Insulin receptor substrate-1 (IRS-1) is a major substrate of insulin and insulin-like growth factor-I receptor, which upon phosphorylation on tyrosine docks several signaling molecules. Recently, IRS-1 was found to interact with αβ integrins upon insulin stimulation. Integrins are transmembrane proteins that play an important role in adhesion between cells and between cells and extracellular matrix. One of the major proteins implicated in integrin signaling is pp125FAK, a cytosolic tyrosine kinase, which upon integrin engagement becomes tyrosine-phosphorylated and subsequently binds to c-Src. Here, we established a mammalian two-hybrid system to show that pp125FAK binds to IRS-1. This association depends largely on the C terminus of pp125FAK but not on pp125FAK tyrosine kinase activity. Furthermore, we observed co-immunoprecipitation of pp125FAK with IRS-1 in 293 cells, suggesting a possible biological function of this association. When IRS-1 was expressed in 293 cells together with pp125FAK or Src, we found extensive IRS-1 tyrosine phosphorylation. In pp125FAK expressing cells, this was concomitant with increased association of IRS-1 with Src homology 2-containing proteins such as growth factor receptor-receptor-bound protein 2, phosphatidylinositol (PI) 3-kinase p85α subunit, and Src homology 2-containing protein-tyrosine phosphatase-2. In addition, pp125FAK-induced association of IRS-1 with PI 3-kinase resulted in increased PI 3-kinase activity. In contrast, no change in mitogen-activated protein kinase activity was observed, indicating that pp125FAK-induced association between IRS-1 and growth factor receptor-bound protein 2 does not affect the mitogen-activated protein kinase pathway. Moreover, we found that engagement of integrins induced IRS-1 tyrosine phosphorylation. Considering our results together, we suggest that integrins and insulin/insulin-like growth factor-I receptor signaling pathways converge at an early point in the signaling cascade, which is the IRS-1 protein.

Integrins are transmembrane proteins expressed in most tissues. They are involved in key biological functions including cell migration and adhesion, embryonic development, prevention of programmed cell death, wound repair, and angiogenesis (1–7). Protein phosphorylation on tyrosine is an immediate event after integrin engagement following cell interaction with the extracellular matrix. One of the major phosphorylated proteins is the cytosolic tyrosine kinase (focal adhesion kinase) pp125FAK,1 which upon integrin engagement becomes phosphorylated on tyrosine and activated (8–10). pp125FAK contains a central kinase domain flanked by large N- and C-terminal regions, but it lacks canonical interaction motifs such as pleckstrin homology (PH), phosphotyrosine binding (PTB), and SH2 and SH3 (Src homology) domains. The pp125FAK sequence, which allows targeting of the kinase to the focal adhesions, is located in the C terminus (11). The potential physiological substrates of pp125FAK are the cytoskeletal proteins paxillin (12–14) and tensin (15) and p130Cas (Crk-associated substrate) (16, 17). The major autophosphorylation site of pp125FAK is tyrosine 397, which upon phosphorylation becomes a binding site for the cytosolic tyrosine kinase c-Src (18, 19). Association of c-Src leads to pp125FAK tyrosine phosphorylation, creating binding sites for SH2-containing proteins such as growth factor receptor-bound protein 2 (GRB2) and possibly for the p85α regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase) (20–22). Evidence for a role of pp125FAK in cell migration has been provided by the use of pp125FAK knockout mice. Indeed, cells from pp125FAK-deficient mice have reduced mobility and enhanced focal adhesion contact formation (23). In addition to integrins, a number of growth factors and neuropeptides, including platelet-derived growth factor, bombesin, endothelin, and lysophosphatidic acid (24–26) have been reported to induce tyrosine phosphorylation and activation of pp125FAK. Together, these data have led to the suggestion that pp125FAK is a key protein linking integrin and growth factor signaling pathways (27). However, the molecular basis underlying this cooperation remains ill defined. Recent studies suggest that synergistic interactions between growth factor and integrin signaling pathways are involved in regulation of cell proliferation, adhesion, and migration (28–30). Experiments performed in our laboratory have shown that insulin stimulation induces phosphorylation or dephosphorylation of pp125FAK, depending on the adhesion state of the cells (31). Moreover, these studies suggest that pp125FAK is a direct substrate of insulin and insulin-like growth factor-I (IGF-I) receptors. Recently, it was found that αβ integrin associates with activated insulin receptor and platelet-derived growth factor-β receptor and potentiates the biological responses induced by platelet-derived growth factor-β receptor.

The abbreviations used are: FAK, focal adhesion kinase; IRS-1, insulin receptor substrate-1; IGF-I, insulin-like growth factor-I; GRB2, growth factor receptor-bound protein 2; PI, phosphatidylinositol; SHP-2, SH2-containing protein-tyrosine phosphatase-2; MAP, mitogen-activated protein; PH, pleckstrin homology; PTB, phosphotyrosine binding; SH2 and SH3, Src homology 2 and 3, respectively; CAS, Crk-associated substrate; IR, insulin receptor; βIR, IR β-subunit; PAGE, polyacrylamide gel electrophoresis; DBD, DNA binding domain; AD, activation domain; SOS, son of sevenless; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; aa, amino acids.

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IR-1 as a Signaling Molecule for pp125FAK and pp60src

growth factor (32). In addition, Vuori and Rusolatih (33) have reported that insulin receptor substrate-1 (IRS-1) co-immunoprecipitates with αβ3 integrin following insulin stimulation. IRS-1 is a docking protein implicated in the insulin and IGF-I signaling pathways (34). Its tyrosine phosphorylation, induced upon insulin and IGF-I stimulation, creates binding sites for SH2-containing proteins such as the p85 regulatory subunit of PI 3-kinase, the adaptor GRB2, and the SH2-containing protein-tyrosine phosphatase-2 (SHP-2), resulting in activation of the PI 3-kinase and the MAP kinase pathways (35–41).

The aim of our study was to investigate the potential cross-talk between the insulin receptor (IR/IRS-1 signaling pathway and the integrin/pp125FAK signaling circuitry. We first looked at a putative interaction between pp125FAK and IRS-1. To do this, we established a mammalian two-hybrid system, which allows for the analysis of direct protein/protein interactions. In addition, we searched for the occurrence of an interaction between pp125FAK and IRS-1 in intact cells.

