An Epidermal Growth Factor Receptor/Gab1 Signaling Pathway Is Required for Activation of Phosphoinositide 3-Kinase by Lysophosphatidic Acid*

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Phosphoinositide 3-kinase (PI3K) has been shown to play an essential role in G protein-induced signaling even in non-myeloid cells where few agonists of G protein-coupled receptors are known to activate PI3K. We have identified adherent cell lines where lysophosphatidic acid (LPA) strongly and rapidly activates the accumulation of PI3K lipid products. The process is not modified by expression of a kinase-dead mutant of the Gβγ-responsive PI3K p110γ. In contrast, it is inhibited by genistein or expression of a dominant negative mutant of p85 and potentiated by overexpressing wild-type p110α or -β but not -γ. By using a specific chemical inhibitor of the epidermal growth factor receptor (EGFR) and expression of a dominant negative mutant, we have observed that recruitment of p85/p110 PI3Ks occurs through transactivation of the EGFR by LPA and downstream mobilization of the docking protein Gab1 that associates with p85 upon LPA stimulation. Finally, we show that LPA cannot activate PI3K in cell lines lacking the EGFR/Gab1 pathway, including cells that transactivate the PDGF receptor. Altogether, these results demonstrate that activation of PI3K by LPA is conditioned by the ability of LPA to transactivate an EGFR/Gab1 signaling pathway.

One of the major discovery of the 1990s in proliferative signaling has been the emergence of the Ras/mitogen-activated protein kinase (MAPK) cascade as the main pathway used by growth factors for non-hematopoietic cells. However, the early mechanisms of activation of this pathway by agonists of G protein-coupled receptors (GPRC) remain elusive (1). Intensive researches have recently focused on the identification of proteins that could make the link between G proteins and the Ras/MAPK cascade, leading to the identification of various protein tyrosine kinases involved in this process, such as Pyk2 (2), Src (3), receptor tyrosine kinases (RTKs) (4, 5), and even Syk in myeloid-derived cells (6). However, the mechanisms of activation of these kinases by GPCR have not been elucidated, except for Pyk2 that is recruited by a Gαi2/Ca2+-dependent pathway (2). On the other hand, phosphoinositide 3-kinase (PI3K) has been recently shown to play a major role in the early mechanisms of GPCR-mediated activation of the Ras/MAPK pathway (7–11). However, the fact that PI3K inhibitors interfere with GPCR-induced signaling even in non-hematopoietic cell lines is difficult to interpret since GPCRs agonists are not known to induce the synthesis of PI3K lipid products in these cells. In contrast, neutrophils for example produce large amounts of phosphatidylinositol 3,4-bisphosphate (PI3,4P2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3) upon stimulation with GPCR agonists (12). Similarly, stimulation of cells by RTK agonists such as platelet-derived growth factor (PDGF) generate PI3K lipid products through well known pathways, and PI3K inhibitors interfere with RTK-mediated activation of the Ras/MAPK pathway, although the mechanisms have to be clarified (13). Thus, one important question still remains concerning the regulation of PI3K in GPCR-induced signaling in non-myeloid cells.

Recently, two groups have identified a novel isoform of PI3K, p110γ, which contains a pleckstrin homology domain in its N terminus region. Interestingly, p110γ can be directly activated by G protein subunits (14, 15), due both to a constitutive association with a p101 βγ-sensitive protein (15) and to a direct interaction with the βγ complex (16). Although this isoform of PI3K plays a role in the activation of the Ras/MAPK pathway by G proteins (17–19), it is not clear yet whether this enzyme is involved in any GPCR-induced PI3K production in non-hematopoietic cell lines (15, 17, 18). On the other hand, p110β was recently found to play a role in GPCR-induced signaling and mitogenesis (20, 21).

