JAK1-dependent Phosphorylation of Insulin Receptor Substrate-1 (IRS-1) Is Inhibited by IRS-1 Serine Phosphorylation*

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Serine phosphorylation of insulin receptor substrate-1 (IRS-1) reduces its ability to act as an insulin receptor substrate and inhibits insulin receptor signal transduction. Here, we report that serine phosphorylation of IRS-1 induces tyrosine phosphorylation by either okadaic acid (OA) or chronic insulin stimulation prevents interferon-α (IFN-α)-dependent IRS-1 tyrosine phosphorylation and IFN-α-dependent IRS-1/phosphatidylinositol 3'-kinase (PI3K) association. In addition, we demonstrate that serine phosphorylation of IRS-1 renders it a poorer substrate for JAK1 (Janus kinase-1). We found that treatment of U266 cells with OA induced serine phosphorylation of IRS-1 and completely blocked IFN-α-dependent tyrosine phosphorylation of IRS-1 and IFN-α-dependent IRS-1/PI3K association. Additionally, IRS-1 from OA-treated cells could not be phosphorylated in vitro by IFN-α-activated JAK1. Chronic treatment of U266 cells with insulin led to a 50% reduction in IFN-α-dependent tyrosine phosphorylation of IRS-1 and IFN-α-dependent IRS-1/PI3K association. More importantly, serine-phosphorylated IRS-1-(511–722) could not be phosphorylated in vitro by IFN-α-activated JAK1. Taken together, these data indicate that serine phosphorylation of IRS-1 prevents its subsequent tyrosine phosphorylation by JAK1 and suggest that IRS-1 serine phosphorylation may play a counter-regulatory role in pathways outside the insulin signaling system.

Tyrosine phosphorylation of IRS-1 transfers tyrosine-phosphorylated IRS-1 coordinates the intracellular signaling and the American Diabetes Association (all to G. G. F.).

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The abbreviations used are: IRS, insulin receptor substrate; IL, interleukin; IFN, interferon; PI3K, phosphatidylinositol 3'-kinase; OA, okadaic acid; PAS kinase, PI3K-associated serine kinase; PAGE, polyacrylamide gel electrophoresis; GST, glutathione S-transferase.

EXPERIMENTAL PROCEDURES

Materials—The myeloma cell line U266 was purchased from American Type Culture Collection (Manassas, VA). [γ-32P]ATP and 32P, were...
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Purchased from Amersham Pharmacia Biotech. Anti-Pi3K p85 (catalog no. 06-195), anti-phosphotyrosine (catalog no. 05-321), and anti-IRS-1 (catalog no. 06-248C) antisera were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-JAK1 antiserum (catalog no. 15296E) was purchased from Pharmingen (San Diego, CA). Neutrophil cytosol was purified from U266 cells purchased from American Type Culture Collection (Rockville, MD). Protein G-Sepharose, glutathione-Sepharose, Precission protease, and pGEX6P3 vector were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Recombinant human IFN-α was purchased from Intergen Co. (Purchase, NY). Polyvinylidene difluoride membrane was purchased from Bio-Rad. Cellulose-coated TLC plates were purchased from Schleicher & Schuell (Keene, NH). Minifilter columns (catalog no. Q8O) were purchased from Midwest Scientific (Valle Park, MO). All other cell culture reagents and chemicals were purchased from Sigma. Oligonucleotide primers were purchased from Operon Technologies, Inc. (Alameda, CA). All other molecular biology reagents and chemicals were purchased from Promega.

Cell Culture—U266 cells were grown in growth medium (RPMI 1640 medium supplemented with 10% neonatal bovine serum, 2 g/liter glucose, 100,000 units/liter penicillin, and 100 g/liter streptomycin). Cells were passaged 1:1 with fresh medium every 3 days. For OA treatment, cells were washed twice and resuspended in growth medium supplemented with 1 μM OA. For insulin treatment, cells were washed twice and resuspended in growth medium supplemented with 1 nM insulin.