EXPERIMENTAL PROCEDURES

Materials

The reporter vector pElbLUC was kindly provided by Richard A. Maurer (Oregon Health Sciences University, Portland, OR). pp125FAK cDNA, inserted at the EcoRI site into the pBluescript (KS−), was a generous gift from Thomas J. Parsons (University of Virginia, Charlottesville, VA). The constitutive active and kinase-dead Src cDNAs, subcloned into pSGT vector, were kindly provided by Sara Courtneidge (EMBL, Heidelberg, Germany). Culture media and Geneticin were from Life Technologies, Inc. Bovine fibronectin, vitronectin, and poly-l-lysine were from Sigma. 125I and [32P]ATP were from ICN Pharmaceuticals, Inc. (Aurora, France). 125I-protein A was labeled using the chloramine-T method as described previously (42). Triton X-100, Nonidet P-40, leupeptin, benzamidine, pepstatin, and l-α-phosphatidylidyinositol were from Sigma. Aprotinin was from Bayer (Argentina, Germany), and phenylmethylsulfonyl fluoride was from Serva (Heidelberg, Germany). Protein A and protein G-Sepharose were from Amersham Pharmacia Biotech Inc. (Uppsala, Sweden). Enzymes for molecular biology were purchased from New England Biolabs (Beverly, MA). Antiserum to IRS-1 was prepared in our laboratory and was raised against a synthetic peptide corresponding to the C-terminal sequence comprising amino acids 1223–1235 of rat IRS-1. Immunoblotting of p42 MAP kinase was performed with rabbit antibodies purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and antibodies against phosphotyrosine/ phospho-threonine p42/p44 MAP kinases were from New England Biolabs. Immunoprecipitation and immunoblotting of pp125FAK were performed, respectively, with a mouse monoclonal antibody from Upstate Biotechnology, Inc. (Uppsala, Sweden). Antibodies to phosphotyrosine and to the p85 subunit of PI 3-kinase were from Novo Nordisk (Copenhagen, Denmark).

293 Cell Culture and Transfection

293 EBNA cells are human embryo kidney cells that constitutively express the EBNA-1 protein from the Epstein-Barr virus (Inviron, San Diego, CA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS) and 500 μg/ml Geneticin. Transfection was performed by the calcium phosphate precipitation method of Chen and Okayama (43) (5 μg of DNA per 100-mm diameter dish). 18 h after transfection, cells were starved in DMEM supplemented with 0.2% (v/v) bovine serum albumin for 20 h before use.

Preparation of Extracellular Matrix Protein-coated Dishes

150-mm culture dishes were incubated with poly-L-lysine (10 μg/ml) or fibronectin (10 μg/ml) together with vitronectin (3 μg/ml) in PBS at 4 °C overnight. The dishes were rinsed twice with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 6.5 mM NaH2PO4, 1.5 mM KH2PO4, pH 7.4) and dried 1 h at 37 °C before use.

NHIR Cell Adherence on Extracellular Matrix

NHIR cells are NIH 3T3 cells stably transfected with the insulin receptor. They are maintained in DMEM containing 10% FCS and 0.5 μg/ml Geneticin. Confluent cells were detached with trypsin and plated on the extracellular matrix protein-coated dishes in DMEM, 10% FCS. 24 h after the plating, NIHIR cells were starved in 0.2% bovine serum albumin medium for 4 h. Cells were then stimulated with insulin (10−7 M) and lysed for immunoprecipitation.

Production of pp125FAK Expression Vectors

To produce the pBSK/pp125FAK-3C, pBSK/pp125FAK was cleaved by Nhel and ClaI. After a fill-in, religation was performed at 16 °C for 4 h. Kinase-deficient pp125FAK was produced by site-directed mutagenesis using the Transformer® kit from CLONTECH Laboratories, Inc. (Palo Alto, CA). The two primers (Genetet, Paris, France) were 5′-CCG CTC TAG AAG TAG TGG GCC CCC CCG GCT GC-3′, which changes the EcoRI site to Apol in the pBSK polylinker, and 5′-GGG GTA AAT CAG CAG AAT TAA AAA CTT C-3′, which changes Lex4′ to Arg in pBSK. pBSK/pp125FAK cDNAs were subcloned into pCEP by excision of pBSK constructs at the XhoI and Xhol sites and ligation with the Nhel and XhoI sites of pCEP.

The Y397F mutant of pp125FAK was made in the pBSK vector using Quick Change™ kit from Stratagene (San Diego, CA). The two primers were 5′-GAA ACA GAT GAC TTT GCA GAG-3′ and 5′-CTC TGG AAA GTC ATC TTC TGC-3′ (Eurogentec, Seraing, Belgium). The Y397F cDNA was then excised from pBSK using NotI and XhoI and inserted into pCEP cleaved by the same enzymes.

To subclone constitutive active and kinase-dead Src cDNAs into pCEP vector, the Src cDNAs were excised of pSGT constructs at the SpeI/BglII sites and ligated into pCEP at the NcoIBamHI sites.

Immunoprecipitation/ Western Blotting

Cells were washed with buffer A (50 mM Hepes, 150 mM NaCl, 100 mM NaF, 10 mM EDTA, 10 mM NaPO4, pH 7.5) supplemented with 2 mM sodium orthovanadate (NaVO4). Cells were then lysed for 15 min on ice with buffer A supplemented with 2 mM NaVO4, 1% (v/v) Nonidet P-40, 20 mM leupeptin, 4 mM benzamidine, 2 mM pepstatin, 1 mM phenylmethylsulfonyl fluoride, and 100 units/ml aprotinin (lysis buff. B). After centrifugation at 4 °C for 15 min, cell lysates were added to antibodies (pp125FAK (1 μg of Ig/sample) or IRS-1 (serum dilution 1:50) preadsorbed on protein G-Sepharose (mouse monoclonal antibodies) or protein A-Sepharose (rabbit polyclonal antibodies). Immunoprecipitation was performed at 4 °C for 4 h for pp125FAK and 2 h for IRS-1. Pellets were then washed three times with buffer A supplemented with 2 mM NaVO4 and resuspended in Laemmli buffer (44). The samples were analyzed by SDS-PAGE under reducing conditions, and proteins were transferred to an Immobilon membrane (Immobilon polyvinylidine difluoride; Millipore Corp.) in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3). The membrane was blocked with TBS (10 mM Tris, pH 7.4, 140 mM NaCl) containing 5% bovine serum albumin (w/v) for 1 h and incubated with antibodies to pp125FAK (1:200), phospho-GRB2 (1.6 μg/ml), p85α (1.6 μg/ml), or IRS-1 (1 μg/ml), or phosphotyrosine (1 μg/ml) for 90 min at room temperature. When necessary, an incubation with rabbit anti-mouse immunoglobulins was performed for 1 h at room temperature. After each incubation, the membrane was washed four times with TBS containing 1% (v/v) Nonidet P-40 and then twice with TBS. The membrane was finally incubated with 1/1000-protein A (250,000 cpm/ml) or with chemiluminescent antibodies (Enhanced chemiluminescence, Pierce) for 1 h at room temperature; washed five times with TBS, 1% (v/v) Nonidet P-40 and twice with TBS; and exposed to sensitive films.