Therefore, this led us to study the activation of PI3K by GPCR in adherent cell lines. We have measured PI3K lipid products upon stimulation with lysophosphaticid acid (LPA), and we have identified cell lines producing large amounts of PI3,4P2 and PIP3 upon treatment with a GPCR agonist. The mechanism does not seem to involve the p110γ isoform of PI3K but recruits the p85/p110 isoforms through LPA-induced mobilization of the EGFR receptor (EGFR) and subsequent engagement of the docking protein Gab1. Finally, we show that LPA cannot activate PI3K in various cell types lacking the EGFR/Gab1 pathway, thereby demonstrating the pivotal role of this transactivation pathway for PI3K activation by LPA in non-myeloid-derived cell lines.
EXPERIMENTAL PROCEDURES

Materials—Rabbit anti-active Erk1/2 antibody was from Promega (catalog number V6671). Rabbit anti-phospho-specific Akt (Ser-473) antibody was from New England Biolabs (catalog number 9271S). Sheep anti-human EGFR (catalog number 06-129), rabbit anti-rat p85 (catalog number 06-195), rabbit anti-human Gab1 (catalog number 06-579), and monoclonal 4G10 anti-phosphotyrosine (catalog number 05-321) antibodies were purchased from Upstate Biotechnology, Inc. Rabbit anti-human βPDGF receptor antibody was from Pharmingen (catalog number 15746E). Rabbit anti-human ErbB3 antibody was from Santa Cruz Biotechnology (catalog number sc-255-G). Monoclonal 12CA5 anti-HA and anti-Myc antibodies were from Roche Molecular Biochemicals and Invitrogen, respectively. Rabbit anti-GST antibody was from Sigma, New England Biolabs, and Rockland, respectively. Rabbit anti-human ErbB3 antibody was from Santa Cruz Biotechnology. Rabbit anti-human ErbB4 antibody was from Santa Cruz Biotechnology. AG1478 were from Biomol. Tyrphostin AG1296 was from Calbiochem. Tyrphostin AG1478 were from Biomol. Tyrphostin AG1296 was from Calbiochem.

Cell Culture and Transfection—Cos, Vero, Rat1, and IMR90 cells were maintained in DMEM supplemented with 10% fetal bovine serum and antibiotics. For B82 L cells, 10% dialyzed newborn calf serum was used. For transfection, cells were incubated 4 h with 10 µl of LipofectAMINE (Life Technologies, Inc.) and 2 µg of plasmid DNA per ml of Opti-MEM (Life Technologies, Inc.). The transfection mixture was then replaced by DMEM supplemented with 10% serum for 24 h. Before stimulation, cells were serum-starved for 24 h.

Plasmid Constructs—Full-length wt-p110γ was subcloned into the Nhe1/Not1 sites of pCDNA3.1/Myc-His (Invitrogen) using polymerase chain reaction with appropriate primers, Pfu DNA polymerase (Stratagene), and PBS-p110-wt as a template (14). The construct was verified by sequencing. The plasmid for bacterial expression of GST-p85wt and other mammalian expression constructs has already been described: pGEX-p85wt (22), SRα-Δp85 (23), pSG5-p110wt (24), pSRK5-p110βwt (25), pCMV-p110-κδ322R (17). The EGFRe688 mutant (26) was subcloned into the pCDNA3.1/Myc-His (Invitrogen) using polymerase chain reaction with appropriate primers, Pfu DNA polymerase (Stratagene), and PBS-p110-wt as a template (14). The construct was verified by sequencing. The plasmid for bacterial expression of GST-p85wt and other mammalian expression constructs has already been described: pGEX-p85wt (22), SRα-Δp85 (23), pSG5-p110wt (24), pSRK5-p110βwt (25), pCMV-p110-κδ322R (17).

Analysis of PI Polyphosphate—Cells grown in 10-cm plates were then serum-starved for 24 h upon reaching 80–90% confluence and then labeled for 5 h with 0.2 mCi of [32P]H3PO₄ (Amersham Pharmacia Biotech) per ml in phosphate-free DMEM. Cells were then stimulated for the indicated time and washed once with ice-cold phosphate-buffered saline before addition of 3.75 ml of 2.4 h HCl solution. Then lipid extraction was performed as described previously (27). Briefly, lipids were solubilized by addition of 3 ml of chloroform and 4.5 ml of methanol followed by vortexing. After centrifugation, the lower phase containing the lipids was collected, and the upper phase was washed with 4.5 ml of chloroform. The lower phases were then combined and evaporated under nitrogen, and lipid extracts were solubilized in 250 µl of chloroform/methanol (1/1, v/v) and finally resolved by thin layer chromatography (TLC) using chloroform/methanol/acetic acid/water (80/30/26/4, v/v). The spots corresponding to PI4,5P2 and PI3,4P2 were identified using a 254-nm UV lamp and were scraped off, deacylated by 20% methanolic acid, and analyzed by HPLC on a Whatman Partisil 5 SAX column. For measurements of PI polyphosphate after transfection of Cos cells with dominant negative mutants, the results represent the mean ± S.E. of three independent experiments. For each experiment, the inhibitory effect of the mutant has been normalized for the percentage of transfected cells. This was determined concurrently using β-galactosidase as reporter and following a standard procedure. The efficiency of transfection was routinely around 40%.

