Insulin-induced activation of a phosphatidylinositol 3-kinase. Demonstration that the P85 subunit binds directly to the COOH terminus of the insulin receptor in intact cells

Rachel Levy-Toledano, Mohammed Taouis, Derek H. Blaettler, Phillip Gorden, Simeon I. Taylor

To cite this version:
Rachel Levy-Toledano, Mohammed Taouis, Derek H. Blaettler, Phillip Gorden, Simeon I. Taylor. Insulin-induced activation of a phosphatidylinositol 3-kinase. Demonstration that the P85 subunit binds directly to the COOH terminus of the insulin receptor in intact cells. Journal of Biological Chemistry, 1994, 269 (49), pp.31178-31182. hal-02713277

HAL Id: hal-02713277
https://hal.inrae.fr/hal-02713277
Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Insulin activates the insulin receptor tyrosine kinase to phosphorylate signaling molecules such as insulin receptor substrate-1 (IRS-1). Phosphorylated IRS-1 binds to SH2 domains in the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase), thereby stimulating the catalytic activity of PI 3-kinase. For most growth factor receptor tyrosine kinases (including receptors for epidermal growth factor and platelet-derived growth factor), the p85 regulatory subunit of PI 3-kinase binds directly to phosphorylated YXXM motifs contained in the cytoplasmic domain of the receptor itself. Previous studies in cell-free systems have shown that the phosphorylated YHTM sequence (amino acid residues 1322-1325) in the COOH terminus of the insulin receptor has the ability to bind to the p85 subunit of PI 3-kinase, thereby activating the enzyme. In this investigation, we demonstrate the occurrence of the same direct binding interaction in intact cells. Subsequent to insulin-stimulated phosphorylation of the insulin receptor, a complex is formed that contains the insulin receptor and PI 3-kinase. This complex can be immunoprecipitated by antibodies directed against either the insulin receptor or the p85 subunit of PI 3-kinase. The Δ43 mutant insulin receptor that lacks 43 amino acids at the COOH terminus does not bind p85. In addition, the Δ43 truncation impairs the ability of the receptor to mediate the activation of PI 3-kinase. Thus, by binding directly to p85, the phosphorylated YHTM motif in the COOH terminus of the insulin receptor contributes partially to mediating the effect of insulin to activate PI 3-kinase.

The tyrosine kinase activity of the insulin receptor plays a necessary role in mediating insulin action (1, 2). Like other receptor tyrosine kinases, ligand binding leads to autophosphorylation of the receptor (3, 4). Autophosphorylation of the insulin receptor is functionally significant in that it activates the receptor tyrosine kinase to phosphorylate other intracellular proteins such as insulin receptor substrate-1 (IRS-1) (5, 6). IRS-1 serves as an intermediate docking protein that contains multiple phosphotyrosine residues, providing binding sites for multiple SH2 domain-containing proteins (7). However, unlike most other growth factor receptor tyrosine kinases, phosphotyrosine residues in the insulin receptor are generally not thought to serve as major binding sites for SH2 domain-containing proteins in intact cells. Nevertheless, one of the phosphorylation sites in the COOH-terminal domain of the insulin receptor is located in a YHTM sequence (amino acid residues 1322-1325) that conforms to the YXMX motif which defines binding sites for the p85 subunit of PI 3-kinase (8, 9). Furthermore, in vitro studies in cell-free systems have demonstrated that p85 can bind directly to phosphorylated insulin receptors (10, 11). In the present work, we conducted immunoprecipitation studies demonstrating that p85 binds directly to phosphorylated insulin receptors and that this interaction requires the COOH-terminal 43 amino acids of the insulin receptor. These data support the hypothesis that p85 binds directly to the phosphotyrosine residue in the YHTM sequence in the COOH-terminal domain of the β-subunit of the insulin receptor. Furthermore, deletion of the COOH-terminal 43 amino acids partially impairs the ability of the receptor to mediate the action of insulin to activate PI 3-kinase. Nevertheless, these data do not necessarily contradict the hypothesis that IRS-1 is required for optimal activation of PI 3-kinase. For example, the presence of two SH2 domains in p85 may permit the molecule to bridge between the phosphorylated YHTM sequence in the insulin receptor and a phosphorylated YXXM motif in IRS-1.

