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Author: Chiba, Kazuyoshi

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[REVIEW]

Meiosis Reinitiation in Starfish Oocyte

Kazuyoshi Chiba*

Department of Biology, Ochanomizu University, 2-1-1 Ohtsuka, Tokyo 112-8610, Japan

Starfish

Starfish, *Asterina pectinifera* (cover picture), is easily found in shallow waters of the Pacific and the Japan Sea. The pretty five armed shape is familiar to almost everyone. Fishermen, however, hate the animal because it attacks and consumes scallops or clams.

In the breeding season (from April to December, depending on the area), starfish have several million fully grown oocytes. At this stage, oocytes are "immature" and have a large nucleus called a germinal vesicle (GV). They are arrested in prophase of meiosis I. Meiosis is reinitiated by the hormone, 1-methyladenine (1-MA), which is released from surrounding follicle cells (Kanatani *et al*., 1969). Germinal vesicle breakdown (GVBD) is the first easily observable event in meiosis reinitiation after hormonal stimulation. After GVBD, oocytes are called "mature". Then, they are spawned and fertilization occurs in the seawater.

Starfish are ideal animals for the study of meiosis reinitiation. They are easy to keep in marine aquaria. Also, oocyte maturation is easily induced by experimental application of 1-MA to the isolated ovary or oocytes. Usually synchronous GVBD of *Asterina pectinifera* occurs within 30 min after 1-MA addition, in which period protein synthesis is not required. The first and second meiotic cycles are completed without arrest of meiosis. In this review, I would like to summarize the pathway of 1-MA signal transduction.

Properties of 1-MA receptors

Intracellularly injected 1-MA does not cause GVBD (Kanatani and Hiramoto, 1970), suggesting that 1-MA receptors exist on the plasma membrane. Indeed, 1-MA specifically binds to the oocyte surface (Yoshikuni *et al*., 1988).

The surface receptors coupling to guanosine nucleotide binding (G) proteins have two different affinities for the agonists (Gilman, 1987). The high-affinity receptors are converted to the low-affinity ones by GTP or GTPγS which can activate the G protein. To determine whether 1-MA receptors couple to G proteins, we measured the specific binding of ^{3}H-radiolabeled 1-MA to a oocyte membrane with or without GTPγS. As expected, the binding activity decreased, when GTPγS was added. Scatchard plot analysis showed that there were two affinities for 1-MA binding with apparent Kd's of approximately 30 nM and more than 1 μM. The lower value of Kd appeared to be reasonably high as a character of G protein-coupled membrane receptors for the agonist. The high Kd value of micromolar range was consistent with the value reported by Yoshikuni *et al*. (1988). In the presence of GTPγS, the high-affinity fraction was completely abolished. The conversion of the binding activity from high-affinity to low-affinity was not induced by ATP or its analogue (Tadenuma *et al*., 1992). These results suggest that starfish oocyte membranes have 1-MA receptors coupling to G proteins. The next step of this study is to identify the receptor.

* Corresponding author: Tel. +81-3-5978-5370;
FAX. +81-3-5978-5369.
E-mail: kchiba@cc.ocha.ac.jp