Immunoblotting, Immunoprecipitation, and GST Pull-down Experiments—Stimulations were carried out at 37 °C in serum-free DMEM containing 20 mM Heps. Cells were washed once with ice-cold phosphate-buffered saline before lysis. For immunoblotting of crude lysates, cells were scraped off in SDS-PAGE sample buffer and boiled 5 min, then resolved by SDS-PAGE, and analyzed by immunoblotting using an enzyme-linked antibody (ECL, Amersham Pharmacia Biotech). Results obtained with anti-phospho-Akt, and Erk antibodies were quantified by densitometry. We have verified that results obtained with the anti-phospho-Erk antibody did not differ from in vitro kinase assays of immunoprecipitated HA-Erk1 (construct kindly provided by Dr. J. Pouyssegur) using myelin basic protein as a substrate. For immunoprecipitations, cells were scraped off in lysis buffer containing 150 mM NaCl, 20 mM Tris-HCl, pH 7.4, 1% Brij (Sigma catalog number P9641), 1 mM Na3VO4, and 10 µg/ml aprotinin and leupeptin. After gentle shaking during 20 min at 4 °C and centrifugation (13,000 rpm for 10 min), the supernatants were incubated 1 h with antibodies followed by addition of 10% (w/v) protein A-Sepharose CL4B (Sigma) for 1 h. The immunocomplexes were washed twice with 1 ml of lysis buffer containing 0.1% Brij, 100 µM Na3VO4, and 1 µg/ml aprotinin and leupeptin and finally boiled in SDS-PAGE sample buffer. For GST pull-down experiments, cells were processed similarly to the immunoprecipitation protocol, except that cells were incubated 1 h with 2 µg of GST fusion protein immobilized on glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech).

RESULTS

LPA Rapidly Activates PI3 Kinase Independently of p110γ but Recruits p85/p110 Isoforms—To gain insight into the regulation of PI3K by LPA, we have measured the amount of PI3K lipid products in serum-starved cells stimulated with LPA. 1 µM LPA induced an important accumulation of PI3,4P2 and PI3,5P2 in both Vero and Cos cells, at a level up to 10-fold higher than control, with a maximum after 2 min stimulation (Fig. 1, A and B). Dose-response assays indicated that the effect was detectable with as low as 0.1 µM LPA and increased up to 10 µM LPA (Fig. 1C). In contrast, another PI3K protein, phosphatidylinositol 3-monophosphate, was found in relative abundance in resting cells and poorly accumulated upon LPA stimulation (counts in resting, 5600 ± 1980; stimulated 2 min, 8850 ± 1280). To elucidate the mechanism of PI3K activation by LPA, we have first studied p110γ, which can directly activated by G protein subunits. The accumulation of PI3,4P2 and PI3,5P2 upon LPA was measured in Cos cells transiently transfected with a kinase-dead mutant of p110γ (K832R) that inhibits the Gβγ-induced activation of the Ras/MAPK pathway (Fig. 2B). Expression of this mutant somewhat reduced the number of

FIG. 1. LPA induces the accumulation of PI3K lipid products in Vero and Cos cells. A, serum-starved Vero cells were labeled 5 h with [32P]H3PO4 and then stimulated with 1 µM LPA. After extraction of lipids and purification on TLC, the PI4,5P2 region was deacylated and analyzed by HPLC. Left graph, measurements at 0 and 2 min expressed in crude counts from the radioactivity detector. Right graph, time course measurements expressed in percent of the maximum value. B, same as A with Cos cells. C, dose-response of PI3K activation by LPA in Cos cells.
 Activation of PI 3-Kinase by LPA