PI3K Assays—PI3K assays were performed as described previously (36). In brief, 20 × 10⁶ cells/ml were treated as indicated and lysed in 1 ml of ice-cold lysis buffer (1% Nonidet P-40, 100 mM NaCl, 50 mM NaF, 1 mM dithiothreitol, 25 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 2 mM sodium orthovanadate, 250 μM okadaic acid, and 50 mM Tris, pH 7.4). IRS-1 was immunoprecipitated from lysates with 4 μl of anti-IRS-1 antiseraum/test, and the resultant immune complexes were washed extensively. Kinase reactions were performed in 100 μl of buffer containing 0.33 mg/ml l-α-phosphatidylinositol, 7.5 mM MgCl₂, 0.4 mM EGTA, 0.4 mM NaPO₄, 7.5 μM [γ-³²P]ATP (13 Ci/nmol), and 20 mM HEPES, pH 7.1, at 22 °C for 15 min. The assay conditions used were linear with respect to time and amount of kinase. Phospholipids were extracted with 1.1 chloroform/methanol and resolved on silica gel plates by TLC in chloroform/methanol/4% ammonium hydroxide (75:58:17). Results were analyzed by autoradiography on a Molecular Dynamics PhosphorImager system.

Western Analysis—Western analysis was performed as described previously (37). In brief, 20 × 10⁶ cells/ml were treated as indicated and lysed in 1 ml of ice-cold lysis buffer. Proteins of interest were immunoprecipitated with the indicated antiseraum/test, and the resultant immune complexes were washed extensively. Proteins were resolved by SDS-PAGE under reducing conditions on 10% gels, electrotransferred to polyvinylidene difluoride membrane, and probed with the indicated antiseraum. Immunoreactive proteins were visualized by secondary detection using an 125I-labeled goat anti-rabbit antibody, followed by autoradiography and densitometry.

Oligonucleotide primers were purchased from Operon Technologies, Inc. (Alameda, CA). All other molecular biology reagents and chemicals were purchased from Promega.

Whole Cell Phosphorylation/Phosphoamino Acid Analysis—Whole cell phosphorylation was performed as described previously (37). In brief, 20 × 10⁶ cells were suspended in 1 ml of phosphate-free RPMI medium supplemented with 0.75 mM CaCl₂, 0.33 mM MgCl₂, and 20 mM HEPES, pH 7.4, at 37 °C for 1.5 h. For OA treatment, 1 μM OA was added at 1.5 h for 30 min. Cells were lysed, and IRS-1 was immunoprecipitated as described above. Phosphoproteins were resolved by SDS-PAGE under reducing conditions on 8% gels. For phosphoamino acid analysis, phosphoproteins were separated on cellulose-coated plates by high voltage TLC, and standards were visualized with ninhydrin. Results were analyzed by autoradiography and densitometry.

Lysate Kinase Assays—Lysate kinase assays were performed as described previously (37). In brief, PAS kinase was purified from 20 × 10⁶ cells by affinity chromatography using glutathione-Sepharose-bound GST-p85 protein. Kinase reactions were performed in 100 μl of reaction buffer containing 5 μg/ml IRS-1 (511–772), 0.4 mM EGTA, 0.4 mM NaPO₄, 1 μM [γ-³²P]ATP (100 μCi/nmol), and 20 mM HEPES, pH 7.1, at 22 °C with or without 10 mM MgCl₂, as a cofactor. For JAK1 kinase assays, IRS-1 (511–772) was prephosphorylated in the absence of [γ-³²P]ATP and recovered by filtration through minifilter columns. IFN-α-activated JAK1 was isolated as described above, and JAK1 kinase assays using prephosphorylated IRS-1 (511–772) as a substrate were performed as described above. Reactions were terminated using SDS-PAGE loading buffer and were run in parallel with respect to time and amount of kinase. Phosphoproteins were resolved by SDS-PAGE under reducing conditions on 7–20% gradient gels and examined by autoradiography and densitometry.