MATERIALS AND METHODS

Expression of Insulin Receptors by Transfection of cDNA in Cultured Cells—NIH-3T3 cells were stably transfected with expression vector for human insulin receptor cDNA (12) or a truncated receptor lacking the 43 amino acid residues from the COOH terminus of the β-subunit as described previously (13). Expression of insulin receptors was assayed by measuring 125I-insulin binding (14) and/or immunoblotting (15). Based upon Scatchard analysis of insulin binding data, we estimate that there are approximately 400,000 wild type human insulin receptors/cell or approximately 300,000 Δ43 truncated receptors/cell expressed on the cell surface of the transfected cells.

Immunoprecipitation—Confluent cells in 15-cm Petri dishes, grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, were incubated in the presence or the absence of 17B-estradiol for 3 min at 37 °C. The cells were quickly washed once with ice-cold phosphate-buffered saline followed by two washes with washing buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, 100 mM NaVO4). Thereafter, the cells were solubilized in 1 ml of washing buffer containing Nonidet P-40 (1%), glycercol (10%), phenylmethylsulfonyl fluoride (2 mM). After normalization for protein concentration, about one-fifth of the cell lysate was immunoprecipitated using either a monoclonal antiphosphotyrosine antibody (Upstate Biotechnology Inc., Lake Placid, NY) at a concentration of 2.5 μg/ml, or a polyclonal antibody directed against the p85 regulatory subunit of PI 3-kinase (Upstate Biotechnology Inc.) at a dilution of 1:250, or B-10, an anti-insulin receptor antibody directed against the α-subunit at a dilution of 1:100 or a mixture of antibodies directed against IRS-1 or a non-immune rabbit serum (1:100 dilution). To achieve efficient immunoprecipitation of IRS-1, we used a mixture of two different polyclonal antibodies, at a final dilution of 1:300 (Upstate Biotechnology Inc.) and a monoclonal antibody, at a final concentration of 250 ng/ml (Transduction Laboratories). The immune complexes were precipitated with protein A-agarose.
(Life Technologies, Inc.) in which nonspecific sites were saturated by washing with a buffer containing Tris-HCl (10 mM, pH 7.5) and albumin (10 mg/ml). The immunoprecipitates were washed once with phosphate-buffered saline containing Nonidet P-40, and vanadate (100 μM), twice with a buffer containing Tris-HCl (100 mM, pH 7.5), LiCl (600 mM), vanadate (100 μM), and once with a buffer containing Tris-HCl (10 mM, pH 7.5), NaCl (100 mM), EDTA (1 mM), and vanadate (100 μM).

Phosphatidylinositol 3-Kinase Activity—After the washings described above, the pellet was resuspended in 40 μl of a buffer containing Tris-HCl (10 mM, pH 7.5), NaCl (100 mM), EDTA (1 mM). To each tube was added 10 μl of MnCl2 (100 mM) and 20 μg of phosphatidylinositol (Sigma). The phosphorylation reaction was started by the addition of 10 μl of ATP (40 μM) containing 30 μCi of [γ-32P]ATP. After 10 min, the reaction was stopped by the addition of 20 μl of HCl (8 M) and 160 μl of CHCl3/methanol (1:1). The organic phase was extracted and applied to a silica gel thin layer chromatography plate (Merck). Thin layer chromatography plates were developed in CHCl3/CH3OH/H2O/NH4OH (60:47:11.3:2), dried, and visualized by autoradiography. The radioactivity was quantitated using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) (16, 17).

Immunoblotting—After immunoprecipitation, the complexes were boiled in 40 μl of Laemmli sample buffer containing dithiothreitol (80 mM) for 3 min. The samples were analyzed by SDS-polyacrylamide gel electrophoresis. Proteins were transferred from the gel to nitrocellulose sheets by electroblotting at 90 V for 1 h at 4 °C in a solution containing Tris (25 mM), glycine (192 mM), and methanol (20%). The immunoblots were probed either with a monoclonal antiphosphotyrosine antibody (Upstate Biotechnology Inc.) (16, 17), or a rabbit antibody (rAb-53) directed against a peptide corresponding to the amino acids 1142-1157 of the β-subunit of the human insulin receptor (1:2000), and proteins were detected by Enhanced Chemiluminescence using horseradish peroxidase-labeled anti-mouse γ-globulin (Amersham Corp.). Thereafter, where indicated, the blots were stripped off as described elsewhere (15) and reprobed as described above.