A. cells were transiently transfected with a kinase-dead mutant of p110γ (K532R) or vector as a control. After serum starvation, PI3K lipid products were measured as described in Fig. 1 following a 2-min stimulation with 10 μM LPA. The results represent the percentage of inhibition of PI polyphosphate production in transfected cells normalized for the percentage of transfection, as described under “Experimental Procedures.” B, the effect of the K532R mutant on G8γ-induced phosphorylation of hemagglutinin (HA)-tagged Erk1 was measured in Cos cells transfected with the indicated constructs (V, empty vector). C, Cos cells were labeled with [32P]H3PO4, incubated or not (control) with 100 μM genistein for 15 min, and then treated for 2 min with 10 μM LPA before analysis of PI3K lipid products. D, Cos cells were transiently transfected with a dominant negative mutant of p85 (Δp85) or vector as a control. After serum starvation, cells were stimulated 2 min with 10 μM LPA and analyzed for their content in PI3K lipid products. E, Cos cells were transfected with constructs expressing wild-type p110α, -β, and -γ or vector as indicated. The cellular contents of PI4,5P2 and PI3,4P2 was determined following a 2-min stimulation with 1 μM LPA. For each transfection, the level of PI4,5P2 and PI3,4P2 in unstimulated cells has been subtracted. Inset, immunoblots of cells transfected with empty vector (-) or the various p110 (+) using their respective anti-tag antibodies.

FIG. 2. Activation of PI3K by LPA is independent of p110γ but involves tyrosine phosphorylations and p110p85 isoforms. A, Cos cells were transiently transfected with a kinase-dead mutant of p110γ (K532R) or vector as a control. After serum starvation, PI3K lipid products were measured as described in Fig. 1 following a 2-min stimulation with 10 μM LPA. The results represent the percentage of inhibition of PI polyphosphate production in transfected cells normalized for the percentage of transfection, as described under “Experimental Procedures.” B, the effect of the K532R mutant on G8γ-induced phosphorylation of hemagglutinin (HA)-tagged Erk1 was measured in Cos cells transfected with the indicated constructs (V, empty vector). C, Cos cells were labeled with [32P]H3PO4, incubated or not (control) with 100 μM genistein for 15 min, and then treated for 2 min with 10 μM LPA before analysis of PI3K lipid products. D, Cos cells were transiently transfected with a dominant negative mutant of p85 (Δp85) or vector as a control. After serum starvation, cells were stimulated 2 min with 10 μM LPA and analyzed for their content in PI3K lipid products. E, Cos cells were transfected with constructs expressing wild-type p110α, -β, and -γ or vector as indicated. The cellular contents of PI4,5P2 and PI3,4P2 was determined following a 2-min stimulation with 1 μM LPA. For each transfection, the level of PI4,5P2 and PI3,4P2 in unstimulated cells has been subtracted. Inset, immunoblots of cells transfected with empty vector (-) or the various p110 (+) using their respective anti-tag antibodies.

activation of transfection, we have observed that expression of Δp85 nearly abolished the accumulation of PI3,4P2 and PIP3 induced by 10 μM LPA (Fig. 2D). In addition, we have determined whether overexpression of wild-type p110α, -β, or -γ had any potentiating effect on PI3K activation triggered by a submaximal dose of LPA (1 μM). Analysis of PI3K stimulation limited for convenience to measurements of PI3,4P2 showed that p110α and p110β to a greater extent potentiated PI3K activation by LPA, whereas overexpression of p110γ had no effect (Fig. 2E). Altogether, these results demonstrate that the activation of PI3K by LPA is mediated by the tyrosine kinase-dependent p85/p110 isoforms of PI3K.

Activation of PI3K by LPA Occurs through Recruitment of the EGFR Receptor—To identify the tyrosine kinase(s) involved in this process, we have searched for major tyrosine-phosphorylated proteins in crude lysates from cells treated 2 min with LPA. The major phosphotyrosine signal induced by LPA in Vero or Cos cells was found in the 180-kDa region (Fig. 3A), suggesting an involvement of the two major RTKs expressed in
fibroblasts, i.e. the receptors for PDGF and EGF. First, we have determined that the PDGFR-specific inhibitor tyrphostin AG1296 had no effect on LPA-induced tyrosine phosphorylation of the 180-kDa protein, whereas the EGFR inhibitor AG1478 nearly abolished the signal in both Vero and Cos cells (Fig. 3A). In addition, anti-EGFR immunoblotting of cell lysates confirmed that the 180-kDa protein colocalized with EGFR, whereas an antibody against βPDGFR gave no signal in Vero cells and a very faint band in Cos cells (Fig. 3B). Finally, following immunoprecipitation, the EGFR was found to be tyrosine-phosphorylated after treatment of Cos cells with LPA (Fig. 3C). Thus, these data demonstrate that phosphorylation of the EGFR is the major early tyrosine phosphorylation event induced by LPA in Cos and Vero cells.