RESULTS

OA Blocks IFN-α-dependent IRS-1/Pi3K Activation and Association—Serine phosphorylation of IRS-1 induced by OA treatment of 3T3-L1 adipocytes stops insulin-dependent activation of PI3K (26). To determine if IFN-α-mediated PI3K activation was prevented by OA, IRS-1-associated PI3K activity was examined in U266 cells pretreated with 1 μM OA for 30 min. Fig. 1A demonstrates that 1000 units/ml IFN-α induced 20-, 22-, and 10-fold increases in IRS-1-associated PI3K activity at 5, 10, and 30 min, respectively, and that pretreatment of cells with OA blocked this response. OA did not inhibit PI3K activity directly because PI3K activity in Pi3K p85 immune complexes from OA-treated cells was no different than that from non-OA-treated cells (data not shown). To determine if this failure to activate PI3K was due to a loss of IFN-α-dependent IRS-1/Pi3K association, Western analysis was performed.
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Fig. 1. OA blocks IFN-α-dependent IRS-1/PI3K activation and association. A, U266 cells were pretreated with (OA) or without (Control) 1 μM OA for 30 min and then treated with 1000 units/ml IFN-α for the times indicated. PI3K (PIP) activity was measured in IRS-1 immunoprecipitates. B, cells were treated as described for A, and Western analysis was used to detect PI3K p85 (p85) in IRS-1 immunoprecipitates (IP) using an anti-p85 antibody. C, cells were treated as described for A, and Western analysis was used to detect IRS-1 tyrosine phosphorylation using an anti-phosphotyrosine antibody (pY). D, cells were pretreated with (+) or without (−) 1 μM OA for 30 min, and Western analysis was performed on IRS-1, p85, and JAK1 immunoprecipitates using the antibodies indicated. E, cells were pretreated with (+) or without (−) OA for 30 min as indicated and then treated with or without 1000 units/ml IFN-α for 5 min. JAK1 was immunoprecipitated, and JAK1 kinase assays were performed in the presence (right panel) or absence (left panel) of IRS-1-(511–772). All data are representative of triplicate experiments.

Fig. 1B shows that IFN-α-dependent IRS-1/PI3K association was increased at 5, 10, and 30 min (as measured by Western detection of PI3K p85) and that OA inhibited this association. To determine if this OA-dependent decline in IRS-1/PI3K association was due to an inhibition of IFN-α-dependent tyrosine phosphorylation of IRS-1, Western analysis was again performed. Fig. 1C shows that OA increased IRS-1 tyrosine phosphorylation at 5, 10, and 30 min and that OA blocked detectable tyrosine phosphorylation of IRS-1. To confirm that OA did not measurably alter IRS-1, p85, and JAK1 protein levels and the ability of these proteins to be immunoprecipitated by their respective antibodies, Western analysis was performed. Fig. 1D demonstrates that IRS-1, p85, and JAK1 protein levels and their ability to be immunoprecipitated were unaffected by OA. Finally, to show that OA did not affect JAK1 autophosphorylation or its ability to phosphorylate in vitro substrates, JAK1 kinase assays were performed. Fig. 1E demonstrates that JAK1 isolated from OA-treated cells phosphorylated recombinant IRS-1-(511–772) as well as JAK1 recovered from non-OA-treated cells and that JAK1 autophosphorylation was unchanged. Taken together, these findings indicate that OA blocks IFN-α-dependent IRS-1/PI3K association by a mechanism that inhibits tyrosine phosphorylation of IRS-1, but does not alter JAK1 kinase activity.

