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Induction of Starfish Oocyte Maturation by the $\beta\gamma$ Subunit of Starfish G Protein and Possible Existence of the Subsequent Effector in Cytoplasm

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$\beta\gamma$ subunits of G proteins were purified from starfish oocytes, and their role in the induction of oocyte maturation by 1-methyladenine was investigated. When injected into starfish oocytes, the purified $\beta\gamma$ subunit of the starfish G protein induced germinal vesicle breakdown (GVBD) faster than that of bovine brain G protein. Injection of the starfish $\beta\gamma$ into cytoplasm near the germinal vesicle (GV) induced GVBD earlier than when injected into the GV or the cytoplasm near the plasma membrane. Fluorescent-labeled $\beta\gamma$ was retained in the injected area even after GVBD. Injected $\beta\gamma$ also induced the formation of maturation-promoting factor as well as an increase of histone H1 kinase activity. These results suggest that $\beta\gamma$ dissociates from α-subunit by the stimulation of 1-methyladenine and interacts with a cytoplasmic effector, which results in formation of active cdc2 kinase.

INTRODUCTION

Oocyte maturation of starfish is induced by the hormone 1-methyladenine (1-MeAde)[1] that is released from surrounding follicle cells (Kanatani *et al.*, 1969). There are apparently two forms of 1-MeAde receptors with different affinities in the plasma membrane. The high affinity form can be converted into the low affinity one in the presence of GTPγS (Tadenuma *et al.*, 1992), suggesting the presence of a G protein coupled with the receptor. Also, when pertussis toxin (PTX) is microinjected into oocytes, it ADP-ribosylates the 39-kDa α subunit of the G protein (Chiba *et al.*, 1992) and inhibits 1-MeAde-induced oocyte maturation (Shilling *et al.*, 1989; Chiba *et al.*, 1992).

The purified G protein from cortices of starfish oocytes has an $\alpha\beta\gamma$-trimeric structure consisting of 39-kDa α, 37-kDa β, and 7-kDa γ subunits, and the properties of the α and $\beta\gamma$ subunits are quite similar to those of mammalian G protein in terms of guanine nucleotide-binding and GTPase reaction, substrate activity for PTX, and recognition by antibodies against mammalian Gi-α and $\beta\gamma$ subunits (Tadenuma *et al.*, 1991). Also, the deduced amino acid sequence of the cDNA of starfish Gα is 89% identical to mammalian Gi-1 α (Chiba *et al.*, 1992). The coupling of 1-MeAde receptor and the 39-kDa Gα is also demonstrated by the finding that the Gα in the membrane is ADP-ribosylated by cholera toxin only when 1-MeAde is added to the membrane (Tadenuma *et al.*, 1992).

Although the effector of the G protein remains to be identified, it is known that the signal of 1-MeAde is transduced into a drop of cAMP concentration (Meijer and Zarutskie, 1987) and the production of maturation-promoting factor (MPF) activity (Kishimoto and Kanatani, 1976), eventually resulting in the breakdown of the nuclear envelope of the germinal vesicle (GVBD). MPF, which is composed of cdc2 protein kinase and cyclin B, induces not only starfish and *Xenopus* oocyte maturation (Dunphy *et al.*, 1988; Gautier *et al.*, 1988, 1990; Labbe *et al.*, 1988, 1989; Lohka *et al.*, 1988) but also regulates G2- to M phase transition of mitosis from yeast to mammals (Kishimoto *et al.*, 1982; Lee and Nurse, 1987; Tachibana *et al.*, 1987). The cdc2 kinase in *Xenopus* oocytes is activated by dephosphorylation of cdc2 kinase/cyclin complex, which is mediated by cdc25 protein phos-

[1] Abbreviations used: G protein, GTP-binding protein; Gi, G protein of adenylate cyclase that mediates inhibition; GVBD, germinal vesicle breakdown; 1-MeAde, 1-methyladenine; MPF, maturation-promoting factor; PAGE, polyacrylamide gel electrophoresis; PTX, pertussis toxin; SDS, sodium dodecyl sulfate.

phatase (Dunphy and Kumagai, 1991; Gautier *et al.*, 1991; Kumagai and Dunphy, 1992). A similar dephosphorylation is also suggested in the starfish cdc2/cyclin complex (Ookata *et al.*, 1992).

Recently Jaffe *et al.* found that $\beta\gamma$ subunits of mammalian G protein from retina and brain induced GVBD when they were microinjected into starfish oocytes (Jaffe *et al.*, 1993). In this study, we find that purified $\beta\gamma$ subunit from starfish oocytes is somewhat more effective for induction of GVBD than the similar proteins from mammalian brain. We also present evidence suggesting that the effector of the $\beta\gamma$ subunit exists in the cytoplasm near the germinal vesicle (GV).