We have then determined whether this event was important for PI3K recruitment by LPA. In both Cos and Vero cells, the accumulation of PI3,4P2 and PIP3 induced by LPA was dramatically inhibited by the EGFR inhibitor AG1478 (Fig. 3D). This compound also abolished the activation of Akt by LPA but did not interfere with Akt stimulation by insulin in Vero cells (not shown). In addition, AG1478 did not alter PI3K activity itself in Rat1 cells stimulated with PDGF (counts of PI3,4P2, control <200; PDGF 9255 ± 475; PDGF +AG1478 100 nm, 8728 ± 836). Furthermore, Cos cells were transfected with an EGFR mutant truncated at amino acid 688 (EGFRec688) which has a strong ability to dimerize upon activation. This mutant inhibited by over 70% the LPA-induced accumulation of PI3,4P2 and PIP3 after normalizing the results for the percentage of transfection (Fig. 3E). Although these results demonstrate that the EGFR plays a crucial role in LPA-induced PI3K activation, EGFR is not a typical agonist of PI3K, and PIP3 levels are insensitive to EGF in various cell types. Nevertheless, we have observed that 10 ng/ml EGF induced an important accumulation of PI3,4P2 and PIP3 in Cos cells (Fig. 3F). In addition, the increase in PI3K lipid products occurred earlier upon EGF stimulation (Fig. 3F) than in the presence of LPA (Fig. 1B), which is compatible with a recruitment of PI3K by LPA occurring downstream the EGFR. Finally, expression of Δ85 in Cos cells suppressed the synthesis of PI3K lipid products induced by EGF to an extent similar to that upon LPA stimulation (Figs. 3G and 2D). This suggested that both LPA and EGF use a same p85-dependent pathway to stimulate PI3,4P2 and PIP3 production.

The EGFR-dependent Activation of PI3K by LPA Mobilizes Gab1—Since the activation of PI3K by EGF seems to differ from one cell type to another, we have studied various pathways possibly involved in the EGFR-dependent activation of PI3K by LPA. Using pull-down experiments with GST-p85 fusion protein, the EGFR and p85 were found to coprecipitate upon stimulation with LPA (Fig. 4A), thereby corroborating that p85 is recruited by LPA through an EGFR-dependent pathway. Although one of the major mechanisms of EGFR-mediated recruitment of p85 is the heterodimerization of the EGFR with ErbB3 (28), we did not find any LPA- or EGF-induced association of ErbB3 in p85 immunoprecipitates or GST-p85 pull-downs (not shown). To identify other candidates possibly involved in recruitment of p85, we have looked for tyrosine-phosphorylated proteins in GST-p85 pull-downs and p85 immunoprecipitates from LPA- or stimulated cells. The major phosphotyrosine signal appearing upon stimulation was located close to the 115-kDa marker (Fig. 4B). This molecular mass led us to consider the adaptor protein Gab1 as a candidate. By performing both p85 immunoprecipitates and GST-p85 pull-downs, Gab1 was found to associate with p85 following cell stimulation with LPA or EGF (Fig. 4B). To confirm this observation, we have performed anti-Gab1 immunoprecipitates, and p85 was found to coprecipitate with Gab1 upon stimulation with LPA or EGF (Fig. 4C). Finally, the EGFR inhibitor AG1478 was found to abolish the association of Gab1 with p85 (Fig. 4D). Altogether, these results demonstrate that activation of PI3K by LPA occurs through an EGFR/Gab1 pathway.