RESULTS

Insulin Receptor Coimmunoprecipitates with PI 3-Kinase—The p85 regulatory subunit of PI 3-kinase binds to phosphotyrosine residues in the context of YXXM or YXXY sequences (9, 18). Binding of p85 to phosphotyrosine residues leads to activation of PI 3-kinase (9, 19). Because the YHTM sequence (amino acid residues 1322-1325) in the COOH terminus of the insulin receptor represents a YXXM motif, we inquired whether PI 3-kinase binds to phosphorylated insulin receptor in intact cells. NIH-3T3 cells transfected with human insulin receptor cDNA were incubated in the presence or absence of 100 nM insulin (Fig. 1). Receptors were immunoprecipitated with anti-receptor antibody, and immunoblots were probed with either antiphosphotyrosine antibody (Fig. 1, panel A) or anti-p85 antibody (panel B). As expected, insulin increased the content of phosphotyrosine in the wild type insulin receptor (Fig. 1A, lanes 3 and 4). In addition, insulin increased the amount of p85 regulatory subunit of PI 3-kinase coimmunoprecipitated with the insulin receptor (Fig. 1B, lanes 3 and 4). Comparable studies were carried out in cells expressing the p43 truncated mutant form of the insulin receptor that lacks the 43 amino acids at the COOH terminus of the human insulin receptor (11, 20). The p43 mutant lacks Tyr1322 and the YXXM motif (20-22). Consistent with previous observations (20, 21), insulin increased the content of phosphotyrosine in the p43 mutant insulin receptor (Fig. 1A, lanes 5 and 6). Although, both the 3T3-WT and the 3T3-p43 cells expressed a similar amount of insulin receptor (Fig. 1B, lanes 4 and 6; Fig. 3C, lanes 1 and 2), the p85 subunit of PI 3-kinase was not coimmunoprecipitated with the p43 mutant insulin receptor (Fig. 1B, lanes 5 and 6). These observations are consistent with the conclusion that the p85 subunit of PI 3-kinase binds to the phosphotyrosine at position 1322 in the phosphorylated insulin receptor.

Similar results were obtained when the same cell extracts were immunoprecipitated with antibody directed against p55 (Fig. 2). In cells expressing full-length human insulin receptors, exposure of cells to insulin increased the quantity of insulin receptors coimmunoprecipitated by anti-p85 antibody (Fig. 2A, lanes 3 and 4). In contrast, phosphorylated p43 mutant insulin receptors were not coimmunoprecipitated by anti-p85 antibody (Fig. 2A, lanes 5 and 6). Immunoblots probed with anti-p85 antibody demonstrated that p85 was expressed in comparable levels in the transfected cells expressing either full-length insulin receptors (Fig. 2B, lanes 3 and 4) or p43 mutant insulin receptors (Fig. 2B, lanes 5 and 6).
phosphotyrosine content of both full-length insulin receptors (Fig. 3A, lanes 3 and 4) and Δ43 mutant insulin receptors (Fig. 3A, lanes 5 and 6). In addition, both forms of the insulin receptor (i.e. full-length and Δ43 mutant) were capable of phosphorylating IRS-1 (Fig. 3A, Fig. 3A). Therefore, 100 ng insulin led to comparable increases in the content of p85 that bound to IRS-1 in cells expressing either full-length insulin receptors (Fig. 3B, lanes 3 and 4) or Δ43 mutant receptors (Fig. 3B, lanes 5 and 6). Thus, deletion of 43 amino acids from the COOH terminus of the receptor as well as the COOH terminus of the insulin receptor was not required for insulin to stimulate binding of PI 3-kinase to the IRS-1 molecule.

Quantitative Comparisons of Immunoprecipitations with Various Antibodies—We carried out sequential immunoprecipitation studies to estimate the efficiencies of immunoprecipitation with various antibodies (Fig. 4). When two sequential immunoprecipitation steps were carried out with either anti-p85 antibody (Fig. 4, lane 7) or anti-IRS-1 antibody (Fig. 4, lane 8), we did not detect the cognate antigen in the pellet of the second immunoprecipitation. Thus, we conclude that, under our conditions, these two antibodies have immunoprecipitated ~100% of the antigen (i.e. either p85 or IRS-1, respectively). In contrast, when samples were subjected to two sequential immunoprecipitations with anti-insulin receptor antibody B-10, approximately 30% of the insulin receptors were recovered in the pellet of the second immunoprecipitate (Fig. 4, lane 9 and data not shown). Thus, under our experimental conditions, antibody B-10 immunoprecipitated ~60–70% of the insulin receptors contained in the cell extract.