MATERIALS AND METHODS

Animals and Oocytes

Starfish *Asterina pectinifera* were collected on the Pacific coast of Honsyu Island and kept in laboratory aquaria supplied with circulating sea water at 17°C. Ovaries were removed and allowed to release oocytes into ice-cold calcium-free seawater (460 mM NaCl, 10 mM KCl, 36 mM $MgCl_2$, 17 mM $MgSO_4$, 10 mM N-2-hydroxyethylpiperazine-N'-3-propanesulfonic acid (HEPES), [pH 8.2]). To remove follicle cells, oocytes were washed twice in ice-cold calcium-free seawater and stored in artificial seawater (460 mM NaCl, 10 mM KCl, 36 mM $MgCl_2$, 17 mM $MgSO_4$, 9.2 mM $CaCl_2$, 10 mM HEPES [pH 8.2]) at 20°C.

Purification of $\beta\gamma$-Subunits

G proteins of starfish oocyte and bovine brain were purified as described previously (Katada *et al.*, 1986; Kobayashi *et al.*, 1989; Tadenuma *et al.*, 1991). They were incubated with 10 mM $MgCl_2$ and 50 μM GTPγS for 40 min at 20°C and then applied to a phenyl sepharose column to obtain their dissociated subunits. The resolved $\beta\gamma$ subunits were further applied to a Mono Q HR 5/5 (Pharmacia-LKB, Sweden) column that had been equilibrated with 20 mM tris(hydroxymethyl)aminomethane (Tris)-HCl pH 7.5, 5 mM $MgCl_2$, and 0.7% (3-[(3-cholamidopropyl)dimethylammonio]-1-propamesulfonate (Chaps). The column was eluted with a 10 ml linear gradient of 0–300 mM NaCl in the equilibration buffer and 500-μl fractions were collected. The $\beta\gamma$ subunits were eluted from the column at a low concentration of NaCl. The fraction of $\beta\gamma$ subunits was concentrated by a Centricon-10 (Amicon, Dancers, MA) to 50 μl and gel filtrated through a Sephadex G-50 column that had been equilibrated with 10 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 100 mM NaCl, and 0.5% Na-Cholate. This gel filtration step was essentially required for the removal of the concentrated Chaps present in the applied sample, because a high concentration of Chaps disturbed the action of $\beta\gamma$ subunits.

AMC (7-amino-4-methylcoumarin-3-acetic acid) Labeling of Bovine Brain $\beta\gamma$ Subunits

Approximately 10 mg/ml of $\beta\gamma$ subunits were incubated at 20°C for 3 h in 40 μl of a reaction mixture consisting of 20 mM NaH_2PO_4-Na_2HPO_4 buffer (pH 8.0), 100 mM NaCl, 1% Na-cholate, 400 μg/ml AMC succinimidyl ester. The reaction was stopped by adding 1 μl of 1 M Tris-HCl (pH 8.0) to the reaction mixture, followed by centrifugation (100 000 × *g*, 20 min) to remove insoluble materials. The clear supernatant thus obtained was applied to a column (0.5 × 3 cm) of Sephadex G-50 that had been equibrated with 3 ml of 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.6% Na-cholate. The AMC-conjugated $\beta\gamma$ subunits (34 μM) were recovered in the void fractions and subjected to the assay of microinjection.

PTX Solution

PTX (Kakenseiyaku, Tokyo) was dissolved in 2 M urea, 0.1 M phosphate buffer pH 7.0 at the concentration of 1 mg/ml and stored at 4°C. Before use, PTX was activated by incubation at 37°C for 15 min with an equal volume of 40 mM Tris-HCl pH 7.5, 20 mM dithiothreitol (DTT), and 0.2 mM ATP.

Microinjection

Microinjection of $\beta\gamma$, PTX, and MPF was carried out as described previously (Shilling *et al.*, 1989). Rapid microinjection of $\beta\gamma$ was carried out as described previously (Chiba *et al.*, 1992).

