Heterodimeric Phosphoinositide 3-Kinase Consisting of p85 and p110β Is Synergistically Activated by the βγ Subunits of G Proteins and Phosphotyrosyl Peptide*

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Phosphoinositide 3-kinase (PI 3-kinase) is a key signaling enzyme implicated in the regulation of a broad array of biological responses including receptor-stimulated mitogenesis, oxidative burst, membrane ruffling, and glucose uptake (1, 2). The activation of PI 3-kinase results in an increase in cellular levels of b3-phosphorylated phosphoinositides, such as PtdIns(3)P, PtdIns(3,4)P2, and PtdIns(3,4,5)P3. These products, however, do not serve as the substrates of phospholipase C (3) and thus have been proposed to act as second messengers. In this regard, recent studies have indicated that PtdIns(3,4)P2 can directly activate certain protein kinase C and Akt (4, 5) and PtdIns(3,4,5)P3 is capable of binding to the Pleckstrin homology domain of guanine nucleotide exchange factor of the small GTP-binding protein ARF1 (6–8).

At least two types of PI 3-kinase, in terms of mode of the activation, have been described in mammalian cells (2). One is stimulated by membrane-bound receptors activating tyrosine kinase, whereas the other is under the direct control of the heterotrimeric GTP-binding proteins. The well known former type has been structurally characterized as a heterodimer consisting of a 110-kDa catalytic subunit (p110) and an 85-kDa regulatory subunit (p85). We separated a PI 3-kinase that could be stimulated by the βγ subunits of G protein (Gβγ) from rat liver. The Gβγ-sensitive PI 3-kinase appeared to be a heterodimer consisting of p110β and p85 (or their related subunits). The stimulation by Gβγ was inhibited by the GDP-bound α subunit of the inhibitory GTP-binding protein. Moreover, the stimulatory action of Gβγ was markedly enhanced by the simultaneous addition of a phosphotyrosyl peptide synthesized according to the amino acid sequence of the insulin receptor substrate-1. Such enzymic properties could be observed with a recombinant p110β/p85α expressed in COS-7 cells with their cDNAs. In contrast, another heterodimeric PI 3-kinase consisting of p110α and p85 in the same rat liver, together with a recombinant p110α/p85α, was not activated by Gβγ, although their activities were stimulated by the phosphotyrosyl peptide. These results indicate that p110β/p85 PI 3-kinase may be regulated in a cooperative manner by two different types of membrane receptors, one possessing tyrosine kinase activity and the other activating GTP-binding proteins.

Phosphoinositide 3-kinase (PI 3-kinase)† is a key signaling enzyme implicated in the regulation of a broad array of biological responses including receptor-stimulated mitogenesis, oxidative burst, membrane ruffling, and glucose uptake (1, 2). The activation of PI 3-kinase results in an increase in cellular levels of b3-phosphorylated phosphoinositides, such as PtdIns(3)P, PtdIns(3,4)P2, and PtdIns(3,4,5)P3. These products, however, do not serve as the substrates of phospholipase C (3) and thus have been proposed to act as second messengers. In this regard, recent studies have indicated that PtdIns(3,4)P2 can directly activate certain protein kinase C and Akt (4, 5) and PtdIns(3,4,5)P3 is capable of binding to the Pleckstrin homology domain of guanine nucleotide exchange factor of the small GTP-binding protein ARF1 (6–8).

At least two types of PI 3-kinase, in terms of mode of the activation, have been described in mammalian cells (2). One is stimulated by membrane-bound receptors activating tyrosine kinase, whereas the other is under the direct control of the heterotrimeric GTP-binding proteins. The well known former type has been structurally characterized as a heterodimer consisting of a 110-kDa catalytic subunit (p110) and an 85-kDa regulatory subunit (p85); the regulatory subunit contains one SH3 and two SH2 domains. Stimulation of tyrosine kinase receptors by extracellular signals phosphorylates specific tyrosine residues located in the YMM motif of their own receptors or adaptor molecules, such as insulin receptor substrate-1. These phosphorylated proteins bind to the SH2 domains of p85 and stimulate the lipid kinase activity. The stimulatory effect of these proteins can thus be mimicked in vitro by a synthetic tyrosine-phosphorylated peptide possessing the YMM motif (9, 10). Although several subtypes of p85 and p110 (α, β, and δ) have been identified (11–13), differences in their functions have not been well described.