In the same experiment, we compared the quantity of p85 immunoprecipitated by anti-p85 and anti-insulin receptor antibodies (Fig. 4, lanes 1–4). Under our experimental conditions, both antibodies immunoprecipitated similar quantities of p85. However, it remains possible that IRS-1 is present in the complex that contains p85 and the insulin receptor. For example, it is possible that one SH2 domain of p85 binds IRS-1 while the second SH2 domain binds the insulin receptor.

Δ43 Truncation Impairs the Ability of Insulin to Activate PI 3-Kinase in Intact Cells—Insulin stimulates phosphorylation of YXXM motifs in the COOH terminus of the receptor as well as in IRS-1. As shown above, the p85 subunit of PI 3-kinase binds to both the receptor and IRS-1. If the association of PI 3-kinase with IRS-1 were the only mechanism involved in mediating the increase in PI 3-kinase activity, then deleting the YXXM motif from the COOH terminus of the receptor would not be predicted to alter the ability of insulin to activate PI 3-kinase. Thus, we designed an experiment to test this prediction (Fig. 5). Cells were incubated for 3 min in the presence or absence of 100 ng insulin. Although insulin had no detectable effect upon PI 3-kinase in cells transfected with only the neomycin resistance gene,
transfection with full-length human insulin receptors allowed for insulin to stimulate PI 3-kinase activity 10-fold. In contrast, the effect of insulin upon PI 3-kinase activity was much smaller (~1.2-fold) in cells expressing the Δ43 truncated receptor.

**DISCUSSION**

The p85 regulatory subunit of PI 3-kinase contains two SH2 domains that bind to phosphorylated YMXM and YXXM motifs in several growth factor receptors, non-receptor tyrosine kinases, and other docking proteins such as IRS-1 (9, 19, 28). Indeed, it has been clearly demonstrated that PI 3-kinase binds to IRS-1 through one or more of its nine YXXM/YMXM motifs (7, 28). Nevertheless, the presence of a phosphorylated YHTM motif in the COOH-terminal domain of the insulin receptor raises the possibility that this may also provide a binding site for SH2 domains of the p85 subunit of PI 3-kinase. In this study, we confirm the existence of signaling complexes containing insulin receptors, IRS-1, and p85 subunit of PI 3-kinase in intact cells. By using a coimmunoprecipitation technique, we characterize the nature of the binding interactions that stabilize these signaling complexes in intact cells. We demonstrate that deletion of the COOH-terminal YHTM sequence markedly decreased the coimmunoprecipitation of p85 by antibodies directed against the insulin receptor as well as the coimmunoprecipitation of the insulin receptor by antibodies directed against p85. These data strongly suggest that the COOH-terminal YHTM sequence is required for optimal binding of p85 to the insulin receptor in intact cells. Furthermore, deletion of the COOH-terminal 43 amino acids partially impairs the ability of the insulin receptor to mediate insulin action to activate PI 3-kinase. This suggests that binding of p85 to the COOH-terminal YHTM sequence has physiological significance.

**Fig. 4.** Quantitative comparison of immunoprecipitations with various antibodies. Extracts of cells expressing wild type human insulin receptor were immunoprecipitated with one of the following antibodies: anti-IRS-1 (lanes 1 and 2), anti-human insulin receptor (B-10; lanes 3 and 4), or anti-p85 (lanes 5 and 6). In several cases, the supernatant of the immunoprecipitation was subjected to a second immunoprecipitation with the same antibody (lanes 7–9). Finally, the immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis followed by immunoblotting with either anti-p85 (lanes 1–7) or antiphosphotyrosine antibodies (lanes 8 and 9). These experiments were carried out as described under "Materials and Methods" with two exceptions. First, we used a polyclonal anti-IRS-1 antibody directed against the COOH terminus of rat IRS-1 (1:100 dilution). Second, we used Ultra-Link Immobilized Protein A Plus supplied by Pierce rather than protein A-agarose supplied by Life Technologies, Inc.