JAK1-dependent Phosphorylation of IRS-1 Is Inhibited by OA—Serine phosphorylation of IRS-1 blocks insulin receptor-dependent tyrosine phosphorylation of IRS-1 (26–35). To determine if OA inhibited JAK1-dependent IRS-1 tyrosine phosphorylation, JAK1 kinase assays were performed. Fig. 2A demonstrates that when IRS-1 isolated from OA-treated cells was used as a substrate for JAK1, IFN-α-dependent JAK1 phosphorylation was not observed. In contrast, when IRS-1 from non-OA-treated cells was used as a substrate for JAK1, IFN-α induced a 5-fold increase in JAK1-dependent IRS-1 phosphorylation. To examine the phosphorylation state of IRS-1 isolated from OA-treated cells, phosphorylmo acid analysis was performed. These experiments showed that IRS-1 was predominantly phosphorylated on serine residues and that no tyrosine phosphorylation was detected (Fig. 2B). Taken together, these findings indicate that serine phosphorylation of IRS-1 induced by OA renders IRS-1 a poorer substrate for JAK1.

Chronic Insulin Treatment Inhibits IFN-α-dependent IRS-1/PI3K Activation and Association—Chronic hyperinsulinemia induces serine phosphorylation of IRS-1 and reduces insulin signaling (33, 35, 40–42). To determine if chronic insulin treatment inhibited IFN-α-dependent activation of PI3K, IRS-1-associated PI3K activity was examined in U266 cells pretreated with 1 nM insulin for 18 h. Fig. 3A demonstrates that 1000 units/ml IFN-α induced a 10-fold increase in IRS-1-associated PI3K activity at 5 min and that pretreatment of cells with insulin reduced this response by 50%. Chronic insulin treatment did not inhibit PI3K activity directly because PI3K activity in PI3K p85 immune complexes from insulin-treated cells was not different than that from non-insulin-treated cells (data not shown). To determine if this reduction in PI3K activation was due to a loss of IFN-α-dependent IRS-1/PI3K association, Western analysis was performed. Fig. 3B shows that IFN-α increased IRS-1/PI3K association at 5 min (as measured by detection of PI3K p85) and that chronic insulin treatment reduced this association by 50%. To further examine the impact of chronic insulin treatment on IRS-1/PI3K association, IRS-1 present in PI3K immune complexes was examined by Western analysis (Fig. 3C). As in Fig. 3B, chronic insulin treatment
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Reduction of IFN-α-dependent IRS-1/PI3K association, and comparably low amounts of IRS-1 were associated with PI3K before and after chronic insulin treatment in cells not treated with IFN-α. To determine if this insulin-dependent decline in IRS-1/PI3K association after IFN-α treatment was due to an inhibition of IFN-α-dependent tyrosine phosphorylation of IRS-1, Western analysis was again performed. Fig. 3D shows that IFN-α increased IRS-1 tyrosine phosphorylation at 5 min and that chronic insulin treatment reduced IFN-α-dependent tyrosine phosphorylation of IRS-1 by 50%. Additionally, chronic insulin treatment did not alter JAK1 activity in that IFN-α-activated JAK1 isolated from chronically insulin-treated cells phosphorylated recombinant IRS-1-(511–772) as well as JAK1 recovered from non-insulin-treated cells (data not shown).

Finally, to confirm that chronic insulin treatment did not measurably alter IRS-1, p85, and JAK1 protein levels and the ability of these proteins to be immunoprecipitated by their respective antibodies, Western analysis was performed. Fig. 3E demonstrates that IRS-1, p85, and JAK1 protein levels and the ability to be immunoprecipitated were unaffected by chronic insulin treatment. Taken together, these findings indicate that chronic insulin treatment inhibits IFN-α-dependent IRS-1/PI3K association by a mechanism that reduces tyrosine phosphorylation of IRS-1, but does not alter JAK1 kinase activity.