The EGFR/Gab1 Pathway Is Essential to PI3K Activation by LPA—To determine whether LPA could activate PI3K using other mechanisms, we have first studied IMR90 human fibroblasts where activation of MAPK by LPA is independent of RTK activities (Fig. 5A). Although LPA and PDGF stimulated Erk to similar levels in these cells, LPA produced only a minor accumulation of PI3K lipid products, whereas they readily accumulated upon treatment with PDGF (Fig. 5A), suggesting that specific RTK transactivation is required for activation of PI3K by LPA. To determine if RTKs other than the EGFR could participate in the process, we have studied mouse B2 L fibroblasts that do not express the EGFR and where transactivation of specific RTKs is required for activation of PI3K by LPA (Fig. 5B) (29). In these cells, LPA produced only a faint accumulation of PI3K lipid products that readily accumulated upon PDGF,
whereas both growth factors activated Erk to comparable levels (Fig. 5B).

Finally, in Rat1 cells where transactivation of the EGFR is required for Erk activation by LPA similarly to Vero cells (4, 29), we have observed that PI3,4P2 and PIP3 were hardly detectable in LPA-treated cells (Fig. 6A). As a control, PDGF induced a massive accumulation of PI3K lipid products, and both LPA and PDGF activated Erk to comparable levels. To gain insight about the missing mechanism in Rat1 cells, we have also measured PI3K activation upon stimulation with EGF. Interestingly, levels of PI3,4P2 and PIP3 were not modified by EGF, although Erk activation by EGF was comparable to the PDGF response (Fig. 6A). This suggested that the EGFR-dependent pathway of PI3K activation present in Vero and Cos cells was deficient in Rat1 cells. By using GST-p85 pull-downs assays, we have observed that Gab1 did not associate with p85 in Rat1 cells stimulated with LPA or EGF (Fig. 6B). Therefore, we have compared the recruitment of Gab1 in Cos and Rat1 cells. By immunoblotting lysates of Cos cells stimulated with EGF, Gab1 was found to undergo a shift in its apparent molecular weight that is typical of this adaptor (30) (Fig. 6C, top). In contrast, Gab1 migration was hardly modified in Rat1 cells stimulated with EGF. Similarly, following immunoprecipitation, we have found that Gab1 was not tyrosine-phosphorylated, and its migration was unchanged in LPA- or EGF-treated Rat1 cells (Fig. 6C, bottom).

**DISCUSSION**

The recent discovery of p110γ that can be directly activated by G protein subunits (14) led us first to evaluate the role of this enzyme in LPA-mediated activation of PI3K. However, our results demonstrate that the synthesis of PI3K lipid products induced by LPA occurs independently of p110γ, based on the lack of inhibitory effect of a kinase-dead mutant and supported by the non-potentiating effect of the overexpressed wild-type enzyme. This observation was rather surprising in light of recent well documented reports showing that p110γ is involved in Gβγ-induced signaling, such as activation of the Ras/MAPK pathway (17, 31). However, we have investigated the natural signaling resources of cells stimulated by endogenous LPA receptor(s), whereas the studies by Lopez-Ilasaca and co-workers (17, 31) were based mainly on transient expression of Gβγ subunits and cotransfection of effectors. Substantial differences might exist between these two complementary models in terms of recruitment of effectors, such as G protein subunits and cotransfection of effectors. Substantial differences might exist between these two complementary models in terms of recruitment of effectors, such as G protein subunits for example. In addition, although p110γ is not apparently involved in generation of PI3K lipid products, a recent report demonstrated that the protein kinase activity of p110γ is essential for MAPK activation (18), in agreement with our observation that a catalytically dead mutant of p110γ moderately inhibited cell growth without influencing PIP3 levels. However, it is also important to consider that the expression of p110γ in fibroblasts is marginal in comparison to blood platelets,3 a cell

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3 M. Laffargue, P. Raynal, A. Yart, C. Peres, R. Wetzker, S. Roche, B. Payrastre, and H. Chap, unpublished data.
type where activation of PI3K by the thrombin GPCR has been reported to engage p110β (32, 33). Similarly, in neutrophils where p110β is readily expressed (15), Wortmann and colleagues have shown to inhibit GPCR-induced signaling independently of p85 (34). Therefore, the facts that p110β seems preferentially expressed in myeloid-derived cells and the inability of most adrenocorticotropic hormones to activate PI3K αβγ given that PI3K interacts directly by the receptor.