**Fig. 5.** Δ43 truncation impairs the ability of insulin to activate PI 3-kinase in intact cells. Antiphosphotyrosine immunoprecipitates from quiescent or insulin-treated cells expressing either the neomycin resistance gene alone, the wild type (WT) insulin receptor, or the Δ43 truncated insulin receptor were assayed for PI 3-kinase activity. Radioactivity was quantitated using a PhosphorImager. Data represent the mean ± S.E. and are representative of three separate experiments. Results are expressed as a percentage of the PI 3-kinase activity measured in insulin-stimulated cells expressing the wild type receptor.
of the Δ43 truncation upon the ability of the insulin receptor to mediate insulin’s action to increase PI 3-kinase activity in intact cells (11, 20, 22). Nevertheless, in our experimental system, the Δ43 truncation partially impaired the ability to activate PI 3-kinase despite the fact that the truncation did not impair phosphorylation of IRS-1. These observations are consistent with the hypothesis that the direct binding interaction between p85 and the insulin receptor contributes importantly to the ability of insulin to activate PI 3-kinase. It is not clear how to explain apparent discrepancies between our data and previously reported observations. However, variations in the levels of expression of PI 3-kinase, IRS-1, and insulin receptors may modulate the relative contributions of the two mechanisms of PI 3-kinase activation, i.e., the direct mechanism mediated by binding of p85 to insulin receptors versus the indirect mechanism mediated by binding of p85 to IRS-1. In order to fully assess the physiological significance of these binding interactions, it will be necessary to carry out similar studies in physiologically relevant target tissues such as skeletal muscle, liver, and adipose tissue. Nevertheless, the present studies demonstrate that it is possible for insulin receptors to bind directly to p85 in intact cells and suggest that this binding interaction may contribute to the mechanism whereby insulin activates phosphatidylinositol 3-kinase in transfected cells grown in tissues culture cells. It is now possible to express the Δ43 truncated receptor in transgenic mice; this would allow for experiments to address the physiological significance of direct binding interactions between the insulin receptor and p85 in vivo.

Conclusions—Over the past several years, considerable progress has been made in elucidating the signaling pathways downstream from tyrosine phosphorylation. It is becoming increasingly clear that these pathways are complex and contain many branches. From the beginning, it was obvious that the branches often diverged from a common point. One tyrosine kinase may phosphorylate many proteins; for example, in addition to autophosphorylation, the insulin receptor phosphorylates IRS-1 (5, 7), s6c (29, 30), ecto-ATPase (31), etc. Similarly, a single phosphoprotein contains phosphotyrosine residues that bind multiple SH2 domain containing proteins; for example, phosphorylated IRS-1 binds PI 3-kinase (32), growth factor receptor binding protein-2 (GRB-2) (29), SH2-containing phosphotyrosine phosphatase two-dimensional (33), and nck (34). More recently, it has become clear that there are also converging branches in the pathways. For example, there are two pathways from the insulin receptor that lead to activation of GRB-2/m-SOS; one pathway involves phosphorylation of IRS-1 while the other involves phosphorylation of Sch (29). Similarly, in the present study, we demonstrate that there are two pathways whereby the insulin receptor can contribute to the activation of PI 3-kinase. In addition to the previously recognized indirect pathway that requires phosphorylation of IRS-1, our data suggest that the phosphorylated YXXM motif in the COOH terminus of the insulin receptor binds directly to the p85 regulatory subunit of PI 3-kinase and that this binding contributes to the activation of PI 3-kinase. This direct interaction between a phosphorylated receptor and PI 3-kinase closely resembles the major pathway whereby most growth factor receptor tyrosine kinases directly activate PI 3-kinase. It seems likely that this type of redundancy in the pathways may provide additional opportunities for regulation of crucial enzyme activities.

Acknowledgments—We thank Dr. Efrat Wertheimer for critical reading of the manuscript. In addition, we are grateful to Dr. Axel Ullrich for generously providing insulin receptor cDNA.