Histone H1 Kinase Assay

Bovine $\beta\gamma$ was injected into 13 oocytes by using rapid microinjection procedure. They were collected with 1 μl of artificial seawater 5 min after GVBD and suspended in 7 μl of buffer containing 80 mM Na-β-glycerophosphate, 20 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 15 mM $MgCl_2$, 1 mM DTT, 20 μg/ml leupeptin, and 0.3 mM phenylmethanesulfonyl fluoride pH 7.3. After lysed by freeze-thawing, they were incubated with 1 μl of reaction mixture containing 7 μg histone H1, 2 mM ATP, and 10 μCi [γ-^{32}P]ATP for 20 min at 20°C. The reaction was terminated by the addition of sodium dodecyl sulfate (SDS)-sample buffer and boiling for 5 min. They were analyzed by SDS polyacrylamide gel electrophoresis (PAGE) on 12.5% gel and stained with Coomasie blue. Sections of the gel containing histone H1 were transferred into scintillation vials for radioactivity counting with a scintillation counter.

RESULTS

Induction of GVBD by Microinjected $\beta\gamma$ Subunit

Purified $\beta\gamma$ subunit from the plasma membrane of starfish oocytes (see MATERIALS AND METHODS and Figure 1, lane 1) was microinjected into starfish oocytes. GVBD occurred when 5.0×10^{-16} mol $\beta\gamma$ subunit or more was injected (Figure 2A). If the injected $\beta\gamma$ diffused uniformly in the oocyte, the final concentration would be over 0.12 μM, which is comparable to the effective concentration seen by Jaffe *et al.* (1993). Lower concentration of $\beta\gamma$ (1.8×10^{-16} mol/oocyte, n = 11) did not induce GVBD. Similar results were obtained when the $\beta\gamma$ from bovine brains was injected (Figure 2A). The effectiveness of starfish and bovine $\beta\gamma$ was different, however, because 1 h after injection, 5.0×10^{-16} mol or 1.0×10^{-15} of starfish and bovine $\beta\gamma$ induced 65 and

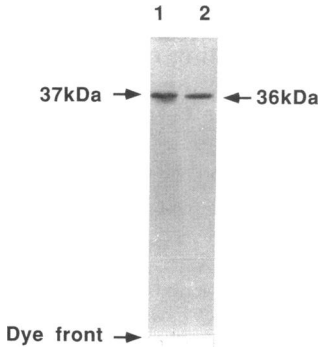

Figure 1. SDS-PAGE of starfish and bovine brain $\beta\gamma$ subunits. Each protein (4 μg) was applied on 15% gel of SDS-PAGE and stained with Coomassie blue. Lane 1, starfish $\beta\gamma$; lane 2, bovine brain $\beta\gamma$. The β-subunits from starfish oocytes (37 kDa) and bovine brains (36 kDa) were apparent. The γ subunits were migrated near dye front.

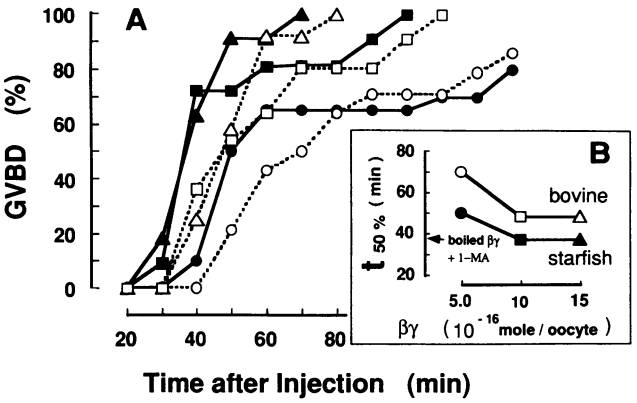

Figure 2. Time course of GVBD induced by injection of starfish and bovine $\beta\gamma$ into the central region of the oocyte (A), and the time required for 50% GVBD (B). Injection volume of $\beta\gamma$ (12 μM) was 42–126 pl (1–3% volume of oocytes). ●, starfish $\beta\gamma$ (5.0×10^{-16} mol); ○, bovine $\beta\gamma$ (5.0×10^{-16} mol); ■, starfish $\beta\gamma$ (1×10^{-15} mol); □, bovine $\beta\gamma$ (1×10^{-15} mol); ▲, starfish $\beta\gamma$ (1.5×10^{-15} mol); △, bovine $\beta\gamma$ (1.5×10^{-15} mol). Each symbol represents the results with 11–20 oocytes. Arrow indicates the 1-MeAde-induced (1 μM) GVBD of oocytes preinjected with boiled starfish $\beta\gamma$ (1.5×10^{-15} mol). Data shown are a representative experiment of three experiments.

43%, or 81 and 64% GVBD, respectively. In agreement, when the time for 50% GVBD was estimated from Figure 2A and plotted against the amount of injected $\beta\gamma$ in Figure 2B, it is seen that starfish $\beta\gamma$ induced GVBD faster than bovine $\beta\gamma$. Control injection of boiled $\beta\gamma$ (90°C, 5 min, 15×10^{-16} mol/oocyte, n = 12) did not cause GVBD. When 1-MeAde was applied to the oocytes that had been injected with boiled $\beta\gamma$, the time for 50% GVBD was comparable to that of oocytes injected with starfish $\beta\gamma$ (Figure 2B).