Two-hybrid System in Mammalian Cells

DNA Constructs—The mammalian two-hybrid system was purchased from CLONTECH. It contains two expression vectors, pM and pVP16. The reporter vector pELUC was kindly provided by Richard A. Maurer. pM expression vector contains the DNA binding domain (DBD) of Gal4, and the other vector contains the VP16 activation domain (AD). We subcloned the cDNAs of the proteins of interest in-frame downstream of the DBD or the AD. The cDNAs were first amplified by polymerase chain reaction using the Pfu DNA polymerase (Stratagene, La Jolla, CA). The two primer sets of genes for GAL4 DBD, 5′ CCAG CGT CGA ATG AGC AGG CCT CCG GCT GC-3′ and 5′-CCCG AAG CTT CTA TGT ACC GTC CTC TGG TGT G-3′; DBP-IRS-1, 5′-GGA ATT CAT GCC GAC CCC TGA TAC CG-3′ and 5′-CCCG AAG CTT CTA TGT ACC GTC CTC TGG TGT G-3′ for the C-terminal part; and ΔPH-IRS-1, 5′-GGG CGT CTA GAT CAG CCA ATG GAT AAC GCG CAC C-3′.
and 5'-CCC AAG CTT CTA TGT ACG GTC TCT TGG-3'; pp125FAK and pp125FAK: KD, 5'-CCG GGA TCC GTA TGG AGC GTP CCC CGG GGG CC-3' and 5'-CCG GGA TCC TTA GTG GGG CCT GGA CTG GC-3'; constitutively active c-Src, 5'-CCG GAA TTC CGG ATG GGG AGC AGC AAC AGC GGC-3' and 5'-CCG GAA TTC CGG CTG CTA TAG GGT CTC TGG AGG-3'. The restriction sites are underlined.

Inserts and vectors pM and pVP16 were digested with the corresponding enzymes, and ligation was performed using the Rapid DNA Ligation Kit from Boehringer Mannheim. pM/pp125FAKΔC, pM/IR, and pVP16/p85α were obtained by excising pp125FAKΔC, IR-β subunit (pIR), and p85α cDNAs from pBTM116/pp125FAKΔC, PCDNA3/pIR, and pLex/p85α, respectively, and religated in-frame into pM vector or pVP16 vector using appropriate enzymes. All the constructions were verified by sequencing the open reading frame between the AD or the DBD and the insert (TT sequence kit, Pharmacia, and SEQUAGEL, National Diagnostic, Atlanta, GA).

Transfection of 293 Cells—Cells were cultured in DMEM supplemented with 5% (v/v) FCS and 50 μg/ml Geneticin. Transfections were performed by the calcium phosphate precipitation method as described previously (500 ng of each expression vector and 100 ng of reporter vector/17-mm diameter dish). 18 h after being transfected, cells were starved in DMEM supplemented with 0.5% FCS for 20 h.

Luciferase Assay—36 h after transfection, cells were solubilized in 100 μl of Reporter lysis buffer from Promega (Madison, WI). 10 μl of cell lysate was used to measure the luciferase activity with 50 μl of luciferin substrate purchased from Promega. Substrate degradation was followed by production of photons, and this chemiluminescent reaction was measured using a luminometer.

PI 3-Kinase Assay

To measure PI 3-kinase activity, 293 cells were transfected with pp125FAK, IRS-1, or both and were stimulated or not stimulated with 10 ng/ml insulin for 5 min. Cells were washed with buffer A, supplemented with 2 mM Na3VO4 and lysed with 500 μl of lysis buffer, and cell extracts were subjected to immunoprecipitation with pp125FAK (1 μg of Ig/sample) or IRS-1 antibodies (serum dilution 1:50). The immunoprecipitates were washed twice with each of the following buffers: 1) 0.1 M Tris, 0.05 M LiCl, pH 7.4; 2) 0.1 M Tris, 0.05 LiCl, pH 7.4; and 3) 100 mEq Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4. The pellets were resuspended in 30 μl of 20 mM Hepes, pH 7.4, 0.4 mM EGTA, 0.4 mM Na3HPO4, and the kinase reaction was started by the addition of 0.2 mg/ml phosphatidylinositol (0.2 mg/ml), 10 mM MgCl2, and 50 μg [γ-32P]ATP (7000 Ci/mmol) and was performed for 15 min at room temperature. After 15 min, the reaction was stopped by the addition of 15 μl of 4 M HCl, and the phosphoinositides were extracted with 130 μl of chloroform/methanol (v/v). The phospholipids were analyzed by thin layer chromatography and Cerenkov counting.

RESULTS

Detection of Protein/Protein Interactions in Mammalian Cells—We have set up a two-hybrid system in mammalian embryonic kidney cells, 293 cells (Fig. 1). We used pM and pVP16 vectors (CLONTECH) and the reporter vector pElbLUC obtained from R. Maurer. The pM vector contains the SV40 promoter followed by the sequence coding for the DBD of Gal4. The pVP16 vector is similar to pM but contains the AD of VP16. Transfection of 293 cells was performed using the calcium phosphate precipitation method as described previously.