In addition to the tyrosine phosphorylation-dependent activation of PI 3-kinase, it has been reported that the βγ subunits of G proteins also stimulate the lipid kinase activity (14–18). One report showed that a partially purified PI 3-kinase, which was immunologically distinct from p110α and not associated with p85, is activated by Gβγ (19). Thus, a novel catalytic subunit of PI 3-kinase, designated as p110γ, has been cloned and shown to be activated in vitro both by the α and βγ subunits of G proteins (14). Receptor-induced translocation of p110γ to a cytoskeletal fraction has also been reported (20). This isozyme lacks the binding site to p85 and thus does not interact with the regulatory subunit. Quite recently, Stephens et al. (15) reported a heterodimeric PI 3-kinase consisting of a p110γ-related 120-kDa (or 117-kDa) catalytic subunit and a 101-kDa adaptor protein, which is distinct from p85. PI 3-kinase activity of the novel heterodimer was markedly stimulated by Gβγ but not by a tyrosine-phosphorylated peptide.

In contrast to these reports, two groups including us showed
that Gβγ is capable of stimulating PI 3-kinase activity immunoprecipitated with anti-p85 antibodies (16, 18). Interestingly, insulin-induced accumulation of PtdIns(3,4,5)P3 in the presence of Gβγ or absence of Gβγ was synergistically enhanced by activation of a pertussis toxin-sensitive G protein (18). This observation suggests the presence of an isozyme that is regulated by both phosphotyrosyl proteins and G proteins. In the present study, we have separated a PI 3-kinase responsible for such synergistic activation from rat liver and identified its structure as a heterodimer consisting of p110β and p85 (or their related catalytic subunits). The same synergistic activation was also observed in a recombinant form of p110β/p85 expressed with their cDNAs.

**EXPERIMENTAL PROCEDURES**

**Materials—**[γ-32P]ATP, 125I-labeled protein A, and 125I-labeled protein D were purchased from NEN Life Science Products. Protein A-Sepharose and anti-mouse IgG-agarose resin were from Pharmacia Biotech Inc. and American Qualex, respectively. Tyrosine-phosphorylated and nonphosphorylated peptides synthesized according to the sequence of insulin receptor substrate-1, NGDYMPSPK5, were obtained from Kuraibou Co. (Osaka, Japan). A monoclonal antibody against the N-terminal SH2 domain of p85α (1N4R) and a polyclonal antibody against the whole p85α (p85PAS) were purchased from Seikagaku Corp. (Tokyo, Japan). Polyclonal antibodies against p110α (α and β) were purchased from Santa Cruz Biotechnology, Inc. All other reagents were from commercial sources and of analytical grade.

**DNA Constructs—**The bovine p85α cDNA was subcloned into pcDNA3. The bovine p110β cDNA was subcloned between the HindIII and SalI sites of the pCMV5. The pCMV5 plasmid was a gift from D. W. Russell, University of Texas Southwestern Medical Center. The human p110β cDNA was obtained by reverse transcriptase polymerase chain reaction with mRNAs of Jurkat cells that had been treated with dibutyryl cAMP and ligated between the HindIII and SalI sites of the pCMV5. To modify the C-terminal end of p110α (α and β), the fragments were assayed for PI 3-kinase activity as the substrate of PtdIns(4,5)P2.

**Immunoprecipitation—**The transfected COS-7 cells were washed twice with ice-cold phosphate-buffered saline, fixed in 400 μl of a lysis buffer consisting of 20 mM Na-HEPES (pH 7.4), 75 mM NaCl, 15 mM NaF, 1 mM Na2VO4, 10 mM Na3P2O7, 2 mM EDTA, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 2 μM aprotinin. The cell extracts, after being precleared with Sepharose 4B resin, were incubated with 9E10 (1 μg) and anti-mouse IgG-agarose resin. After the incubation, the beads were washed three times with the lysis buffer and then three more times with 20 mM Tris-HCl (pH 8.0) and 150 mM NaCl.
Comparison between duplicate determinations in a typical study.