In contrast, a scavenger of reactive oxygen species partly inhibited PI3K activation by LPA, due to an inhibitory effect on tyrosine phosphorylation of the EGFR, as described recently in HeLa cells (43). This suggests that in unstimulated cells, protein tyrosine phosphatases involved in maintaining RTK in an inactivated state could play a role in the mechanism of transactivation.

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REFERENCES

1. Gutkind, J. S. (1998) J. Biol. Chem. 273, 1839–1842
2. Lev, S., Moreno, H., Martinez, R., Canell, P., Pelis, E., Muserchio, J. M., Plowman, G. D., Rudy, B., and Schlessinger, J. (1995) Nature 376, 737–745
3. Luttrell, L. M., Hawes, B. E., van Biesen, T., Luttrell, D. K., Lansing, T. J., and Lefkowitz, R. J. (1996) J. Biol. Chem. 271, 19443–19450
4. Duan, H., Weiss, P. U., Wallach, C., and Ulrich, A. (1996) Nature 379, 557–560
5. Luttrell, L. M., Delioro, G. J., van Biesen, T., Luttrell, D. K., and Lefkowitz, R. J. (1997) J. Biol. Chem. 272, 4637–4644
6. Han, Y., Kurosaki, T., and Huang, X. Y. (1996) Nature 380, 541–544
7. Ferby, I. M., Waga, I., Sakacana, K., Kume, K., and Shimizu, T. (1994) J. Biol. Chem. 269, 30465–30468
8. Sakacana, C., Ferby, I., Waga, I., Bito, H., and Shimizu, T. (1994) Biochem. Biophys. Res. Commun. 205, 18–23
9. Hawes, B. E., Luttrell, L. M., Vanbiesbroeck, T., and Lefkowitz, R. J. (1996) J. Biol. Chem. 271, 12133–12136
10. Kranenburg, O., Verlaan, I., Nordijk, P. L., and Moelenaar, W. W. (1997) EMBO J. 16, 3097–3105
11. Hu, Z.-W., Shi, K.-Y., Lin, R. Z., and Hoffman, B. B. (1996) J. Biol. Chem. 271, 8977–8982
12. Stephens, L. R., Jackson, T. B., and Hawkins, P. T. (1993) Biochem. Biophys. Acta 1179, 27–75
13. Duckworth, B. C., and Cantley, L. C. (1997) J. Biol. Chem. 272, 27663–27670
14. Stoyanov, B., Volinia, S., Hanck, T., Rubio, I., Loubchouenkov, M., Malek, D., Stoyanov, S., Vanhaesebroeck, B., Dhand, R., Nurnberg, B., Gierschik, P., Seedorf, K., Hauan, J. J., Waterfield, M. D., and Wetzker, R. (1995) Science 269, 690–693
15. Stephens, L. R., Gueinoua, A., Erdjument-Bromage, H., Lui, M., Cooke, F., Goodwell, J., Smirna, A. S., Thelen, M., Cadwallader, K., Tempst, P., and Hawkins, P. T. (1997) Cell 89, 105–114
16. Leopold, D., Hanck, T., Exner, T., Maier, U., Wetzker, R., and Nurnberg, B. (1998) J. Biol. Chem. 273, 7024–7029
17. Lopez-Haseara, M., Crespo, P., Pellici, P. G., Gutkind, J. S., and Wetzker, R. (1997) Science 275, 394–397
18. Bondova, T., Pirla, L., Bulgarrele-Leva, G., Rubio, I., Wetzker, R., and Wymann, M. P. (1998) Science 282, 293–296
19. Takeda, H., Matozaki, T., Takada, T., Noguchi, T., Yamao, T., Tsuda, M., Ochi, F., Fukunaga, K., Inagaki, K., and Kasuga, M. (1999) EMBO J. 18, 386–395
20. Kurosu, H., Maehama, T., Okada, T., Yamamoto, T., Hoshino, S., Fukui, Y., Ui, M., Hazeki, O., and Katada, T. (1997) J. Biol. Chem. 272, 24252–24256
21. Roche, S., Downward, J., Raynal, P., and Courtneidge, S. (1998) Mol. Cell. Biol. 18, 7119–7129