PTX-sensitive G protein in starfish oocytes

G proteins mediate signal transduction from a receptor to an effector enzyme in many systems. In mammals, pertussis toxin (PTX) ADP-ribosylates the α subunit of Gi-type G protein and prevents the α subunits from accepting the signal of the receptor (Gilman, 1987; Ui and Katada, 1990). When we injected PTX into starfish oocytes, 1-MA-induced GVBD was completely inhibited (Shilling *et al*., 1989). These results suggest that 1-MA receptors interact with PTX-sensitive G proteins. To demonstrate whether PTX ADP-ribosylates the G protein *in vivo*, [^{32}P]NAD and PTX were injected into immature oocytes. Then, the oocytes were analyzed by SDS-PAGE and autoradiography. As shown in Fig. 1, a 39-kDa protein was radiolabeled. When [^{32}P]NAD without PTX was injected, the band was not found (Fig. 1). Thus, it is likely that microinjected PTX ADP-ribosylated the 39-kDa G protein α subunit in the oocytes. Indeed, the purified G protein from oocyte plasma membranes had an αβγ-trimeric structure consisting of 39-kDa α, 37-kDa β, and 8-kDa γ subunits. During purification, a PTX-substrate G protein (39-kDa α) was always recovered as a single peak, indicating that there is the only one class of PTX substrate in starfish oocyte membranes, in contrast to mammalian G protein having three distinct forms of α subunit, Gi-1, Gi-2 and Gi-3 (Tadenuma *et al*., 1991).

The α subunit of the starfish G protein was purified and digested with trypsin. The resulting peptides were fractionated by HPLC and purified peptides were microsequenced, which revealed their high degree of identity with mammalian Gi-α. Thus we screened a cDNA library constructed from the

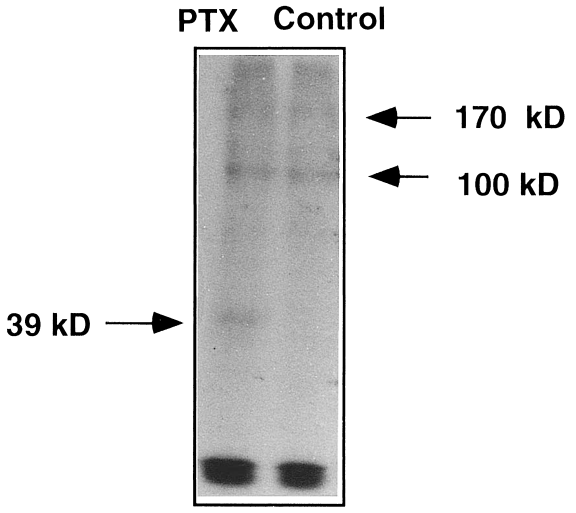

Fig. 1. *In vivo* ADP-ribosylation of G protein α subunit. [α-32P]NAD with PTX (lane 1, PTX) or without PTX (lane 2, control) were microinjected into immature oocytes. To obtain enough oocytes for autoradiography, we had developed a rapid injection technique that enables us to prepare 500 PTX-injected oocytes within an hour (Chiba *et al*., 1992). In this experiment, 50 oocytes were used and oocyte proteins were separated by SDS-PAGE and autoradiographed. The 39-kDa protein was radiolabeled only in the presence of PTX. Other radiolabeled proteins (170 and 100 kDa) were polyADP-ribosylated by endogenous polyADP-ribose polymerase (data not shown).

starfish ovary with rat Giα cDNA. A positive cDNA clone contained an open reading frame of 1,062 bases. The deduced amino acid sequence contained microsequences, indicating that the isolated cDNA clone encodes the α subunit of the PTX-sensitive G protein in oocytes (Chiba *et al*., 1992). Identity of starfish Gα was 89% with rat Gi-1α, 85% with rat Gi-2α, 86% with rat Gi-3α, and 72% with rat Goα. Similarly, high identity of starfish Gβ with mammalian one was suggested, since the 37-kDa β subunit strongly reacted to an antibody against mammalian 36-/35-kDa β subunits (Tadenuma *et al*., 1991).

Usually, cholera toxin (CTX) cannot ADP-ribosylate the PTX-substrate G proteins. However, when the agonist stimulates the receptor and G proteins, CTX can ADP-ribosylate the PTX-sensitive G proteins (Iiri *et al*., 1991). Thus, coupling of the PTX-sensitive G protein with the receptor can be demonstrated using CTX-dependent ADP-ribosylation. As expected, the starfish 39-kDa G protein became a CTX substrate in the presence of 1-MA. We thus concluded that the G protein in starfish oocyte couples to the 1-MA receptors (Tadenuma *et al*., 1992).