G Protein βγ-Sensitive Phosphoinositide 3-Kinase

PI 3-kinase activity in the immunocomplex was determined as described below. In some cases, proteins in the immunecomplex were separated by SDS-PAGE and probed with the antibody against p85α, p110γ, or epitope tag.

Assay of PI 3-Kinase Activity—PI 3-kinase activity was assayed with 0.1 mM PtdIns(4,5)P2 as described previously (17). When the effects of GTP-binding proteins were examined, GDP-bound Gα and/or Gβγ subunits were mixed and incubated on ice for 5 min before addition of the phosphatidylinositol 3,4,5-triphosphate (PtdIns(4,5)P3) as substrate. The assay was performed in the presence or absence of the phosphoryrosyl peptide (pY-pep., 100 μM), Gβγ (0.5 μM), and/or wortmannin (0.1 μM). Bars represent differences between duplicate determinations in a typical study.

PI 3-kinase activity was purified from rat liver according to a purification protocol of Carpenter et al. (22) with modifications. Rat livers were homogenized in 25 volumes of a buffer consisting of 20 mM Tris-HCl (pH 7.4), 2 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 10 μg/ml leupeptin, 0.5 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, and 10% glycerol (buffer B). The mixture was then loaded on a 5-ml column of blue Sepharose, and eluted with a linear gradient of 0–400 mM KCl in buffer B.

Preparation of GTP-binding Protein Subunits—The Gβγ subunits of GTP-binding proteins were purified from rat brain and stored at −80 °C.

RESULTS

When rat liver cytoplasmic proteins were applied to a DEAE-Sepharose column and eluted with a linear gradient of KCl, there were several peaks of fractions containing PI 3-kinase activity (17). In the previous paper (17), we have identified that a 100-kDa/46-kDa heterodimeric form of PI-3 kinase, which was not tightly retained on the column, was activated by Gβγ.

Preparation of GTP-binding Protein Subunits—The Gβγ subunits of GTP-binding proteins were further purified by means of sequential chromatography with CM-Sepharose, Sephacryl S-300/HR, blue Sepharose, and Mono Q HR5/5, which had been equilibrated with buffer D containing 50 mM KCl. The PI 3-kinase eluted at a flow rate of 0.25 ml/min, and the fractions containing the activity were concentrated to 2 ml using a Centricon-30. The concentrate was diluted with 10 volumes of buffer B and loaded on a 5-ml column of blue Sepharose, CL-6B equilibrated with buffer B containing 25 mM NaCl.

Preparation of GTP-binding Protein Subunits—The Gβγ subunits of GTP-binding proteins were further purified by means of sequential chromatography with CM-Sepharose, Sephacryl S-300/HR, blue Sepharose, and Mono Q columns. Fig. 1 shows the PI 3-kinase activity eluted from the Mono Q column. The kinase activity eluted to a concentration of 20 μM in 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, and 0.5% CHAPS (23). Rat recombiant Gαs was purified from Escherichia coli and stored at a concentration of 20 μM in 50 mM Na-HEPES (pH 7.4), 1 mM EDTA, and 1 mM dithiothreitol (24).

The Gβγ-sensitive PI 3-kinase activity eluted from the Mono Q column was equilibrated with 20 mM Tris-HCl (pH 7.4), 1 mM EGTA, 1 mM EGTA, 0.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 10 μg/ml leupeptin, 0.5 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, and 10% glycerol (buffer C). The PI 3-kinase was eluted at a flow rate of 1.25 ml/min, and the fractions containing the activity were concentrated to 2 ml using a Centricon-30. The concentrate was diluted with 10 volumes of buffer B and loaded on a 5-ml column of blue Sepharose, CL-6B equilibrated with buffer B containing 25 mM NaCl. The column was washed with 50 ml of buffer B containing 25 mM NaCl and eluted with a linear gradient of 25–1000 mM NaCl in buffer B over 25 ml. The PI 3-kinase eluted with 400 mM NaCl was concentrated to 1 ml using a Centricon-30, and diluted with 10 volumes of 20 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.5 mM EGTA, 1 mM DTT, 10% glycerol (buffer D). The mixture was loaded on a 1-ml column of Mono Q HR5/5, which had been equilibrated with buffer D containing 50 mM KCl. The column was washed with 10 ml of buffer D containing 50 mM KCl and eluted with a linear gradient of 50–300 mM KCl in buffer D over 25 ml. The PI 3-kinase eluted with 170 mM KCl was pooled and concentrated to 0.2 ml using the Centricon-30. The sample was supplemented with 0.1% (w/v) bovine serum albumin before being aliquoted, frozen in liquid N2, and stored at −80 °C.