293 cells were transfected with the reporter vector pElbLUC, pp125FAK WT, or several mutants subcloned into pM and constitutively active c-Src subcloned into pVP16. Direct interaction between the proteins was revealed by a lumimetric assay, and results have been calculated as a function of the mock condition (Fig. 2). The mock condition is the highest basal luciferase activity given by either the construct in pM or the construct in pVP16 alone. As shown in Fig. 2, and in accordance with previous reports of co-immunoprecipitations (18, 19), we found in our mammalian two-hybrid system that wild-type pp125FAK interacted with c-Src. More precisely, interaction between pp125FAK and c-Src induced a 6-fold increase in luciferase activity compared with the mock condition. Since it is known that c-Src binds to the autophosphorylated tyrosine 397 of pp125FAK, our observations suggest that the kinase activity of pp125FAK is functional in our mammalian system. This was further confirmed using the kinase-deficient mutant of pp125FAK (obtained by mutation of lysine 454 in the ATP binding site), which did not associate with c-Src. Next we looked at the possible participation of another domain of pp125FAK. The pp125FAK mutant deleted of the C-terminal domain (Δ aa 965–1065) interacted with c-Src to the same extent as wild-type pp125FAK. This is expected, since the c-Src binding site is not included in the deleted part. In summary, our mammalian two-hybrid system is able to detect the interaction between pp125FAK and constitutively active c-Src. This interaction is subject to modulation by changes in the structure and kinase activity of pp125FAK. Taken together, our results confirm the validity of this mammalian system.

pp125FAK Interacts with IRS-1—The cDNAs of pp125FAK or the βIR were subcloned into pM. The cDNAs of IRS-1 or the p85aa subunit of PI 3-kinase (p85α) were subcloned into pVP16. 293 cells were transfected with pElbLUC, pM/βIR, and pVP16/ IRS-1; pM/pp125FAK and pVP16/p85α; or pM/pp125FAK and pVP16/IRS-1. Interestingly, as shown in Fig. 3, we found that pp125FAK and IRS-1 interact strongly in this system, since their coexpression led to a 48-fold increase in luciferase activity compared with the mock condition. This is the first evidence demonstrating a direct molecular interplay between these two molecules. Moreover, coexpression of βIR and IRS-1 led to a 15-fold increase in luciferase activity, indicating that the two proteins interact in our mammalian system. Such interaction has been previously demonstrated using the classical yeast two-hybrid system (45). Indeed, IRS-1 contains a PTB (aa 144–318), which binds to phosphotyrosine 960 of βIR (46, 47). In addition, as described previously (20, 21), pp125FAK interacts directly with p85α, since we found a 20-fold increase in luciferase activity when pp125FAK and p85α are coexpressed. The interactions between pp125FAK and p85α, and between
Fig. 2. Interaction of wild-type and mutant forms of pp125FAK with c-Src using the mammalian two-hybrid system. 293 cells were co-transfected with 500 ng of pVP16/constitutively active c-Src, pp125FAK wild type (WT) or mutants subcloned into pM vector (500 ng), and 100 ng of the reporter vector encoding luciferase. Cell lysates were prepared and used for a luciferase assay. Results are presented as fold induction compared with the mock condition. The ΔC mutant corresponds to pp125FAK deleted of its last 100 amino acids, and KD is a kinase-deficient mutant of pp125FAK (K454R). Results are representative of four independent experiments each performed in triplicate.

Fig. 3. Interaction of pp125FAK with IRS-1 in a mammalian two-hybrid system. 293 cells were co-transfected with the indicated constructs: pM/βIR expressing constitutively active insulin receptor β-subunit and pVP16/IRS-1; pM/pp125FAK and pVP16/IRS-1; and pM/pp125FAK and pVP16/p85α encoding the p85α subunit of PI 3-kinase. 500 ng of each expression vector and 100 ng of the luciferase reporter vector were transfected. After cell lysis, a luciferase assay was performed. The results are presented as fold induction compared with the mock condition and represent three independent experiments each performed in triplicate.

IRS-1 and βIR, indicate that these proteins are functional and correctly expressed in 293 cells.

Characterization of pp125FAK/IRS-1 Interaction—Next, we searched for the domains of pp125FAK and IRS-1 potentially involved in the interaction between the two molecules. Neither pp125FAK nor IRS-1 contains SH2 or SH3 domains (35, 48). However, pp125FAK has various putative tyrosine phosphorylation sites, which can become binding sites for SH2 domain-containing proteins (20–22, 37–41) and for the PTB domain of IRS-1. To characterize the interaction between pp125FAK and IRS-1, several pp125FAK mutants were tested (see Fig. 2). 293 cells were co-transfected with the reporter vector pElbLUC, pVP16/IRS-1, and pp125FAK, or its mutants subcloned into pM. The interaction was measured by luminescent assay and calculated as a function of the mock condition (Fig. 4A). Similarly to the results shown in Fig. 3, interaction between pp125FAK and IRS-1 induced a 45-fold increase in luciferase activity compared with mock. Interestingly, pp125FAK-ΔC interaction with IRS-1 was decreased about 5-fold compared with the interaction between wild-type pp125FAK and IRS-1 and presented only a 9-fold increased activity compared with mock. This indicates that the pp125FAK C-terminal region is directly involved in this interaction. However, since deletion of the C-terminal domain did not completely abolish the association, additional domain(s) of the protein might be involved. We have also determined that the N-terminal domain of pp125FAK (aa 1–386) is not implicated in the interaction with IRS-1 (data not shown). Next, we tested the interaction of IRS-1 with the kinase-deficient mutant of pp125FAK, pp125FAK-KD, which interacted with IRS-1 similarly to wild-type pp125FAK, indicating that pp125FAK kinase activity is not necessary for the association of pp125FAK with IRS-1. In conclusion, the C-terminal domain of pp125FAK is important for the interaction with IRS-1, and this association is independent of pp125FAK tyrosine kinase activity.

Concerning IRS-1, we envisioned that its PTB domain could interact with phosphotyrosines on pp125FAK. Moreover, IRS-1 also contains a PH domain, which is implicated in the association between protein and phospholipids and is also thought to be involved in protein/protein interactions (49). To evaluate the possibility of IRS-1 PH and PTB domain interaction, we subcloned IRS-1 deleted of its PTB domain (∆PTB, aa 144–316) or its PH domain (∆PH, aa 1–144) into pVP16, whereas wild-type pp125FAK was subcloned into pM. 293 cells were co-transfected with pElbLUC, pp125FAK, IRS-1 wild type, ∆PH, or ∆PTB. Results are presented in Fig. 4B. When pp125FAK was coexpressed with ∆PH-IRS-1 or with ∆PTB-IRS-1, the resulting interaction was comparable with that seen with wild-type IRS-1, indicating that neither the IRS-1 PH domain nor the IRS-1 PTB domain is involved in the interaction. To ensure the viability of our IRS-1 mutants, we verified that in this system ∆PH-IRS-1 interacts with βIR, whereas ∆PTB-IRS-1/βIR does not (data not shown). In summary, neither the PH domain nor the PTB domain of IRS-1 participates in the interaction be-
total cell lysates that the expression level of pp125 FAK and its 
part
A
Moreover, in accordance with results presented in Fig. 4
pp125FAK with IRS-1 in intact cells. 