PTX did not block dithiothreitol-induced maturation

Dithiothreitol is known to induce GVBD of starfish oocytes (Kishimoto and Kanatai 1973), presumably due to its ability to reduce disulfide bonds (Kishimoto *et al*., 1976: Mita *et al*., 1987). Dithiothreitol-treated oocytes, as well as the 1-MA-treated, form a maturation-promoting factor (MPF: see below) in the cytoplasm (Kishimoto *et al*., 1976), yet the primary target of dithiothreitol is an open question. If a target molecule of dithiothreitol is similar to that of 1-MA, PTX should inhibit dithiothreitol-induced maturation as well. However, dithiothreitol induced GVBD of oocytes preinjected with PTX. It is therefore concluded that dithiothreitol acts on a downstream pathway of G protein (Chiba *et al*., 1992).

Induction of GVBD by microinjected starfish G protein βγ-subunit

The binding of 1-MA to the receptor should induce dissociation of Gα and Gβγ. Thus, 1-MA-induced GVBD is likely to be mediated by released Gα and/or Gβγ. Jaffe *et al*. (1993) found that mammalian Gβγ purified from brain or retina induced GVBD when they were microinjected into starfish oocytes. Similarly, when we purified starfish Gβγ from oocyte plasma membrane and microinjected it into starfish oocytes, GVBD was occurred (Chiba *et al*., 1993). Starfish Gβγ seems to be more effective than mammalian one, since it induced GVBD faster. Oocyte maturation induced by starfish Gβγ was quite similar to that induced by 1-MA: MPF or high activity of cdc2 kinase were found in the cytoplasm of Gβγ-injected oocytes. Maturing oocytes formed fertilization envelope after the penetration of a spermatozoon, expelled polar bodies, and proceeded to cleavage. These results indicate that βγ-injection mimicked 1-MA treatment in both cortical maturation (Meijer and Guerrier, 1984; Chiba and Hoshi, 1989; Chiba *et al*., 1990) and nuclear maturation. We concluded that 1-MA induces GVBD through an action of dissociated Gβγ from Gα.

Localization G protein βγ subunit in starfish oocytes

We raised the monoclonal antibody which cross-reacts with purified β subunit from starfish oocytes. The antibody recognized a single band in Western blots of immature and mature oocyte lysate, indicating that it is specific for β subunit.

When isolated plasma membranes of immature and mature oocytes were labeled with the antibody, there was faint punctate staining of β subunit throughout the plasma membrane. Thus, as had been expected, G proteins interacting with the 1-MA receptor exist on the plasma membrane (Chiba *et al*., 1995). To our surprise, whole mount preparation of immature oocytes labeled with the antibody revealed a reticular network throughout the cytoplasm. The fiber exhibited complex striation with a periodicity of 0.7 μm. The thickness of the fibers were about 0.4 μm. The identical staining patterns were obtained, when the oocyte was stained with anti-γ subunit antibodies. Similar networks of filaments in immature oocytes of sea urchin (Boyle and Ernst, 1989) as well as starfish (Schroeder and Otto, 1991) were stained with antibodies against cytokeratin. Indeed, when we double-stained the oocytes with anti-Gβ and anti-cytokeratin antibodies, the networks labeled with both antibodies shared a common feature of looping and branching. However, the pattern of stained striation alternated. These results suggest that G protein βγ subunit distributes segmentally on the continuous cytokeratin filaments (Chiba *et al*., 1995). The role of the cytoplasmic Gβγ remains unclear.