Preparation of GTP-binding Protein Subunits—The Gβγ subunits of GTP-binding proteins were further purified by means of sequential chromatography with CM-Sepharose, Sephacryl S-300/HR, blue Sepharose, and Mono Q columns. Fig. 1 shows the PI 3-kinase activity eluted from the Mono Q column. The kinase activity eluted with about 170 mM KCl (fractions 15–18) was markedly stimulated by Gβγ (closed circles), although the basal activity assayed

FIG. 3. Comparison between the Gβγ-sensitive (p110γ/p85γ) and p110α/p85δ PI 3-kinases of rat liver. The Gβγ-sensitive PI 3-kinase (p110γ/p85γ) in the Mono Q fraction and p110α/p85δ-rich fraction separated from the same rat liver were subjected to immunoprecipitation with polyclonal antibody against p110γ and p110α, respectively. After resolution of the immunoprecipitated proteins by SDS-PAGE (6% acrylamide), the samples were transferred and immunoblotted (IB) with anti-p110γ (left) or p110α (center), or p85Δγ (right) antibody. IR, immunoprecipitate. B, the samples immunoprecipitated with the anti-p110γ (left) or p110α (right) antibody were assayed for the PI 3-kinase activity as the substrate of PtdIns(4,5)P3. The assay was performed in the presence or absence of the phosphoryrosyl peptide (pY-pep., 100 μM), Gβγ (0.5 μM), and/or wortmannin (0.1 μM). Bars represent differences between duplicate determinations in a typical study.
without Gβγ was quite low (open circles). The Gβγ-sensitive PI 3-kinase fractions contained 110- and 85-kDa proteins, which were recognized with anti-p110β and p85 antibodies, respectively (Fig. 1, middle and bottom panels of the inset), and the degree of the Gβγ-stimulated activity was correlated with both protein amounts. There was, however, no protein band immunoreacted with an anti-p110α specific antibody in the Mono Q fractions (Fig. 1, top panel of the inset).

We next investigated whether a signal associated with protein tyrosine phosphorylation may also regulate the kinase activity. A tyrosine-phosphorylated peptide, NGDYMPMSPKS (Y* indicates phosphotyrosine), derived from the sequence of insulin receptor substrate-1 was used for the present kinase assay. In the chromatography on Mono Q column, the phosphotyrosyl peptide produced only a slight increase in the PI 3-kinase (data not shown, but see Figs. 2C and 3B below). However, the combination of Gβγ with the phosphotyrosyl peptide caused marked activation of the PI 3-kinase (Fig. 1, closed triangles).

Fig. 2 shows properties of the Mono Q PI 3-kinase, which was assayed with the various concentrations of Gβγ and the phosphotyrosyl peptide. Gβγ stimulated the kinase activity in a concentration-dependent manner, regardless of whether the phosphotyrosyl peptide was included in the assay mixture; the half-maximal activation was observed with about 0.5 μM Gβγ (Fig. 2A). To confirm that the free Gβγ subunits were responsible for the kinase activation, we examined the effect of GDP-bound Gαs-2 on the Gβγ-sensitive PI 3-kinase (Fig. 2B). As expected, the increasing concentrations of the α subunit induced the progressive inhibition of PI 3-kinase activities stimulated by Gβγ alone and Gβγ plus phosphotyrosyl peptide. Fig. 2C shows the effect of the various concentrations of the phosphotyrosyl peptide, together with a control tyrosine-nonphosphorylated peptide, NGDYMPSPKS. The phosphotyrosyl peptide further enhanced the Gβγ-sensitive PI 3-kinase activity, although the control peptide had no stimulatory effect on the kinase activities assayed in the presence and absence of Gβγ.