expression of wild-type pp125FAK in 293 cells induced pronounced tyrosine 

membrane corresponding to pp125FAK

mids encoding wild-type or mutant forms of pp125FAK. Insulin 
IRS-1 could have consequences on IRS-1 phosphorylation. In 
Since pp125FAK is a tyrosine kinase, we next
examined whether Src could be implicated in IRS-1 tyrosine phosphorylation. To this end, 293 cells 
were co-transfected with IRS-1 and with wild-type or mutant forms of pp125FAK or Src. After immunoprecipitation of cell 
lysates with antibody to IRS-1, phosphoproteins were separated, whereas the kinase-deficient mutant was not. Fig. 6B (upper part) shows that the level of IRS-1 immunoprecipitation is similar in all conditions. Moreover, in accordance with results presented in Fig. 4A, kinase-deficient pp125 FAK (KD) co-immunoprecipitated with IRS-1 to the same extent as pp125 FAK wild type. Fig. 5B (lower part) shows the tyrosine phosphorylation level of pp125 FAK co-immunoprecipitating with IRS-1. Wild type and the C-terminal mutant are phosphorylated on tyrosine, while as expected kinase-deficient pp125 FAK is not. We have verified in total cell lysates that the expression level of pp125 FAK and its mutants was the same. In summary, in intact cells and in the mammalian two-hybrid system, the interaction between pp125 FAK and IRS-1 is independent of pp125 FAK kinase activity but requires the pp125 FAK C terminus.

Expression of pp125 FAK and IRS-1 Induces IRS-1 Tyrosine Phosphorylation—Since pp125 FAK is a tyrosine kinase, we next examined whether the interaction between pp125 FAK and IRS-1 could have consequences on IRS-1 phosphorylation. In brief, 293 cells were cotransfected with IRS-1 and with plasmids encoding wild-type or mutant forms of pp125 FAK. Insulin stimulation was used as a positive control for IRS-1 phosphorylation. Cell lysates were subjected to immunoprecipitation with antibody to IRS-1; proteins were separated on a 7.5% polyacrylamide gel and transferred to a membrane, and phosphotyrosine-containing proteins were revealed with antibody 
to phosphotyrosine (Fig. 6A). When IRS-1 is expressed alone, its basal tyrosine phosphorylation is not detectable in 293 cells. However, IRS-1 tyrosine phosphorylation was seen when cells were stimulated with insulin. Interestingly, the expression of wild-type pp125 FAK in 293 cells induced pronounced tyrosine phosphorylation of IRS-1. In contrast, IRS-1 phosphorylation was low in cells expressing pp125 FAK-ΔC, which is in accordance with the observation that pp125 FAK-ΔC interacts with IRS-1 less efficiently than wild-type pp125 FAK (Fig. 6B, lower part). When 293 cells were transfected with pp125 FAK-KD, no tyrosine phosphorylation of IRS-1 was observed, indicating that the tyrosine kinase activity of pp125 FAK is responsible directly or indirectly for IRS-1 phosphorylation. We also checked in these cells whether wild-type and pp125 FAK-ΔC were indeed phosphorylated, whereas the kinase-deficient pp125 FAK was not. Fig. 6B (upper part) shows that the level of IRS-1 immunoprecipitation is similar in all conditions. Moreover, the amount of pp125 FAK mutants co-immunoprecipitating with IRS-1 (Fig. 6B, lower part) was similar to the wild-type, except with pp125 FAK-ΔC, and thus could not explain the reduced tyrosine phosphorylation of IRS-1 observed with the kinase-deficient mutant. Finally, comparable expression levels of pp125 FAK (wild type and mutants) were also found in total cell lysates (Fig. 6C). In conclusion, pp125 FAK expression in 293 cells leads to an important increase in IRS-1 tyrosine phosphorylation. This phosphorylation requires an intact pp125 FAK C-terminal domain and is dependent on pp125 FAK tyrosine kinase activity. However, we cannot exclude the possibility that another associated tyrosine kinase participates in IRS-1 phosphorylation.

Expression of Src and IRS-1 Induces IRS-1 Tyrosine Phosphorylation—We next examined whether Src could be implicated in IRS-1 tyrosine phosphorylation. To this end, 293 cells were co-transfected with IRS-1 and with wild-type or mutant forms of pp125 FAK or Src. After immunoprecipitation of cell 
lysates with antibody to IRS-1, phosphoproteins were separated and revealed with antibody to phosphotyrosine. As described previously in this paper, expression of pp125 FAK WT in 293 cells induced robust IRS-1 tyrosine phosphorylation (Fig. 7). In cells expressing IRS-1 and pp125 FAK-Y397F, which has lost the ability to bind Src, IRS-1 phosphorylation was strongly

between pp125 FAK and IRS-1.

Co-immunoprecipitation of pp125 FAK with IRS-1 in Intact Cells—To find whether pp125 FAK and IRS-1 interact in intact cells, wild-type and mutant forms of pp125 FAK were overexpressed in 293 cells. After immunoprecipitation of endogenous IRS-1, the proteins were separated by SDS-PAGE and analyzed by Western blotting using antibodies to IRS-1 (Fig. 5A) or to pp125 FAK (Fig. 5B). Fig. 5A shows that IRS-1 immunoprecipitation was comparable in all conditions. As shown in Fig. 5B, we did not detect co-immunoprecipitation of pp125 FAK with IRS-1 when pp125 FAK was not overexpressed in 293 cells (mock). This is due to the very low level of pp125 FAK expression in these cells (data not shown). However, Fig. 5B shows that pp125 FAK co-immunoprecipitated with IRS-1 when cells were transfected with the wild-type pp125 FAK (WT). The C-terminal mutant of pp125 FAK (ΔC) weakly co-immunoprecipitated with IRS-1, a result that further supports the idea that the pp125 FAK C terminus is involved in the association with IRS-1. Moreover, in accordance with results presented in Fig. 4A, kinase-deficient pp125 FAK (KD) co-immunoprecipitated with IRS-1 to the same extent as pp125 FAK wild type. Fig. 5B (lower part) shows the tyrosine phosphorylation level of pp125 FAK co-immunoprecipitating with IRS-1. Wild type and the C-terminal mutant are phosphorylated on tyrosine, while as expected kinase-deficient pp125 FAK is not. We have verified in total cell lysates that the expression level of pp125 FAK and its mutants was the same. In summary, in intact cells and in the mammalian two-hybrid system, the interaction between pp125 FAK and IRS-1 is independent of pp125 FAK kinase activity but requires the pp125 FAK C terminus.
IRS-1 as a Signaling Molecule for pp125FAK and pp60src

Fig. 6. Overexpression of pp125FAK and IRS-1 induces IRS-1 phosphorylation on tyrosine. 293 cells were cotransfected with 5 μg of pCEP only (mock) or pCEP/pp125FAK (wild-type and mutants) and 5 μg of pCEP/IRS-1. Where indicated, cells were stimulated with insulin (10^{-6} M). Immunoprecipitation of IRS-1 was carried out on cell lysates. The immunocomplexes were separated into two fractions, and proteins were separated by 7.5% SDS-PAGE and transferred to membranes. One membrane was incubated with antibody to phosphotyrosine (1 μg/ml) (A), and the second one was incubated with anti-IRS-1 (1:1000) or anti-pp125FAK (1:200) (B). C, immunodetection of pp125FAK was also carried out using a fraction of the total cell lysate by Western blot using the anti-pp125FAK to check expression levels of pp125FAK mutants. A representative experiment out of four is shown performed in duplicate.