PI3K and protein phosphorylation

In mammalian cells, Gβγ directly activates some members of the phosphatidylinositol 3-kinase (PI3K) family (Kurosu *et al*., 1995, 1997; Leopoldt *et al*., 1998; Stephens *et al*., 1997; Stoyanov *et al*., 1995; Thomason *et al*., 1994). PI3K phosphorylates inositides at the D-3 position of the inositol ring to generate such lipid messengers as phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-triphosphate. Sadler and Ruderman (1998) reported that 1-MA-induced oocyte maturation in starfish *Asterina miniata* is blocked by the PI3K inhibitors, wortmannin and LY294002, suggesting that PI3K acts downstream of the 1-MA receptor. We showed that these lipid kinase inhibitors blocked Gβγ-induced oocyte maturation in starfish *Asterina pectinifera* (Nakano *et al*., 1999). These results support the hypothesis that PI3K is the target of the Gβγ. Cytoplasmic distribution of the subsequent effector for Gβγ (Chiba *et al*., 1993) is also consistent with the above hypothesis, since PI3K is demonstrated to exist in the cytoplasm (Leopoldt *et al*., 1998).

To investigate targets for PI3K, we examined endogenous protein phosphorylation (Nakano *et al*., 1999). When starfish oocytes were microinjected with [γ-^{32}P]ATP, the significant enhancement of phosphorylation of a 62-kDa protein from 1-MA-treated oocytes was observed. The stimulation of the phosphorylation was a very early event in the 1-MA signaling pathway, since phosphorylation of the 62-kDa protein started within the first 4 min of 1-MA stimulation. In the cell-free preparations (Chiba *et al*., 1999), the 62-kDa protein was also phosphorylated on serine residue(s) immediately after addition of 1-MA or Gβγ (Nakano *et al*., 1999). If the PI3K is an essential component of the pathway linking the Gβγ to the serine kinase activation, inhibitors of the lipid kinase should block the phosphorylation of the 62-kDa protein. As expected, when we added Gβγ to the immature oocyte supernatant preincubated with wortmannin or LY294002, the phosphorylation of the 62-kDa protein was inhibited. These results indicate that the 62-kDa protein functions downstream of Gβγ and PI3K in the early signaling pathway of 1-MA-induced starfish oocyte maturation. Although it is much more likely that PI3K activation sets off a signal cascade that includes the protein kinase, there is a possibility that the PI3K has a protein kinase activity, since some PI3K family members have protein kinase activity (for review see Hunter, 1995). In particular, DNA-dependent protein kinase (DNA-PK), which lacks detectable lipid kinase activity, is inhibited by wortmannin or LY294002 (Christodoulopoulos *et al*., 1998; Gu *et al*., 1998). If this is the case, the 62-kDa protein may be a direct target of the PI3K.

MPF activity of maturing oocytes

MPF is activated in the cytoplasm about 20 min after the phosphorylation of 62- kDa protein. MPF, which is composed of cdc2 protein kinase and cyclin B, not only induces starfish oocyte maturation, but also regulates G2- to M phase transi-