22. Kotani, K., Yonezawa, K., Ueda, H., Kitamura, Y., Sakaue, H., Ando, A., Chavanieu, A., Calas, B., Grigorescu, F., Nishiyama, M., Waterfield, M. D., and Kasuga, M. (1994) EMBO J. 13, 2313–2321
23. Hara, K., Yonezawa, K., Sakaue, H., Ando, A., Kotani, K., Kitamura, T., Kitamura, Y., Ueda, H., Stephens, L., Jackson, T. R., Hawkins, P. T., Dhand, R., Clark, A. E., Holman, G. D., Waterfield, M. D., and Kasuga, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7415–7419
24. Rodriguez-Viciana, P., Warne, P. H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M. J., Waterfield, M. D., and Downward, J. (1994) Nature 370, 527–532
25. Hu, P., Mondino, A., Skolnik, E. Y., and Schlessinger, J. (1993) Mol. Cell. Biol. 13, 7677–7688
26. Chen, W. S., Lazar, C. S., Lund, K. A., Welsh, J. B., Chang, C. P., Walton, G. M., Der, C. J., Wiley, H. S., Gill, G. N., and Rosenfeld, M. G. (1989) Cell 59, 33–43
27. Ireton, K., Payrastre, B., Chap, H., Ogawa, W., Sakaue, H., Kasuga, M., and Cossart, P. (1996) Science 274, 780–782
28. Soltoff, S. P., Carraway, K. L. R., Prigent, S. A., Gullick, W. G., and Cantley, L. C. (1994) Mol. Cell. Biol. 14, 3550–3558
29. Herrlich, A., Daub, H., Knebel, A., Herrlich, P., Ulrich, A., Schultz, G., and Gudermann, T. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8985–8990
30. Holgado-Madruga, M., Emlet, D. R., Moscatello, D. K., Godwin, A. K., and Wong, A. J. (1996) Nature 379, 560–564
31. Lopez-Iglesias, M., Gutkind, J. S., and Wetzker, R. (1998) J. Biol. Chem. 273, 2505–2508
32. Zhang, J., Benovic, J. L., Sugai, M., Wetzker, R., Gout, I., and Rittenhouse, S. E. (1995) J. Biol. Chem. 270, 6584–6594
33. Tang, X. W., and Downes, C. P. (1997) J. Biol. Chem. 272, 14193–14199
34. Ferby, I. M., Waga, I., Hoshino, M., Kume, K., and Shimizu, T. (1996) J. Biol. Chem. 271, 11684–11688
35. Daub, H., Wallasch, C., Lankenau, A., Herrlich, A., and Ulrich, A. (1997) EMBO J. 16, 7032–7044
36. Habib, A. A., Hognason, T., Ren, J., Stefansson, K., and Ratan, R. R. (1998) J. Biol. Chem. 273, 6885–6891
37. Akimoto, K., Takahashi, R., Moriya, S., Nishioka, N., Takayanagi, J., Kimura, K., Fukui, Y., Osada, S., Mizune, K., Hirai, S., Kazlauskas, A., and Ohne, S. (1996) EMBO J. 15, 788–798
38. Holgado-Madruga, M., Moscatello, D. K., Emlet, D. R., Dieterich, R., and Wong, A. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12419–12424
39. Zwick, R., Daub, H., Aoki, N., Yamaguchi-Aoki, Y., Tinhofer, I., Maly, K., and Ulrich, A. (1997) J. Biol. Chem. 272, 24767–24770
40. Eguchi, S., Namaguchi, K., Iszaki, H., Matsumoto, T., Yamakawa, T., Usunomiya, H., Motley, E. D., Kawakatsu, H., Owada, K. M., Hirata, Y., Marumo, F., and Inagami, T. (1998) J. Biol. Chem. 273, 8890–8896
41. Tsai, W., Morielli, A. D., and Peralta, E. G. (1997) EMBO J. 16, 4597–4605
42. Vlodavsky, I., Folkman, J., Sullivan, R., Pridman, R., Ishai-Michaeli, R., Sasse, J., and Klagesbren, M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2292–2296
43. Cummick, J. M., Dorsey, J. F., Standley, T., Turksen, J., Krakr, A. J., Fry, D. W., Jove, R., and Wu, J. (1998) J. Biol. Chem. 273, 14468–14475