Reduced but was still observed. This result could indicate that, without participation of Src, pp125FAK is able to phosphorylate IRS-1, albeit to a limited extent. However, since pp125FAK, Y397F expression led to a pronounced decrease in IRS-1 phosphorylation compared with the wild-type, we investigated whether Src by itself could be responsible for IRS-1 tyrosine phosphorylation. In 293 cells expressing IRS-1 and a constitutively active form of Src (Src-CA), extensive IRS-1 tyrosine phosphorylation was found. In contrast, expression of kinase-dead Src (Src-KD) did not lead to such phosphorylation. Immunoprecipitation of IRS-1 was the same in all conditions (data not shown). These results indicate that constitutively active Src is able to lead to IRS-1 tyrosine phosphorylation and that this process requires Src’s kinase activity. Moreover, in cells expressing IRS-1 and the two kinases, pp125FAK and Src, there was an additional effect on IRS-1 phosphorylation.

pp125FAK-induced IRS-1 Tyrosine Phosphorylation Results in IRS-1 Docking of p85a, SHP-2, and GRB2—IRS-1 contains multiple sites for tyrosine phosphorylation, of which several are docking sites for SH2 domain-containing proteins such as the p85a subunit of PI-3 kinase, the adaptor protein GRB2, and the phosphotyrosine phosphatase SHP-2 (37–41). Therefore, we were interested in testing the ability of IRS-1-tyrosine phosphorylation. Increased interaction of IRS-1 with GRB2 in the presence of pp125FAK. Moreover, there was also no detectable PI 3-kinase activity associated with transfected pp125FAK. This correlates with our previous experiments (Fig. 8) showing no detectable co-immunoprecipitation of p85α with pp125FAK. Moreover, there was also no detectable PI 3-kinase activity associated with endogenous IRS-1. In contrast, in cells overexpressing IRS-1, PI 3-kinase activity associated with IRS-1 was observed in basal conditions, and a further 2-fold stimulation was obtained after insulin treatment. Transfection with both IRS-1 and pp125FAK induced an 8-fold stimulation of PI 3-kinase activity associated with IRS-1 compared with PI 3-kinase activity from cells overexpressing IRS-1 only. Expression of p85α was verified in all conditions (data not shown). Taken together, our observations show that pp125FAK-induced tyrosine phosphorylation of IRS-1 creates docking sites for p85α, which upon binding to IRS-1 leads to activation of p110 PI 3-kinase.

pp125FAK Increases IRS-1-Associated PI 3-Kinase Activity in 293 Cells—Next we approached the putative biological consequences of IRS-1 phosphorylation in the presence of pp125FAK. Since we found that the interaction between IRS-1 and p85α increases in the presence of pp125FAK, we anticipated that pp125FAK overexpression could stimulate IRS-1-associated PI 3-kinase activity. To test this, 293 cells were overexpressed with pp125FAK, IRS-1, or both. Insulin was used as a positive control of PI 3-kinase activation. pp125FAK or IRS-1 was immunoprecipitated, and the associated PI 3-kinase activity was measured in the immunocomplexes. As shown in Fig. 9, no PI 3-kinase activity was associated with transfected pp125FAK. PP125FAK-induced IRS-1 containing multiple sites for tyrosine phosphorylation, of which several are docking sites for SH2 domain-containing proteins such as the p85a subunit of PI-3 kinase, the adaptor protein GRB2, and the phosphotyrosine phosphatase SHP-2 (37–41). Therefore, we were interested in testing the ability of IRS-1-tyrosine phosphorylation. Increased interaction of IRS-1 with GRB2 in the presence of pp125FAK. Moreover, there was also no detectable PI 3-kinase activity associated with endogenous IRS-1. In contrast, in cells overexpressing IRS-1, PI 3-kinase activity associated with IRS-1 was observed in basal conditions, and a further 2-fold stimulation was obtained after insulin treatment. Transfection with both IRS-1 and pp125FAK induced an 8-fold stimulation of PI 3-kinase activity associated with IRS-1 compared with PI 3-kinase activity from cells overexpressing IRS-1 only. Expression of p85α was verified in all conditions (data not shown). Taken together, our observations show that pp125FAK-induced tyrosine phosphorylation of IRS-1 creates docking sites for p85α, which upon binding to IRS-1 leads to activation of p110 PI 3-kinase.

Increased Interaction of IRS-1 with GRB2 in the Presence of pp125FAK Does Not Lead to MAP Kinase Activation—Tyrosine kinases induce recruitment of GRB2-SOS complexes to the plasma membrane and allow activation of Ras, which then initiates the MAP kinase cascade (50). Thus, pp125FAK-induced tyrosine phosphorylation of IRS-1 and subsequent association with GRB2 could lead to increased MAP kinase activity. To test this possibility, 293 cells were cotransfected with IRS-1, pp125FAK, or both and stimulated or not stimulated with insulin or okadaic acid as controls for MAP kinase activation. A fraction from each lysate was separated on a 10% acrylamide/bisacrylamide gel. As expected, pp125FAK overexpressed in 293 cells was tyrosine-phosphorylated, and this was not modified...
by IRS-1 overexpression. IRS-1 is phosphorylated on tyrosine when cells are stimulated with insulin or when pp125FAK is overexpressed (Fig. 10A). Fig. 10B shows that p42 MAP kinase is present at similar levels in all conditions. Fig. 10C shows activation of p42 and p44 MAP kinases revealed using antibodies, which detect doubly phosphorylated threonine 202/tyrosine 204 of p42/p44 MAP kinases. No activated MAP kinase was seen in mock condition in the absence of induction. Okadaic acid induced the appearance of the phosphorylated and activated form of p42/p44 MAP kinase. In cells overexpressing only pp125FAK, no activation of MAP kinase was detected. In addition, a slight MAP kinase activation was observed in the presence of insulin in cells transfected with IRS-1. However, no activated MAP kinase was observed in 293 cells co-transfected with IRS-1 and pp125FAK. In conclusion, despite increased formation of the complex IRS-1-GRB2 induced by pp125FAK, stimulation of MAP kinase is not observed.