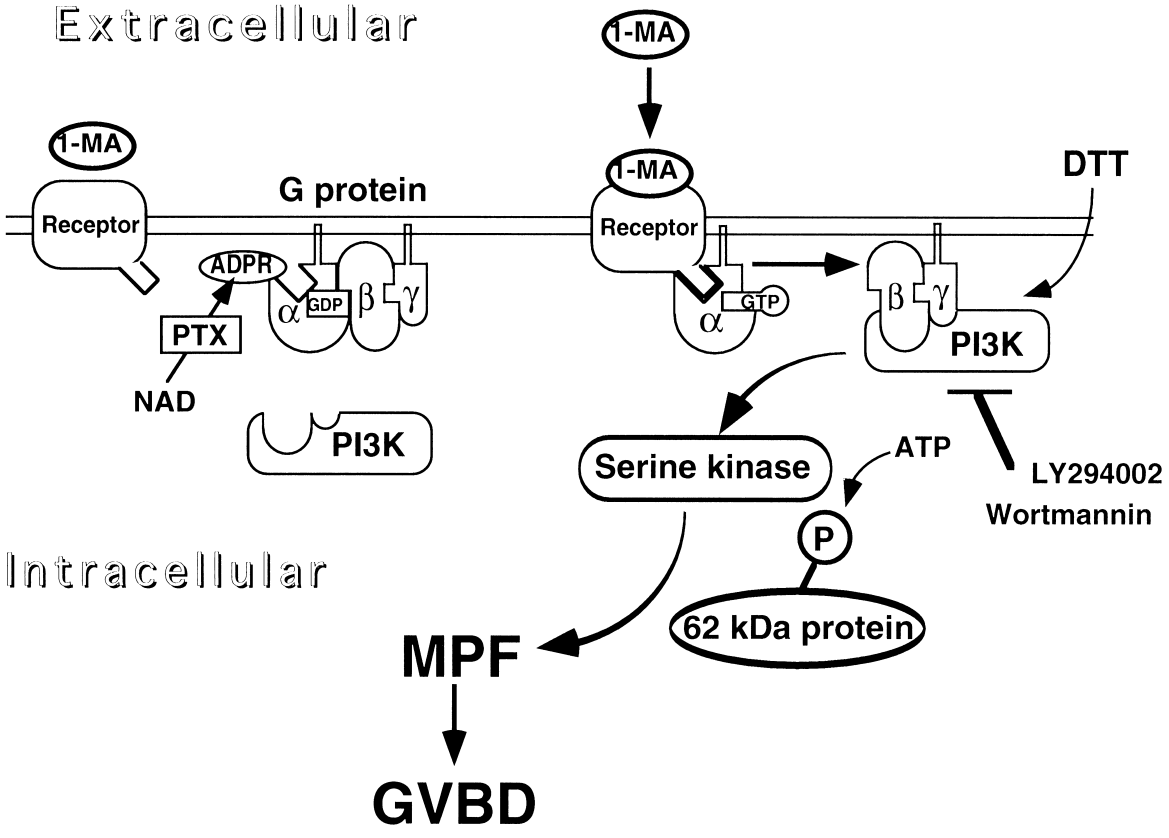

Fig. 2. Model for the process of 1-MA-induced GVBD. See text in detail.

tion of mitosis from yeast to mammalian cells (Kishimoto *et al*., 1982; Tachibana *et al*., 1987; Lee, M. G. and Nurse, P., 1987). The cdc2 kinase in starfish oocytes is activated by dephosphorylation of cdc2 kinase/cyclin complex, which is mediated by cdc25 protein phosphatase (for review see Kishimoto, 1999). Interestingly in starfish oocytes, Okumura *et al.* (1996) indicated that cdc25 phosphatase (90-kDa) is activated by an unknown kinase called “initial kinase”. The serine kinase activated by PI3K is likely to be involved in the activation of the “initial kinase”. Or the phosphorylation of the 62-kDa protein may be required for the activation of the kinase. Studies on the identification and characterization of the 62-kDa protein and the serine kinase are now in progress.

In summary as shown in Fig. 2, the receptor of 1-MA on the plasma membrane of starfish oocytes couples to the $\alpha\beta\gamma$ trimeric G protein. Microinjected pertussis toxin (PTX) catalyzes ADP-ribosylation (ADPR) of Gα. This modification prevents the interaction between the receptor and G protein, and blocks the 1-MA signal transduction. The hormonal stimulation dissociates G$\beta\gamma$ from GTP-bound Gα, and the dissociated G$\beta\gamma$ activates PI3 kinase (PI3K).The PI3K participates in the activation of the protein kinase that phosphorylates the serine residue of the 62-kDa protein, which results in the activation of MPF (cdc2 kinase/cyclin B). MPF eventually induces GVBD.