Serine Phosphorylation of IRS-1-(511–772) Inhibits Its Phosphorylation by JAK1—Phosphorylation of IRS-1 by serine kinases renders it a poorer substrate for the insulin receptor (27–35). To determine if serine phosphorylation of IRS-1 inhibits its ability to act as a JAK1 substrate, kinase assays were performed. Fig. 4A shows that plasma membrane-depleted lysates from U266 cells treated with 1 nM insulin for 18 h contained kinase activity that phosphorylated IRS-1-(511–772) exclusively on serine residues. Fig. 4B demonstrates that phosphorylation of IRS-1-(511–772) by plasma membrane-depleted serine kinase activity reduced by 50% the ability of IRS-1-(511–772) to serve as a substrate for IFN-α-activated JAK1. Fig. 4C demonstrates that IRS-1-(511–772) was a substrate for the serine kinase PAS kinase (34) and that phosphorylation of IRS-1-(511–772) by PAS kinase reduced by 75% the ability of IRS-1-(511–772) to serve as a substrate for IFN-α-activated JAK1 (Fig. 4D). These results indicate that serine phosphorylation of IRS-1 inhibits its ability to act as a JAK1 substrate.

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**Fig. 3.** Chronic insulin treatment inhibits IFN-α-dependent IRS-1/PI3K activation and association. A, U266 cells were pre-treated with (+) or without (−) 1 nM insulin for 18 h as indicated and then treated with (closed bars) or without (open bars) 1000 units/ml IFN-α for 5 min. PI3K activity was measured in IRS-1 immunoprecipitates. Data are representative of triplicate experiments ± S.E. B, cells were treated as described for A, and Western analysis was used to detect PI3K p85 (p85) in IRS-1 immunoprecipitates (IP) using an anti-p85 antibody. C, cells were treated as described for A, and Western analysis was used to detect IRS-1 in PI3K p85 (p85) immunoprecipitates using an anti-IRS-1 antibody. D, cells were treated as described for A, and Western analysis was used to detect IRS-1 tyrosine phosphorylation using an anti-phosphotyrosine antibody (pY). E, cells were treated with (+) or without (−) 1 nM insulin for 18 h as indicated, and Western analysis was performed on IRS-1, p85, and JAK1 immunoprecipitates using the antibodies indicated. Data in B–E are representative of triplicate experiments.

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**Fig. 4.** Serine phosphorylation of IRS-1-(511–772) inhibits its phosphorylation by JAK1. A, Plasma-membrane depleted cell lysates were prepared from U266 cells treated with 1 nM insulin for 18 h. Kinase assays using these lysates were performed with IRS-1-(511–772) as a substrate in the presence (+) or absence (−) of 10 mM MgCl₂ (left panel). Phosphoamino acid analysis was performed on IRS-1-(511–772) from the + lanes (right panel). B, IRS-1-(511–772) was prephosphorylated with (Insulin +) or without (Insulin −) serine kinase activity generated in A and then used as a substrate for JAK1 isolated from U266 cells treated with (+) or without (−) 1000 units/ml IFN-α for 5 min. C, PAS kinase was affinity-purified from U266 cells using GST-p85, PAS kinase assays were performed using IRS-1-(511–772) as a substrate in the presence (+) or absence (−) of 10 mM MgCl₂. D, IRS-1-(511–772) was prephosphorylated as described for C without [γ-32P]ATP and then used as a substrate for IFN-α-activated JAK1 isolated from U266 cells treated with 1000 units/ml IFN-α for 5 min. All data are representative of triplicate experiments.
Thus abrogating direct IRS-1/insulin receptor interaction. Like within the IH1 phosphotyrosine-binding domain appears to IRS-1, the mechanism of this effect is not clearly delineated. In the insulin receptor and now JAK1 to phosphorylate IRS-1-(511–772). Moreover, serine phosphorylation within the IRS-1 IH1 pleckstrin homology do- main inhibits this effect. This is important in that IRS-1-(511–772) does not contain either the IRS-1 IH1 pleckstrin homology or P85 subunit of PI3K through SH2 domain interactions and phosphorylates IRS-1 and inhibit the ability of IRS-1 to serve as a JAK1 substrate, that IRS-1 serine phosphorylation inhibits signal transduction in pathways outside the insulin system, and that hyperinsulinemia may alter signaling of JAK1-dependent cytokine receptors.