The active fractions of the Mono Q column (Fig. 1) were next treated with the specific antibody against the p110γ. The peptides in the immunoprecipitate were separated by SDS-PAGE and then analyzed by immunoblotting with the anti-p85 antibody. As expected, an 85-kDa peptide can be detected in the p110β immunoprecipitate. The PI 3-kinase activity in the immunoprecipitate was again found to be stimulated by Gβγ (Fig. 3B, left panel). The further addition of the phosphopeptide to the assay mixture increased the Gβγ-stimulated activity in the immunocomplex (Fig. 3B, left panel), as is the case in the Mono Q fraction (Fig. 1). The activity in the immunocomplex was completely inhibited by the presence of PI 3-kinase inhibitors, wortmannin (Fig. 3B) (25) or LY294002 (data not shown) (26). Thus, the Mono Q PI 3-kinase activated in a cooperative manner by Gβγ and the phosphotyrosyl peptide appeared to be a heterodimer consisting of p110β and p85 (or their related subunits). This notion is in agreement with the findings that the antibody against the N-terminal SH2 domain of p85 (N1SH2) recognized the 85-kDa polypeptide of the Mono Q fraction and that the Gβγ-sensitive PI 3-kinase migrated as an apparent molecular mass of 220,000 on a gel filtration column (data not shown).

For a comparison, another heterodimeric PI 3-kinase consisting of p110α and p85 was also separated from the same rat liver. The p110α subtype specificity in the separated fraction was confirmed by immunoblot analysis (Fig. 3A, middle and right panels). As shown in the left panel of Fig. 3A, there was no protein band cross-reacted with anti-p110β antibody in the p110α/p85 PI 3-kinase fraction. In contrast to the Mono Q fraction containing p110β/p85, Gβγ had no stimulatory effect on the PI 3-kinase activity of the p110α/p85 PI 3-kinase fraction, although its activity was certainly stimulated by the phosphotyrosyl peptide (Fig. 3B, right panel). As expected, the p110α/p85 PI 3-kinase activity was also inhibited by wortmannin. The phosphotyrosyl peptide alone increased the activity of both p110β/p85 and p110α/p85 only slightly, but this effect can be observed reproducibly and significantly.

To confirm that p110β/p85 PI 3-kinase certainly functions as the target isoform of the synergistic activation by Gβγ and the phosphotyrosyl peptide, we expressed Mhc-tagged p110β/p85α and p110α/p85α in COS-7 cells with their cDNAs. As shown in the left panel of Fig. 4, PI 3-kinase activity of the recombinant p110β/p85α was stimulated by both Gβγ and the phosphotyrosyl peptide and synergistically by the presence of the two activators. The activity of p110α/p85α was increased by the phosphotyrosyl peptide but was unaffected by Gβγ regardless of the presence or absence of the phosphotyrosyl peptide (Fig. 4, right panel). These results suggest that the Gβγ sensitivity of the heterodimer was carried by p110β. This notion is supported by a finding that a PI 3-kinase consisting of an undefined 46-kDa peptide and p110β is also stimulated by Gβγ (Ref. 17).

**DISCUSSION**

In the present study, we have separated the target PI 3-kinase of Gβγ-induced stimulation from rat liver cytosol. Immunological analysis (Figs. 1 and 3) suggested that the rat liver Gβγ-sensitive PI 3-kinase is a heterodimer consisting of p110β and p85 (or their related subunits). In fact, Gβγ could stimulate an epitope-tagged p110β/p85α PI 3-kinase, which was expressed in COS-7 cells with their cDNAs (Fig. 4, left panel). Both the purified and the recombinant p110β/p85 PI 3-kinases were stimulated not only by Gβγ but also by a synthetic phosphotyrosyl peptide (containing the YMXY motif) that binds to the SH2 domain of p85. A surprising feature of the p110β/p85 type is its synergistic activation in the presence of the two effectors. The actions of the two effectors are considered to be specific, since (i) Gβγ-induced activation could be inhibited by the GDP-bound form of Gαs-2 (Fig. 2B) and (ii) a nonphosphorylated peptide with the same sequence had no stimulatory effect (Fig. 2C).