**DISCUSSION**

Previous studies have shown that the focal adhesion kinase pp125FAK is a key player in integrin signaling pathways. Thus, upon integrin engagement pp125FAK is activated (8–10) and becomes implicated in interactions with signaling proteins such as PI 3-kinase and GRB2 (20–22). In addition, neuropeptides and growth factors are also able to activate pp125FAK (8–10), indicating that this kinase is involved in growth factor signaling pathways. However, the molecular basis of this integrin/growth factor receptor cross-talk is unknown. In contrast, a coherent picture is starting to emerge concerning the early events mediated by the insulin and IGF-I receptors (34). A major substrate of these two tyrosine kinase receptors is IRS-1. Vuori and Ruoslahti (33) have reported that insulin stimulation leads to co-immunoprecipitation of IRS-1 with the α,β3 integrin. More recently, evidence for interactions between integrins and insulin receptors and between integrins and platelet-derived growth factor receptors has been provided.

**Engagement of Integrins Induces IRS-1 Phosphorylation**—To address the physiological relevance of our findings, we studied the effect of the engagement of integrins on IRS-1 tyrosine phosphorylation. To this end, NHIR cells were plated onto polylysine or fibronectin plus vitronectin and stimulated or not stimulated with insulin as a positive control for IRS-1 phosphorylation. Cell lysates were subjected to immunoprecipitation with antibodies to pp125FAK (1µg/sample) or to IRS-1 (1:50). Proteins were separated by 10% SDS-PAGE and transferred onto a membrane. Immunoblotting using antibody to phosphotyrosine was performed (Fig. 11). In cells plated onto polylysine, basal tyrosine phosphorylation of IRS-1 was observed. As expected, insulin stimulation induced an important increase in IRS-1 phosphorylation. When NHIR cells were adherent onto fibronectin/vitronectin, integrins were engaged and IRS-1 phosphorylation was increased compared with cells plated onto polylysine. Taken together, these results mean that engagement of integrins induces IRS-1 tyrosine phosphorylation, which can further be stimulated by insulin.
FIG. 10. Increased IRS-1-GRB2 complex formation in the presence of pp125FAK does not lead to MAP kinase activation. 293 cells were transfected with vector only, with pCEP/pp125FAK or pCEP/IRS-1, or with both. After incubation with buffer, 2 × 10⁻⁸ M okadaic acid, or 10⁻⁸ M insulin, cells were lysed. Proteins were separated on a 10% polyacrylamide gel and transferred to a membrane that was blotted with antibodies to phosphotyrosine (1 µg/ml) (A) or to phospho-MAP kinase (1 µg/ml) (C). After stripping, the blot was incubated with antibody to p42 MAP kinase (0.5 µg/ml) (B) to check the amount of MAP kinase in all conditions (enhanced chemiluminescence). This experiment is representative of three performed in duplicate. OKA, 2 × 10⁻⁸ M okadaic acid; INS, 10⁻⁸ M insulin.

FIG. 11. Engagement of integrins induces IRS-1 tyrosine phosphorylation. NHIR cells were plated on poly-l-lysine 10 µg/ml or fibronectin (10 µg/ml) plus vitronectin (3 µg/ml) for 24 h. Then after 4 h of starvation, cells were stimulated or not stimulated with insulin (10⁻⁸ M). Immunoprecipitation of IRS-1 was performed on cell lysates, and proteins were separated by SDS-PAGE. Phosphotyrosine proteins transferred onto a membrane were revealed with antiphosphotyrosine (1 µg/ml). An autoradiogram is shown.

let-derived growth factor receptors has been provided (32). In addition, α,β integrin engagement potentiates platelet-derived growth factor- and insulin-induced DNA synthesis. Taken together, these observations suggest the existence of an interplay between integrin and insulin/IGF-1 signaling pathways. To search for the molecular basis of such a cross-talk, we looked for a possible link between pp125FAK and IRS-1. To do so, we established an experimental system that permits the analysis of direct protein/protein interactions in mammalian cells. Interestingly, we found that the tyrosine kinase pp125FAK actually binds IRS-1. This interaction occurs through the C terminus of pp125FAK and does not require pp125FAK kinase activity, suggesting that tyrosine-phosphorylated sites of pp125FAK may not be involved. This hypothesis is strengthened by the observation that the PTB domain of IRS-1 is not implicated in the interaction with pp125FAK. We also determined that the PH domain of IRS-1 is not involved in this association. Next, we demonstrated an association between pp125FAK and IRS-1 in intact cells. Consistent with such a direct molecular interplay, we found that expression of pp125FAK leads to IRS-1 tyrosine phosphorylation. Our studies also show that pp125FAK kinase activity is required for IRS-1 tyrosine phosphorylation, since a kinase-deficient pp125FAK mutant does not induce IRS-1 phosphorylation. Expression of the pp125FAK-Y397F mutant (mutated on the c-Src binding site) was still associated with increased IRS-1 tyrosine phosphorylation, although to a minute extent compared with that seen with wild-type pp125FAK. This indicates that pp125FAK is able to increase IRS-1 phosphorylation but that most of the phosphorylation obtained with pp125FAK expression is due to c-Src. This corroborates with the fact that expression of a constitutive active Src results in strong IRS-1 tyrosine phosphorylation. This was not observed upon expression of a kinase-dead Src. Moreover, the two kinases, pp125FAK and Src, appear to have an additional effect on this phosphorylation. So far we cannot conclude whether pp125FAK or Src directly phosphorylate IRS-1, since our results were obtained after immunoprecipitation of expressed proteins. The most likely interpretation of our data taken as a whole is that pp125FAK functions as a scaffold protein for both Src and IRS-1 and by doing so allows the phosphorylation of IRS-1 by Src. Whether pp125FAK by itself phosphorylates IRS-1 is not known at present.