Inhibition of PP1 and PP2A serine phosphatases by OA and calycin A increase IRS-1 serine phosphorylation and leads to decreased insulin receptor-mediated IRS-1 tyrosine phosphorylation (26, 33). Chronic insulin treatment has also been shown to induce serine phosphorylation of IRS-1 and to inhibit insulin receptor-dependent phosphorylation of IRS-1 (33, 35, 40–42). Recently, the region of IRS-1 susceptible to chronic insulin treatment-dependent serine phosphorylation has been reported, and it appears to reside between amino acids 530 and 843 (35). The kinase responsible for this phosphorylation is unknown, but appears to be insensitive to inhibitors of protein kinases C and A, PI3K, and mitogen-activated protein kinase (35). We have identified a kinase (PAS kinase) that can serine phosphorylate IRS-1 and inhibit the ability of IRS-1 to serve as an insulin receptor substrate (34, 37). This kinase associates with the pS8 subunit of PI3K through SH2 domain interactions and phosphorylates IRS-1 in IRS-1/PI3K complexes after insulin stimulation (37). Here, we show that IRS kinase can phosphorylate IRS-1-(511–772) and that this phosphorylation inhibits the ability of IFN-α-activated JAK1 to subsequently phosphorylate IRS-1-(511–772).

Although serine phosphorylation of IRS-1 decreases the ability of the insulin receptor and now JAK1 to phosphorylate IRS-1, the mechanism of this effect is not clearly delineated. In the insulin signaling system, serine phosphorylation of IRS-1 within the IH1 phosphotyrosine-binding domain appears to impair NPXY-mediated IRS-1/insulin receptor association (33), thus abolishing direct IRS-1/insulin receptor interaction. Like the insulin receptor, the IL-4 receptor contains an NPXY motif, and this motif appears to coordinate the formation of receptor/JAK/IRS-1 complexes, which result in IRS-1 tyrosine phosphorylation (43). The IFN-α receptor does not contain an NPXY motif and may rely on the IRS-1 IH1 pleckstrin homology domain to coordinate receptor/JAK/IRS-1 association and subsequent IRS-1 phosphorylation (8). This suggests that serine phosphorylation within the IRS-1 IH1 pleckstrin homology domain might be important for preventing IFN-α-activated JAK1-dependent tyrosine phosphorylation of IRS-1. We show here, however, that IFN-α-activated JAK1 can phosphorylate IRS-1-(511–772) and that serine phosphorylation of IRS-1-(511–772) inhibits this effect. This is important in that IRS-1-(511–772) does not contain either the IRS-1 IH1 pleckstrin homology or IH2 phosphotyrosine-binding domain and suggests that other regions of IRS-1 may be important in IRS-1/JAK1 interactions.

Hyperinsulinemia and insulin resistance are prominent features in both syndrome X and the development of type 2 diabetes mellitus (44). However, the pathogenesis of the multiple complications and conditions associated with these diseases is not yet understood (45). We show here that chronic insulin treatment and IRS-1 serine phosphorylation decrease JAK1-mediated IRS-1 tyrosine phosphorylation and IRS-1/PI3K association, suggesting that cytokine signal transduction may be altered during hyperinsulinemia. Currently, a rapidly growing number of hormone and cytokine receptors appear to signal through JAK and IRS family members, and this appears to be critical to hormone/cytokine function (1). This is most clearly understood in IL-4 signaling, where IRS function has been shown to be critical to IL-4-dependent mitogenesis and antiapoptosis (4, 46). Additionally, site-specific mutagenesis of the phosphotyrosine-binding domain motif in the IL-4 receptor reduces both IRS and STAT6 tyrosine phosphorylation and abolishes the effect of IL-4 on the induction of DNA binding activity and CD23 induction (47). Thus, by inducing IRS serine phosphorylation, hyperinsulinemia may potentially contribute to the pathogenesis of syndrome X/type 2 diabetes mellitus complications by disrupting JAK-mediated cytokine and hormone signaling pathways that use IRS. In summary, we show that OA and chronic insulin treatment inhibit IFN-α-dependent IRS-1 tyrosine phosphorylation and IRS-1/PI3K association and activity. More importantly, we show that these effects are mediated by serine phosphorylation of IRS-1. We conclude that IRS-1 serine phosphorylation plays an inhibitory role in signaling pathways outside the insulin system and suggest that hyperinsulinemia may alter signaling of JAK1-dependent cytokine receptors through serine phosphorylation of IRS-1.