Stephens et al. (19) showed that the PI 3-kinase activity distinct from the p110β/p85 heterodimer was activated by Gβγ but was unaffected by a phosphotyrosyl peptide. In agreement with their results, we have observed the presence in THP-1 cells of the PI 3-kinase activity that is regulated solely by Gβγ (18). Because the fractions having these activities are reported not to contain p85, the Gβγ-sensitive activities are considered to be different from the p110β/p85 described in this study. Thus the present paper indicates that there are multiple species of Gβγ-sensitive PI 3-kinase. The possibility that the previously reported enzyme is the p110α subunit freed from p85 is unlikely because the enzyme migrates as an apparent molecular mass of 200,000 on a gel filtration column (19). Furthermore, a very recent report by the same authors showed that the enzyme consisted of the p101 regulatory subunit and the γ-subtype of p110 (15).

Recent studies have revealed that a tyrosine kinase-associated PI 3-kinase plays an important role in cellular signaling mediated by pertussis toxin-sensitive G proteins (27–30). For example, an increase in PI 3-kinase activity was observed in immunoprecipitated fractions with anti-phosphotyrosine and anti-src tyrosine kinase antibodies upon stimulation of the G protein-coupled receptors (27, 28), and mitogen-

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activated protein kinase activation induced by Gβγ was attenuated by the introduction of a dominant negative p85 mutant (29). Furthermore, we previously reported in monocytic THP-1 cells that stimulation of N-formyl-Met-Leu-Phe receptors, which activate pertussis toxin-sensitive G proteins, potentiated the insulin-induced and thus tyrosine kinase-mediated accumulation of PtdIns(3,4,5)P_3 (18). Such synergistic activation has been observed also in Chinese hamster ovary cells expressing both insulin and formyl-Met-Leu-Phe receptors. These results suggested that p110β/p85 PI 3-kinase is regulated in the downstream of a pertussis toxin-sensitive GTP-binding protein-coupled receptor. Thus, the synergistic activation of PI 3-kinase indicated in the present in vitro study may be expected to function in the intact cell systems.