PTX inhibited 1-MeAde-induced GVBD but not DTT-induced GVBD (Chiba *et al.*, 1992). PTX had no effect on $\beta\gamma$-induced GVBD as shown in Figures 3 and 4.

Figure 4 shows that oocyte maturation induced by starfish $\beta\gamma$ is quite similar to that induced by 1-MeAde. Maturing oocytes formed a fertilization envelope after the penetration of a spermatozoon, expelled polar bodies, and proceeded to cleavage (Figure 4, a–c).

Correlation Between $\beta\gamma$-injected Areas in Oocytes and Times Required for Induction of GVBD

We chose three different areas (Figure 5) of the oocyte to evaluate sensitivity to the injected $\beta\gamma$ subunit. If the $\beta\gamma$ quickly diffused throughout the oocyte after the injection or if the effector of $\beta\gamma$ was distributed throughout the oocyte, the areas where $\beta\gamma$ was injected should make no difference. Figure 6, however, shows that injected $\beta\gamma$ into the center of oocytes (near GV, Figure 5, 1) induced GVBD earlier than that injected into periphery of oocytes (near the plasma membrane, Figure 5, 2) or into the GV (Figure 5, 3).

These results suggest that the $\beta\gamma$ did not diffuse quickly or that microinjection near the plasma membrane might cause damage to delay the response. To check the latter possibility, we did the following experiment: In one group of oocytes (group 1), $\beta\gamma$ was injected near the plasma membrane, and boiled $\beta\gamma$ was injected near the GV. In another group of oocytes (group 2), boiled $\beta\gamma$ was injected near the plasma membrane, and nonboiled $\beta\gamma$ was injected near the GV. If damage artifacts were not significant, oocytes in group 1 should mature more slowly than those in group 2. In fact, the data summarized in Figure 6B clearly support the conclusion that possible damage artifacts from injection near the plasma membrane are not significant.

We therefore checked the possibility that diffusion of injected protein was limited. We labeled β and γ with fluorescent dye, as shown in Figure 7, to follow their distribution after injection. When the labeled $\beta\gamma$ was injected into the center of oocyte, GVBD occurred 35 min after injection (Figure 8, c and d), although the $\beta\gamma$ was retained in the central area even 50 min after injection (Fig. 8, e and f). When $\beta\gamma$ was injected into the periphery of oocytes (near the plasma membrane), GVBD occurred 65 min after injection, and $\beta\gamma$ was still retained in this area (Figure 8, m and n). On the contrary, immediately after the injection of $\beta\gamma$ into GV, it dispersed uniformly throughout the GV (Figure 8, o and p). It deposited, however, on the nuclear membrane by 35 min after injection (Figure 8, q and r), and GVBD occurred 45 min after injection (Figure 8, s and t).

MPF and H1 Kinase Activity of Maturing Oocytes

MPF is found in the cytoplasm of 1-MeAde-treated oocytes and cytoplasmic transfer of the factor induces GVBD of 1-MeAde-untreated oocytes (Table 1) (Kishimoto and Kanatani, 1976). To ascertain whether in-

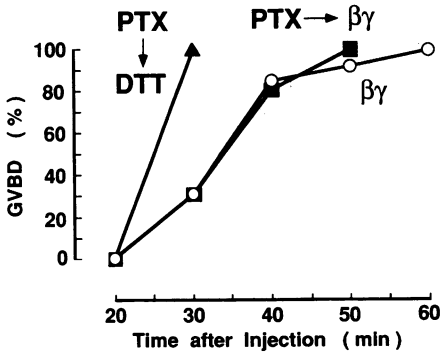

Figure 3. Time course of $\beta\gamma$-induced GVBD of oocytes preinjected with PTX. Oocytes preinjected with PTX (5 μg/ml) were treated with 10 mM DTT (▲). Starfish $\beta\gamma$ (84 pl of 12 μM) was injected into the central region of oocytes preinjected with (■) or without (○) PTX. Data shown are representative of two experiments.