REFERENCES

1. Yenush, L., and White, M. F. (1997) Bioessays 19, 491–500.
2. White, M. F., Maron, R., and Kahn, C. R. (1985) Nature 318, 183–186.
3. Wang, L. M., Keegan, A. D., Paul, W. E., Heidaran, M. A., Gutchik, J. S., and Pierce, J. H. (1992) EMBO J. 11, 4899–4908.
4. Wang, L. M., Myers, M. G., Jr., Sun, X. J., Aaronson, S. A., White, M., and Pierce, J. H. (1993) Science 261, 1591–1594.
5. Johnston, J. A., Kawamura, M., Kirken, R. A., Chen, Y. Q., Blake, T. B., Shibuya, K., Ortadjo, R. J., McVicar, D. W., and OShea, J. J. (1994) Nature 370, 151–153.
6. Burfoot, M. S., Rogers, N. C., Watling, D., Smith, J. M., Pons, S., Paonnass, G., Pellegrini, S., White, M. F., and Kerr, I. M. (1997) J. Biol. Chem. 272, 24183–24190.
7. Uddin, S., Yenush, L., Sun, X. J., Sweet, M. E., White, M. F., and Platanias, L. C. (1995) J. Biol. Chem. 270, 15938–15941.
8. Platanias, L. C., Uddin, S., Yetter, A., Sun, X. J., and White, M. F. (1996) Mol. Biol. Cell 7, 271,278.
9. Uddin, S., Fish, E. N., Sher, D., Gardziole, C., Colamonechi, O. R., Kellum, M., Pitha, P. M., White, M. F., and Platanias, L. C. (1997) Blood 90, 2574–2582.
10. Domanski, P., and Colamonechi, O. R. (1996) Cytokine Growth Factor Rev. 7, 143–151.
11. Pellegrini, S., John, J., Shearer, M., Kerr, I. M., and Stark, G. R. (1989) Mol. Cell. Biol. 9, 4656–4661.
12. Velazquez, L., Fellous, M. G., and Pellegrini, S. (1992) Cell 70, 313–322.
13. Muller, M., Briseo, J., Laxton, C., Gischin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A. G., Barbieri, G., Wittulahn, B. A., Schindler, C., Pellegrini, S., Wilks, A. F., Ille, J. N., Stark, G. R., and Kerr, I. M. (1993) Nature 366, 129–135.
14. Colamonechi, O. R., Uyttendaele, H., Domanski, P., Yan, H., and Krolewski, J. J. (1994) J. Biol. Chem. 269, 3518–3522.
15. Novick, D., Cohen, B., and Rubinstein, M. (1994) Cell 77, 391–400.
16. Barbieri, G., Velazquez, L., Scrubagna, M., Fellous, M., and Pellegrini, S. (1994) Eur. J. Biochem. 223, 427–435.
17. Domanski, P., Witte, M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P., and Colamonechi, O. R. (1995) J. Biol. Chem. 270, 21606–21611.
18. Pfeiffer, J., Mullersman, J., Pfeiffer, S. R., Murti, A., Shi, W., and Yang, C. H. (1997) Science 276, 1418–1420.
19. Kapellner, R., and Cantley, L. C. (1994) Bioessays 16, 555–576.
20. Becker, J. M., Myers, M. G., Jr., Shoelson, S. E., Chinn, D. J., Sun, X. J., Miralpeix, M., Hu, P., Margolis, B., Skolnick, E. Y., Schlessinger, J., and White, M. F. (1992) EMBO J. 11, 3489–3497.
21. Shoelson, S. E., Sivaraj, M., Williams, K. P., Hu, P., Schlessinger, J., and Weiss, M. A. (1995) EMBO J. 14, 785–802.
22. Sun, X. J., Crimmins, D. L., Myers, M. G., Jr., Miralpeix, M., and White, M. F. (1993) Mol. Cell. Biol. 13, 4714–4728.
23. Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A.,
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Kapeller, R., and Soltoff, S. (1991) Cell 64, 281–302