REFERENCES

1. Kapeller, R., and Cantley, L. C. (1994) BioEssays 16, 565–576
2. Fry, M. J. (1994) Biochem. Biophys. Acta 1226, 237–268
3. Serunian, L. A., Haber, M. T., Fukui, T., Kim, J. W., Rhee, S. G., Lowenstein, J. M., and Cantley, L. C. (1989) J. Biol. Chem. 264, 17869–17815
4. Toker, A., Meyer, M., Reddy, K. K., Falck, J. R., Aneja, R., Aneja, S., Parra, A., Burns, D. J., Ballas, L. M., and Cantley, L. C. (1994) J. Biol. Chem. 269, 32358–32367
5. Franke, T. F., Kaplan, D. R., Cantley, L. C., and Toker, A. (1997) Science 275, 665–668
6. Hammond-Odie, L. P., Jackson, T. R., Prifit, A. A., Blader, I. J., Turck, C. W., Prestwich, G. D., and Theibert, A. B. (1996) J. Biol. Chem. 271, 18859–18863
7. Klarlund, J. K., Guiherme, A., Holik, J. J., Virbasius, J. V., Chawla, A., and Czech, M. P. (1997) Science 275, 1927–1930
8. Takana, K., Imai-Shimizu, S., Sasada, T., Shirai, R., Hashimoto, Y., Iwasaki, S., Kajibuchi, K., Kanai, Y., Shirai, T., Terada, Y., Kimura, K., Nagata, S., and Fukui, Y. (1997) Eur. J. Biochem. 245, 512–519
9. Backer, J. M., Myers, M. G., Jr., Shoelson, S. E., Chin, D. J., Sun, X.-J., Miralpeix, M., Hu, P., Margolis, B., Skolnik, E. Y., Schlessinger, J., and White, M. F. (1992) EMBO J. 11, 3469–3479
10. Rordorf, N. T., Van, H. D., Chen, D., White, M. F., and Backer, J. M. (1995) J. Biol. Chem. 270, 3662–3666
11. Hiles, I. D., Otsu, M., Volinia, S., Fry, M. J., Gout, I., Dhand, B., Panayotou, G., Ruiz-Larrea, F., Thompson, A. Totty, N. F., Hsuam, J. J., Courteinige, S. A., Parker, P. J., and Waterfield, M. D. (1992) Cell 70, 419–429
12. Hu, P., Mondino, A., Skolnik, E. Y., and Schlessinger, J. (1993) Mol. Cell. Biol. 13, 7677–7688
13. Vanhaesebroeck, B., Welham, M. J., Kotani, K., Stein, R., Warne, P. H., Zreileh, M. J., Higashi, K., Volinia, S., Downward, J., and Waterfield, M. D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4330–4335
14. Stoyanov, B., Volinia, S., Hasek, T., Rubio, I., Leubtchenkov, M., Malek, D., Stoyanova, S., Vanhaesebroeck, B., Dhand, R., Nurnberg, B., Gierschik, P., Seedorf, K., Hsuam, J. J., Waterfield, M. D., and Wetzker, R. (1995) Science 269, 690–693
15. Stephens, L., Eguinoa, A., Erdjumen-Bromage, H., Lui, M., Cooke, F., Coadwell, J., Smrcka, A., Thelen, M., Cadwallader, J., Tempst, P., and Hawkins, P. T. (1997) Cell 89, 105–114
16. Thompson, P. A., James, S. S., Casey, P. J., and Downes, C. P. (1994) J. Biol. Chem. 269, 16525–16528
17. Kurosaka, H., Hazeki, O., Kukimoto, I., Honzawa, S., Shibasaki, M., Nakada, N., Uji, M., and Katada, T. (1995) Biochem. Biophys. Res. Commun. 216, 655–661
18. Okada, T., Hazeki, O., Uji, M., and Katada, T. (1996) Biochem. J. 317, 475–480
19. Stephens, L., Smrcka, A., Cooke, F. T., Jackson, T. R., Sternweis, P. C., and Hawkins, P. T. (1994) Cell 77, 83–93
20. Zhang, J., Zhang, J., Benovic, J. L., Sugai, M., Wetzker, R., Gout, I., and Rittenhouse, S. E. (1995) J. Biol. Chem. 270, 6589–6594
21. Takana, S., Matsuda, M., Nagata, S., Kurata, T., Nagoshi, K., Shizawa, Y., and Fukui, Y. (1993) Jpn. J. Cancer Res. 84, 279–289
22. Carpenter, C. L., Duckworth, B. C., Auger, K. R., Cohen, B., Schaffhausen, B. S., and Cantley, L. C. (1998) J. Biol. Chem. 273, 19704–19711
23. Kontani, K., Takahashi, K., Inanobe, A., Uji, M., and Katada, T. (1992) Arch. Biochem. Biophys. 294, 527–533
24. Nishina, H., Nimota, K., Kukimoto, I., Mabehama, T., Takahashi, K., Hoshino, S., Kato, Y., and Katada, T. (1995) J. Biol. Chem. 270, 7677–7688
25. Uji, M., Okada, T., Hazeki, K., and Hazeki, O. (1995) Trends Biochem. Sci. 20, 303–307
26. Vlahos, C. J., Matter, W. F., Hui, K. Y., and Brown, R. F. (1994) J. Biol. Chem. 269, 5241–5248
27. Stephens, L., Eguinoa, A., Corey, S., Jackson, T., and Hawkins, P. T. (1993) EMBO J. 12, 2265–2273
28. Ptasznik, A., Traynor-Kaplan, A., and Bokoch, G. M. (1995) J. Biol. Chem. 270, 19969–19973
29. Hawes, B. E., Luttrell, L. M., van Biesen, T., and Lefkowitz, R. J. (1986) J. Biol. Chem. 271, 12133–12136
30. Lopez-Ilasaca, M., Crespo, P., Pellini, P. G., Gutkind, J. S., and Wetzker, R. (1997) Science 275, 384–397

3T. Suzuki, O. Hazeki, and T. Katada, manuscript in preparation.