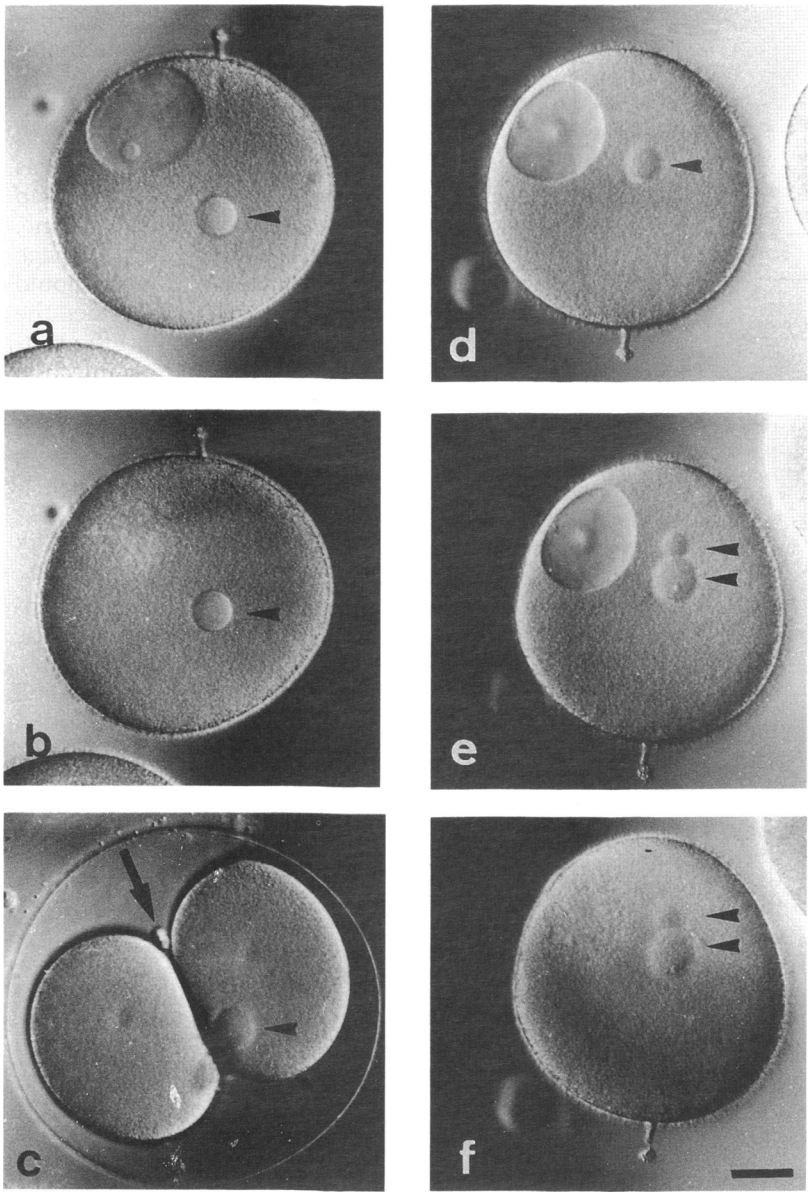

Figure 4. Induction of GVBD by the injection of the starfish $\beta\gamma$. (a) An oocyte was injected with the starfish $\beta\gamma$ (5.0×10^{-16} mol). (b) GVBD occurred 35 min after the injection of $\beta\gamma$. (c) After the GVBD, the oocyte was inseminated. Formation of fertilization envelope, extrusion of polar bodies, and cleavage were normal. (d) PTX-preinjected oocyte was treated with 1 μM 1-MeAde. GVBD was not induced. (e) The PTX-preinjected oocyte was double injected with the starfish $\beta\gamma$. (f) GVBD was induced in the PTX-preinjected oocyte 35 min after the injection of $\beta\gamma$.

jected $\beta\gamma$ formed MPF in the cytoplasm, we sucked the cytoplasm of starfish $\beta\gamma$-injected oocytes after GVBD and then injected it into recipient oocytes. Table 1 shows that the recipient oocytes underwent GVBD. Because diffusion of injected $\beta\gamma$ was quite slow as shown in Figure 8, the sucked cytoplasm might have contained rather a high concentration of $\beta\gamma$. We used labeled $\beta\gamma$ to eliminate this possibility. After injection of labeled bovine $\beta\gamma$ to induce GVBD, we transferred a portion of cytoplasm without detectable fluorescence to the recipients. Table 1 shows that recipients underwent GVBD. These results indicate that both injection of starfish and bovine $\beta\gamma$ increased MPF activity as 1-MeAde did.

The major component of MPF is cdc2 kinase, and histone H1 is a good substrate for this kinase (Table 2) (Ookata *et al.*, 1992). To confirm that the $\beta\gamma$-induced MPF contained cdc2 kinase activity, homogenized oocytes were incubated with radioactive ATP and histone H1. Table 2 shows that histone H1 kinase activity of $\beta\gamma$-injected oocytes was 18 times higher than noninjected control. These results indicate that injection of $\beta\gamma$ increased cdc2 kinase activity and eventually induced GVBD.