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REFERENCES

Boyle JA, Ernst SG (1989) Sea urchin oocytes posses elaborate cortical arrays of microfilaments, microtubules and intermediate filaments. Dev Biol 134: 72–84

Chiba K, Hoshi M (1989) Three phase of cortical maturation during meiosis reinitiation in starfish oocytes. Develop Growth Differ 31: 447–451

Chiba K, Kado RT, Jaffe LA (1990) Development of calcium release mechanism during starfish oocyte maturation. Dev Biol 140: 300–306

Chiba K, Tadenuma H, Matsumoto M, Takahashi K, Katada T, Hoshi M (1992) The primary structure of the α subunit of a starfish guanosine-nucleotide-binding regulatory protein involved in 1-methyladenine-induced oocyte maturation. Eur J Biochem 207: 833–838

Chiba K, Kontani K, Tadenuma H, Katada T, Hoshi M (1993) Induction of starfish oocyte maturation by the $\beta\gamma$ subunit of starfish G protein and possible existence of the subsequent effector in cytoplasm. Mol Biol Cell 4: 1027–1034

Chiba K, Longo FJ, Kontani K, Katada T, Hoshi M (1995) A periodic network of a G protein $\beta\gamma$ subunit coexisting with cytokeratin filament in starfish oocytes. Dev Biol 169: 415–420

Chiba K, Nakano T, Hoshi M (1999) Induction of germinal vesicle breakdown in cell-free preparation from starfish oocytes. Dev Biol 205: 217–223

Christodoulopoulos G, Muller C, Salles B, Kazmi R, Panasci L (1998) Potentiation of chlorambucil cytotoxicity in B-cell chronic lymphocytic leukemia by inhibition of DNA-dependent protein kinase activity using wortmannin. Cancer Res 58: 1789–1792

Gilman AG, (1987) G proteins: Transducers of receptor-generated signals. Annu Rev Biochem 56: 615–649

Gu XY, Weinfeld MA, Povirk LF (1998) Implication of DNA-dependent protein kinase in an early, essential, local phosphorylation event during end-joining of DNA double-strand breaks *in vitro*. Biochemistry 37: 9827–9835

Hunter T (1995) When is a lipid kinase not a lipid kinase? When it is a protein kinase. Cell 83: 1–4

Iiri T, Ohoka Y, Ui M Katada T. (1991) Functional modification by cholera toxin-catalyzed ADP-ribosylation of guanine nucleotide-binding protein serving as the substrate of pertussis toxin. Eur J Biochem. 202: 635–641

Jaffe LA, Gallo CJ, Lee RH, Ho Y-K, Jones TLZ (1993) Oocyte maturation in starfish is mediated by the $\beta\gamma$-subunit complex of a G-protein. J Cell Biol 121: 755–783

Kanatani H, Hiramoto Y (1970) Site of action of 1-methyladenine in inducing oocyte maturation in starfish. Exp Cell Res 61: 280–284

Kanatani H, Shirai H, Nakanishi K, Kurokawa T (1969) Isolation and identification of meiosis-inducing substance in starfish *Asterias amurensis*. Nature 221: 273–274

Kishimoto T (1999) Activation of MPF at meiosis reinitiation in starfish oocytes. Dev Biol 214: 1–8

Kishimoto T, Kanatani H (1973) Induction of starfish oocyte maturation by disulfide-reducing agents. Exp Cell Res 82: 296–302

Kishimoto T, Kanatani H (1976) Cytoplasmic factor responsible for germinal vesicle breakdown and meiotic maturation in starfish oocyte. Nature 260: 321–322

Kishimoto T., Kuriyama R, Kondo H, Kanatani H (1982) Generality of the action of various maturation-promoting factors. Exp Cell Res 137: 121–126

Kurosu H, Hazeki O, Kukimoto I, Honzawa S, Shibasaki M, Nakada M, Ui M, Katada T (1995) Radiolabeling of catalytic subunits of PI3-kinases with 17β-hydroxy-16α-[^{125}I]iodowortmannin: identification of the G$\beta\gamma$-sensitive isoform as a complex composed of 46-kDa and 100-kDa subunits. Biochem Biophys Res Commun 216: 655–661.