REFERENCES
1. Chou, C. K., Dull, T. J., Russell, D. S., Gherzi, R., Lebwohl, D., Ulrich, A., and Rosen, O. M. (1987) J. Biol. Chem. 262, 1842–1847
2. Cam, Q. M., Quon, M. J., Sierra, M. L., and Taylor, S. I. (1993) J. Biol. Chem. 268, 8383–8389
3. Kasuga, M., Zick, Y., Blithe, D. L., Cretzax, M., and Kahn, C. R. (1982) Nature 298, 667–669
4. Rosen, O. M. (1987) Science 237, 1452–1458
5. White, M. F., Maron, R., and Kahn, C. R. (1985) Nature 318, 183–186
6. Sun, X. J., Mirlapixe, M. J., Burt, M. S., Lebwohl, D., Baker, B. G., Birge, R. B., Pazdro, J. E., Chou, M. M., Hanafuss, H., Schaffhausen, B., and Canley, L. C. (1993) Cell 72, 767–778
7. Backer, J. M., Myers, M. J., Shoelson, S. E., Chin, D. J., Sun, X. J., Mirlapixe, M., Hu, P., Margolis, B., Skolnick, E. Y., Schlessinger, J., and White, M. F. (1992) EMBO J. 11, 3469–3479
8. Myers, M. J. J., Backer, J. M., Siddle, K., and White, M. F. (1991) J. Biol. Chem. 266, 396–400
9. Kato, H., Faria, T. N., Stannard, S., Roberts, C. T. J., and LeRoith, D. R. (1993) J. Biol. Chem. 268, 2655–2661
10. Songyang, Z., Shoelson, S. E., Chauhan, M., Ghig, G., Pawson, T., Hafer, W. G., Go, F., Roberts, M. S., Meuser, T., Olefsky, J. M., and Cantley, L. C. (1992) J. Biol. Chem. 267, 440346
11. Canaan, A., Bell, J. R., Chen, K. Y., Harrera, R., Petruzelli, L. M., Dull, T. J., Gray, A., Cossens, L., Liao, Y. C., Taubokawa, M., Mason, A., Seeburg, P. H., Grunfeld, C., Rosen, O. M., and Ramachandran, J. (1985) Nature 313, 756–761
12. Levy-Toledano, R., Caro, L. A., Accili, D., and Taylor, S. I. (1994) EMBO J. 13, 856–862
13. Kadrowski, H., Kadowaki, T., Cama, A., Marcus Samaesa, B., Rovira, A., Bevina, C. L., and Taylor, S. I. (1990) J. Biol. Chem. 265, 21285–21286
14. Levy-Toledano, R., Caro, L. H. F., Hindman, N., and Taylor, S. I. (1993) Endocrinology 133, 1903–1908
15. Endemann, G., Yorozua, K., and Roth, R. A. (1990) J. Biol. Chem. 265, 396–400
16. Kato, H., Faria, T. N., Stannard, S., Roberts, C. T. J., and LeRoith, D. R. (1993) J. Biol. Chem. 268, 2655–2661
17. Songyang, Z., Shoelson, S. E., Chauhan, M., Ghig, G., Pawson, T., Hafer, W. G., Go, F., Roberts, M. S., Meuser, T., Olefsky, J. M., and Cantley, L. C. (1992) J. Biol. Chem. 267, 440346
18. Canaan, A., Bell, J. R., Chen, K. Y., Harrera, R., Petruzelli, L. M., Dull, T. J., Gray, A., Cossens, L., Liao, Y. C., Taubokawa, M., Mason, A., Seeburg, P. H., Grunfeld, C., Rosen, O. M., and Ramachandran, J. (1985) Nature 313, 756–761
19. Levy-Toledano, R., Caro, L. A., Accili, D., and Taylor, S. I. (1994) EMBO J. 13, 856–862
20. Kadrowski, H., Kadowaki, T., Cama, A., Marcus Samaesa, B., Rovira, A., Bevina, C. L., and Taylor, S. I. (1990) J. Biol. Chem. 265, 21285–21286
21. Levy-Toledano, R., Caro, L. H. F., Hindman, N., and Taylor, S. I. (1993) Endocrinology 133, 1903–1908
22. Endemann, G., Yorozua, K., and Roth, R. A. (1990) J. Biol. Chem. 265, 396–400
23. Kate, T., Faria, T. N., Stannard, S., Roberts, C. T. J., and LeRoith, D. R. (1993) J. Biol. Chem. 268, 2655–2661