24. Hiles, I. D., Otsu, M., Volinia, S., Pry, M. J., Gout, I., Dhand, R., Panayotou, G., Ruiz-Larrea, F., Thompson, A., Totty, N. F., Husain, J. J., Courtneidge, S. A., Parker, P. J., and Waterfield, M. D. (1992) Cell 70, 419–429

25. Uddin, S., Fish, E. N., Sher, D. A., Gardziola, C., White, M. F., and Plataniotis, L. C. (1997) J. Immunol. 158, 2390–2397

26. Tanti, J. F., Gremeaux, T., van Obberghen, E., and Le Marchand-Brustel, Y. (1994) J. Biol. Chem. 269, 6051–6057

27. Kanety, H., Feinstein, R., Papa, M. Z., Hemi, R., and Karasik, A. (1995) J. Biol. Chem. 270, 23780–23784

28. Hotamisligil, G. S., Udenfriend, S., Peraldi, P., Budavari, A., Ellis, R., White, M. F., and Spiegelman, B. M. (1996) Science 271, 665–668

29. De Fea, K., and Roth, R. A. (1997) Biochemistry 36, 12939–12947

30. De Fea, K., and Roth, R. A. (1997) J. Biol. Chem. 272, 31400–31406

31. Ricort, J. M., Tanti, J. F., van Obberghen, E., and Le Marchand-Brustel, Y. (1997) J. Biol. Chem. 272, 19814–19818

32. Folli, F., Kahn, C. R., Hansen, H., Beuchel, J. L., and Feener, E. P. (1997) J. Clin. Invest. 100, 2158–2169

33. Paz, K., Hemi, R., LeRoith, D., Karasik, A., Elhanany, E., Kanety, H., and Zick, Y. (1997) J. Biol. Chem. 272, 29911–29918

34. Cengel, K. A., Kason, R. E., and Freund, G. G. (1998) Biochem. J. 335, 397–404

35. Sun, X., Qiao, L., and Goldberg, J. (1998) Diabetes 47, A35 (abstr.)

36. Freund, G. G., Kulas, D. T., Way, B. A., and Mooney, R. A. (1984) Cancer Res. 54, 3179–3185

37. Cengel, K. A., Godbout, J. G., and Freund, G. G. (1998) Biochem. Biophys. Res. Commun. 242, 513–517

38. Smith, D. B., and Johnson, K. S. (1998) Gene (Amst.) 67, 31–40

39. Dulcos, B., Marecandier, S., and Cozzone, A. J. (1991) Methods Enzymol. 201, 10–21

40. Mayor, P., Maianu, L., and Garvey, W. T. (1992) Diabetes 41, 274–285

41. Saad, M. J., Folli, F., and Kahn, C. R. (1995) Endocrinology 136, 1579–1588

42. Heller-Harrison, R. A., Morin, M., and Czech, M. P. (1995) J. Biol. Chem. 270, 24442–24450

43. Keegan, A. D., Nelms, K., White, M., Wang, L. M., Pierce, J. H., and Paul, W. E. (1994) Cell 76, 811–820

44. Reaven, G. M. (1995) Physiol. Rev. 75, 473–486

45. Horton, E. S. (1995) Diabetes Res. Clin. Pract. 28, suppl., S3–S11

46. Zamorano, J., Wang, H. Y., Wang, L. M., Pierce, J. H., and Keegan, A. D. (1996) J. Immunol. 157, 4926–4934

47. Wang, H. Y., Zamorano, J., and Keegan, A. J. (1998) J. Biol. Chem. 273, 989–996