DISCUSSION

We have demonstrated that the stimulation of starfish oocyte maturation by 1-MeAde is mimicked by injection

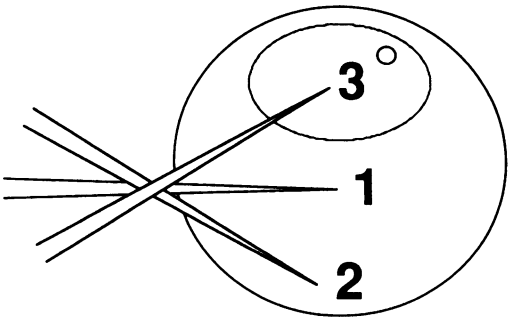

Figure 5. Schematic drawing of the injection points in the oocyte. 1, the central region of the oocyte (cytoplasm near GV); 2, the periphery of oocyte (cytoplasm near the plasma membrane); 3, GV.

of purified $\beta\gamma$ subunits of G proteins from either starfish oocyte or bovine brain. Starfish $\beta\gamma$ induced GVBD appreciably quicker than bovine $\beta\gamma$, although the threshold concentrations for GVBD were not different between them. We concluded that there is a small difference in the effectiveness of starfish and brain $\beta\gamma$ in inducing GVBD. Maturation seemed to proceed similarly in 1-MeAde treated oocytes and $\beta\gamma$-injected ones; timing of GVBD, polar body formation, fertilization, and cleavage appeared normal in $\beta\gamma$-injected oocytes. Therefore it is concluded that $\beta\gamma$ injection mimicked 1-MeAde treatment in both cortical maturation (Meijer and Guerrier, 1984; Chiba and Hoshi, 1989; Chiba *et al.*, 1990) and nuclear maturation.

Our previous data show that 1-MeAde binds to the receptor that couples to an $\alpha\beta\gamma$-trimeric G protein (Tadenuma *et al.*, 1991, 1992). The α subunit of the G protein is ADP-ribosylated by PTX (Tadenuma *et al.*, 1991; Chiba *et al.*, 1992), and this modification blocks the 1-MeAde-induced GVBD (Shilling *et al.*, 1989; Chiba *et al.*, 1992). It is well documented that hormonal stimulation catalyzes the activation of α subunit by enhancing the exchange of GTP for bound GDP and dissociates the $\beta\gamma$ from GTP-bound α (Stryer and Bourne, 1986; Gilman, 1987). Therefore PTX block of GVBD should be because of the inhibition of GTP-α formation and/or dissociation of $\beta\gamma$. Although the role of the GTP-bound starfish α is yet unknown, it has recently been found that injection of GDP-bound α of mammalian αi1 or αt inhibits 1-MeAde-induced GVBD presumably by sequestering the dissociated $\beta\gamma$ (Jaffe *et al.*, 1993). Thus, it is most likely that 1-MeAde induces GVBD through an action of dissociated $\beta\gamma$. Injection of starfish $\beta\gamma$ bypassed the block of PTX, as predicted from this hypothesis.

This hypothesis implies an effector directly related to the $\beta\gamma$ subunit. Because the G protein that mediates 1-MeAde signal transduction is located in the plasma membrane, the plausible effector may also be located in or adjacent to the plasma membrane. If this is the

