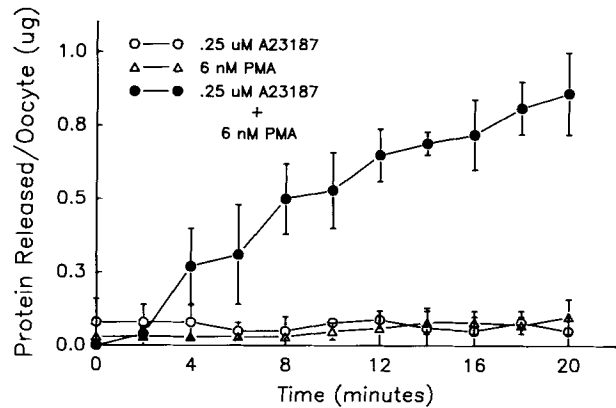


Figure 8. PMA and calcium ionophore A23187 synergistically trigger cortical granule exocytosis in oocytes. The amount of protein released from oocytes incubated in O-R2 containing 6 nM PMA, 0.25 μM A23187, or both was determined spectrophotometrically. Data were analyzed as described in the legend to Fig. 3.

the short incubation times we employed account for the difference in results of this report and the report of Stith and Maller (50).

The synergy between calcium ionophore A23187 and PMA found in this study is typical for a variety of systems in which polyphosphoinositide turnover is thought to transduce extracellular signals (38, 61, 62). The models proposed to explain this synergy suggest that an increase in intracellular free calcium translocates inactive cytosolic PKC to the plasma membrane where it becomes activated by diacylglycerol (2, 31, 61). It is possible that the wavelike increase of intracellular free calcium triggered at fertilization results in the translocation of PKC to the egg plasma membrane, where it is activated by an interaction with diacylglycerol. The diacylglycerol could be provided by the phosphoinositide turnover thought to be activated at fertilization (8, 35). Alternatively, the calcium wave itself could trigger diacylglycerol formation by activating phospholipase C, which is highly sensitive to intracellular calcium increases (42).

Finally, we note that several model systems have been developed wherein the events of the cell cycle are uncoupled by inhibition of cytokinesis (e.g., see 27, 41). Activation of oocytes with PMA provides an interesting counterpoint to these systems in that the signal for cytokinesis is apparently initiated (cleavage furrow formation) in the absence of the nuclear events that normally precede it (dissolution of the germinal vesicle). This system may therefore prove useful in studies of the influence of cytoplasmic and nuclear factors on cell cycling.

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