Kurosu H, Maehama T, Okada T, Yamamoto T, Hoshino S, Fukui Y, Ui M, Hazeki O, Katada T (1997) Heterodimeric phosphoinositide 3-kinase consisting of p85 and p110β is synergistically activated by the $\beta\gamma$ subunits of G proteins and phosphotyrosyl peptide. J Biol Chem 272: 24252–24256

Lee MG, Nurse P (1987) Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. Nature 327: 31–35

Leopoldt D, Hanck T, Exner T, Maier U, Wetzker R, Nürnberg B (1998) G$\beta\gamma$ stimulates phosphoinositide3-kinase-γ by direct interaction with two domains of the catalytic p110 subunit. J Biol Chem 273: 7024–7029

Meijer L, Guerrier P (1984) Maturation and fertilization in starfish oocytes. Int Rev Cytol 86: 130–195

Mita M, Ueta N, Nagahama Y (1987) In vitro induction of starfish oocyte maturation by cystein alkylesters. Dev Growth Differ 29: 607–616

Nakano T, Kontani K, Kurosu H, Katada T, Hoshi M, Chiba K (1999) G-protein $\beta\gamma$ subunit-dependent phosphorylation of 62-kDa protein in early signaling pathway of starfish oocyte maturation induced by 1-methyladenine. Dev Biol 209: 200–209

Okumura E, Sekiai T, Hisanaga S, Tachibana K, Kishimoto T (1996) Initial triggering of M-phase in starfish oocytes: a possible novel component of maturation-promoting factor besides cdc2 kinase. J. Cell Biol. 132: 125–35

Sadler KC, Ruderman JV (1998) Components of the signaling pathway linking the 1-methyladenine receptor to MPF activation and maturation in starfish oocytes. Dev Biol 197: 25–38

Schroeder TE, Otto JJ (1991) Snoods: A periodic network containing

cytokeratin in the cortex of starfish oocytes. Dev Biol 144: 240–247

Shilling F, Chiba K, Hoshi M, Kishimoto T, Jaffe LA (1989) Pertussis toxin inhibits 1-methyladenine-induced maturation in starfish oocytes. Dev Biol 133: 605–608

Stephens LR, Eguinoa A, Erdjument-Bromage H, Lui M, Cooke F, Coadwell J, Smrcka AS, Thelen M, Cadwallader K, Tempst P, Hawkins PT (1997) The Gβγ sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. Cell 89: 105–114

Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nürnberg B, Gierschik P, Seedorf K, Hsuan J J, Waterfield MD, Wetzker R (1995) Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. Science 269: 690–693.

Tachibana K, Yanagishima N, Kishimoto T (1987) Preliminary characterization of maturation-promoting factor from yeast *Saccharomyces cerevisiae*. J Cell Sci 88: 273–281

Tadenuma H, Chiba K, Takahashi K, Hoshi M, Katada T (1991) Purification and characterization of a GTP-binding protein serving as pertussis toxin substrate in starfish oocytes. Arch Biochem Biophys 290: 411–417

Tadenuma H, Takahashi K, Chiba K, Hoshi M, Katada T (1992) Properties of 1-methyladenine receptors in starfish oocyte membranes: Involvement of pertussis toxin-sensitive GTP-binding protein in the receptor-mediated signal transduction. Biochem Biophys Res Commun 186: 114–121

Thomason PA, James SR, Casey PJ, Downes CP (1994) A G-protein βγ-subunit-responsive phosphoinositide 3-kinase activity in human platelet cytosol. J Biol Chem 269: 16525–16528

Ui M, Katada T (1990) Bacterial toxins as probe for receptor-Gi coupling. Adv. Second Messenger Phosphoprotein Res 24: 63–69

Yoshikuni M, Ishikawa K, Isobe M, Goto T, Nagahama Y (1988) Characterization of 1-methyladenine binding in starfish oocyte cortices. Proc Natl Acad Sci USA 85: 1874–1877

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