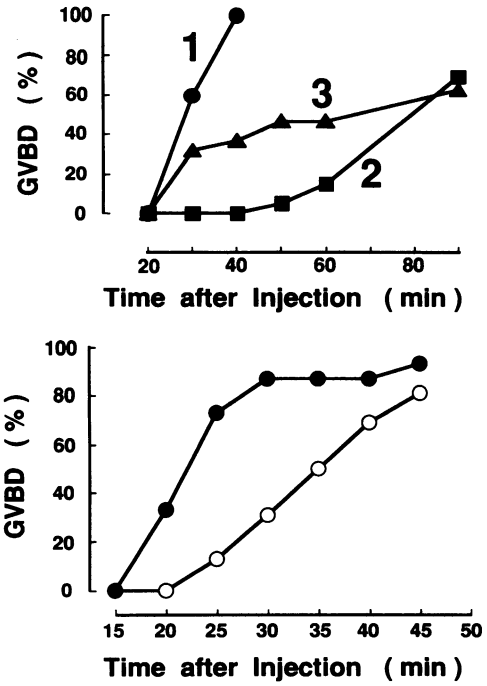

Figure 6. (A) Time course of GVBD induced by the injection of bovine $\beta\gamma$ (10 pl of 0.1 mM, 0.23% volume of oocyte, 1.0×10^{-15} mol $\beta\gamma$/oocyte) into different points of oocyte. Symbols correspond to the injection points: ●, Figure 5, 1; ■, Figure 5, 2; ▲, Figure 5, 3. Each symbol represents the results with 20 oocytes. Data shown are representative of six experiments using different animals, and the total number of oocytes in those experiments was 140. (B) Time course of GVBD induced by double injection of boiled and nonboiled $\beta\gamma$ (22 pl of 0.1 mM) into near the plasma membrane and near the GV. Group 1 (○), $\beta\gamma$ was injected near the plasma membrane and then boiled $\beta\gamma$ was injected near the GV. Group 2 (●), boiled $\beta\gamma$ was injected near the plasma membrane and then nonboiled $\beta\gamma$ was injected near the GV. Each symbol represents the results with 16 oocytes using two animals.

case as generally thought, $\beta\gamma$ injected closer to the plasma membrane will be more effective. To our surprise, the $\beta\gamma$ was most effective when it was injected into the central region of the oocytes (Figure 6). This unexpected result suggests that the effector exists in the central region of oocytes or that $\beta\gamma$ is more quickly de-

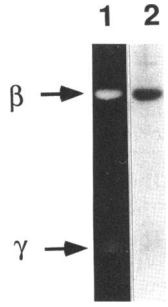

Figure 7. SDS-PAGE of AMC-labeled bovine brain $\beta\gamma$-subunits. Labeled $\beta\gamma$ was applied on 15% gel of SDS-PAGE. Fluorescence image (lane 1), Coomassie blue staining (lane 2).