Several IRS-1 tyrosine residues become docking sites for SH2 domain-containing molecules upon phosphorylation (38–41). We show here that expression of pp125FAK promotes the interaction of IRS-1 with p85α, SHP-2, and GRB2. Furthermore, pp125FAK-induced association of IRS-1 with p85α results in increased PI 3-kinase activity. Data have been accumulating showing that p85α interacts directly with pp125FAK and that upon integrin engagement PI 3-kinase activity associated with pp125FAK is increased (20, 21). We propose that two pathways may lead to activation of PI 3-kinase by pp125FAK, the first one consisting of a direct interaction between pp125FAK and PI 3-kinase, the second one using IRS-1 as a docking molecule. Whether both of these pathways occur in intact cells and what the precise contribution of each one is in a physiological context remain open questions. It has been demonstrated that pp125FAK is an antiapoptotic factor (51, 52). Moreover, pp125FAK cleavage by caspases is one of the earliest events occurring during apoptosis (53, 54). It was previously assumed that the antiapoptotic effect of pp125FAK was mediated by PI 3-kinase (55). The involvement of PI 3-kinase in preventing...
apoptosis has been reported by several groups (56–61). In addition, a series of experiments has shown that IRS-1 expression partially prevents apoptosis induced by interleukin-3 deprivation in 32D cells (62). This is consistent with other studies describing IRS-1 as an antiapoptotic factor (63). As a whole, these observations and our present work favor the idea that pp125FAK, IRS-1, and PI 3-kinase could participate in common signaling pathways, leading for instance to antiapoptotic effects. Although expression of pp125FAK increases the association of GRB2 with IRS-1, it does not lead to MAP kinase activation. This is in accordance with observations suggesting that pp125FAK is not implicated in MAP kinase activation (64). A previous report has shown that GRB2 located at focal adhesions interacts with the cytoskeletal protein dynamin (65). This GRB2 pool may not be capable of interacting with SOS and thus would not be implicated in activation of the MAP kinase cascade. Moreover, the GRB2-dynamin complex is able to bind to tyrosine-phosphorylated IRS-1 (66). We can envision that in our system and in the presence of pp125FAK, IRS-1 associates with this pool of GRB2-dynamin, which is not actively involved in MAP kinase activation.

To address the question of the physiological significance of our results, we investigated whether engagement of integrins could induce IRS-1 tyrosine phosphorylation. Indeed, we find that upon integrin binding to fibronectin and vitronectin, IRS-1 tyrosine phosphorylation is increased. Accordingly, we also found that in attached cells tyrosine phosphorylation of IRS-1 is constantly observed, whereas it disappears in suspended cells (data not shown). Thus cell adhesion clearly controls the level of IRS-1 tyrosine phosphorylation.

Our results are reminiscent of other findings showing the occurrence of converging effects induced by integrin and growth factor signaling pathways (30, 34). Moreover, our recent studies suggest that integrin engagement modulates the effect of insulin on pp125FAK phosphorylation (31). These observations strongly favor the idea that integrin and insulin or growth factor receptor signaling pathways cross-react.

From our results, we propose the following two hypotheses schematized in Fig. 12: 1) IRS-1 participates in signaling mediated by the pp125FAK/pp60src module, which is part of the integrin cascade functioning independently of the insulin or IGF-I circuitry (A); 2) IRS-1 forms a point of convergence between integrin and insulin/IGF-I signaling pathways (B). Previous work from J. Pessin’s laboratory (67) and our own (31) demonstrating that insulin modulates pp125FAK phosphorylation leads us to believe that the latter hypothesis is the more likely one. For both scenarios, the most pressing questions relate to the biological consequences of this interplay and to its participation in physiological processes and, more importantly, in disease states associated with altered cell growth and apoptosis.

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49. Yenush, L., Makati, K. J., Smith-Hall, J., Ishibashi, O., Myers, M. G., Jr., and White, M. F. (1996) J. Biol. Chem. 271, 24300–24306
50. Egan, S. E., Giddings, B. W., Brooks, M. W., Buday, L., Sizeland, A. M., and Weinberg, R. A. (1993) Nature 363, 45–51
51. Hungerford, J. E., Compton, M. T., Matter, M. L., Hoffstrom, B. G., and Otey, C. A. (1996) J. Cell Biol. 135, 1383–1390
52. Frisch, S. M., Vuori, K., Ruoslahti, E., and Chan, H. P. (1996) J. Cell Biol. 134, 793–799
53. Wen, L.-P., Fahrni, J. A., Troie, S., Guan, J.-L., Orth, K., and Rosen, G. D. (1997) J. Biol. Chem. 272, 26056–26061
54. Crouch, D. H., Fincham, V. J., and Frame, M. C. (1996) Oncogene 12, 2689–2696
55. Frisch, S. M., and Ruoslahti, E. (1997) Curr. Opin. Cell Biol. 9, 701–706
56. Ueno, H., Honda, H., Nakamoto, T., Yamagata, T., Sasaki, K., Miyagawa, K., Mitani, K., Yazaki, Y., and Hirai, H. (1997) Oncogene 14, 3067–3072
57. Yao, R., and Cooper, G. M. (1995) Science 267, 2003–2006
58. Yao, R., and Cooper, G. M. (1996) Oncogene 13, 343–351
59. Kulik, G., Klippel, A., and Weber, M. J. (1997) Mol. Cell. Biol. 17, 1595–1606
60. Khwaja, A., Rodriguez, V. P., Wennstrom, S., Warne, P. H., and Downward, J. (1997) EMBO J. 16, 2783–2783
61. Franke, T. F., Kaplan, D. R., and Cantley, L. C. (1997) Cell 88, 435–437
62. Zhou, L. F., Xu, S. Q., Dew, M., and Baserga, R. (1997) Oncogene 15, 961–970
63. Ueno, H., Sasaki, K., Kozutsumi, H., Miyagawa, K., Mitani, K., Yazaki, Y., and Hirai, H. (1996) J. Biol. Chem. 271, 27707–27714
64. Lin, T. H., Aplin, A. F., Shen, Y., Chen, Q., Schaller, M., Romer, L., Aukhil, I., and Juliano, R. L. (1997) J. Cell Biol. 136, 1385–1395
65. Kharbanda, S., Saleem, A., Yuan, Z., Emoto, Y., Prasad, K. V., and Kufe, D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6132–6136
66. Ando, A., Yonezawa, K., Gout, I., Nakata, T., Ueda, H., Har, K., Kitamura, Y., Noda, Y., Takenawa, T., Hirokawa, N., Waterfield, M. D., and Kasuga, M. (1994) EMBO J. 13, 3033–3038
67. Knight, J. B., Yamauchi, K., and Pessin, J. E. (1995) J. Biol. Chem. 270, 10199–10203

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