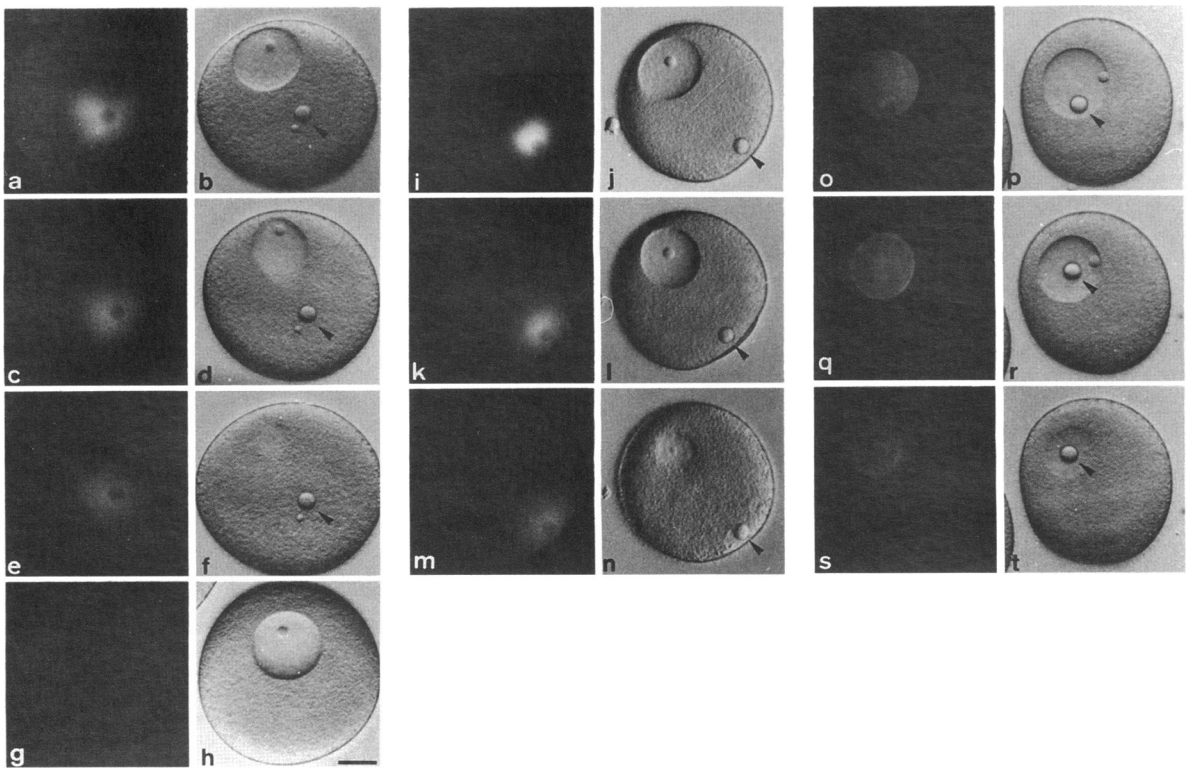

Figure 8. GVBD induced by the labeled $\beta\gamma$. The $\beta\gamma$ of bovine brain was labeled with AMC and injected into the center (a–f), the periphery (i–n), and GV (o–t) of oocytes. The distribution of injected $\beta\gamma$ was shown in a, c, e, i, k, m, o, q, and s. The corresponding Nomarski images of oocytes are shown in b, d, f, j, l, n, p, r and t. Autofluorescence of a control oocyte was shown in g, and the corresponding Nomarski image of the oocyte in h. Images were taken 2 min (a, b, i, j, o, and p), 35 min (c, d, k, l, q and r), 45 min (s and t), 50 min (e and f), and 65 min (m and n) after injection.

livered to the plasma membrane from that region. Using fluorescent-labeled $\beta\gamma$, we found that the diffusion of injected $\beta\gamma$ in cytoplasm was quite slow (Figure 8), indicating that $\beta\gamma$ that was injected into the central region was not quickly delivered to the plasma membrane and suggesting that the effector existed in this central region.

Kiehart *et al.* (1982) showed fluorescent-labeled γ-globulin diffused to homogeneity throughout cytoplasm within 2–4 min when it was injected into starfish eggs. At present, we do not know the reason why $\beta\gamma$ injected in the cytoplasm diffused slowly. One explanation for this observation is that injected $\beta\gamma$ into cytoplasm may be quickly trapped by membrane systems such as the endoplasmic reticulum or vesicles. In fact, immediately after the injection of $\beta\gamma$ in the GV, it dispersed throughout nucleoplasm that did not contain membrane, and within 35 min after injection, it was accumulated and/or deposited on the nuclear membrane without detect-

Table 1. Formation of MPF in $\beta\gamma$-injected oocytes

Treatment of donor	GVBD (%) of recipient	n[a]
1-MeAde (external)	91	11
Starfish $\beta\gamma$[b]	69	13
Bovine $\beta\gamma$[c]	86	14

[a] The number of recipient oocytes injected with cytoplasm from donors.
[b] The starfish $\beta\gamma$ (15×10^{-16} mole) was injected into donor oocytes.
[c] The AMC-labeled bovine $\beta\gamma$ was injected into donor oocytes. After GVBD of donor oocytes, the cytoplasm that did not contain fluorescence was sucked and injected into recipient oocytes.

Table 2. Histone H1 kinase activity of $\beta\gamma$-injected oocytes

	cpm
Control	4453 ± 1610
1-MeAde	78020 ± 8264
$\beta\gamma$	81920 ± 6230

Control, oocytes without 1-MeAde treatment or $\beta\gamma$ injection; 1-MeAde, oocytes treated with 1-MeAde; $\beta\gamma$, oocytes injected with bovine $\beta\gamma$. The data were shown as the means ± SE of three experiments.

able leakage to the cytoplasm (Figure 8). It will be of interest to see if the stimulation by 1-MeAde releases endogenous $\beta\gamma$ from the plasma membrane into cytoplasm or, conversely, it translocates the cytoplasmic effector to the plasma membrane.

To identify the effector of $\beta\gamma$, it is important to know what molecules participate in the $\beta\gamma$-induced GVBD. Our results clearly show that injected $\beta\gamma$ increases the activity of MPF and cdc2 kinase in the oocytes (Tables 1 and 2). Dephosphorylation of cdc2 kinase by cdc25 phosphatase is a prerequisite for activation of the kinase (Gautier *et al.*, 1991; Kumagai and Dunphy, 1992). Thus, $\beta\gamma$ could be involved in the activation of cdc25 either directly or indirectly. Another possible target for $\beta\gamma$ is adenylate cyclase, because 1-MeAde decreases the concentration of cAMP in oocytes 10–30% (Meijer and Zarutskie, 1987) and $\beta\gamma$, by itself, decreases the activity of mammalian Ca^{2+}/calmodulin-stimulated type-I adenylate cyclase (Katada *et al.*, 1987; Tang *et al.*, 1991; Tang and Gilman, 1991; Iniguez-Lluhi *et al.*, 1992). However, forskolin-mediated increase of cAMP (about 3700%) does not block GVBD completely (Meijer and Zarutskie, 1987). Therefore adenylate cyclase is not the sole target of the dissociated $\beta\gamma$ even if it is involved in 1-MeAde-induced oocyte maturation. Identification of the effector will provide a new insight into the molecular mechanisms underlying the $\beta\gamma$-mediated regulation of cell cycle.

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