Protein Phosphatase 4 Interacts with and Down-regulates Insulin Receptor Substrate 4 following Tumor Necrosis Factor-α Stimulation

Received for publication, July 16, 2004, and in revised form, August 23, 2004
Published, JBC Papers in Press, August 24, 2004, DOI 10.1074/jbc.M408067200

Kathie A. Mihindukulasuriya‡§, Guisheng Zhou‡§§, Jun Qin‡, and Tse-Hua Tan‡**
From the ‡Department of Immunology and §Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030

Protein phosphatase 4 (PP4; also named PPX or PPP4) is a PP2A-related protein serine/threonine phosphatase with important roles in a variety of cellular processes such as microtubule growth/organization, apoptosis, tumor metastasis, and c-Jun N-terminal kinase and NF-κB. To further investigate the cellular functions of PP4, we isolated and identified PP4-interacting proteins using a proteomic approach. We found that insulin receptor substrate 4 (IRS-4) interacted with PP4 and that this interaction was enhanced following TNF-α stimulation. We also found that PP4, but not PP2A, down-regulated IRS-4 in a phosphatase activity-dependent manner. Pulse-chase analysis revealed that PP4 decreased the half-life of IRS-4 from 4 to 1 h. Moreover, we found that TNF-α stimulated a PP4-dependent degradation of IRS-4, as indicated by the blockage of the degradation by a potent PP4 inhibitor (okadaic acid) and a phosphatase-dead PP4 mutant (PP4-RL). Taken together, our studies indicate that IRS-4 is subject to regulation by TNF-α and that PP4 mediates TNF-α-induced degradation of IRS-4.

Insulin receptor substrate (IRS)1 proteins play a central role in signal transduction by the receptors for insulin, insulin-like growth factor 1 (IGF-1), and a growing number of cytokines and integrins (1). Four IRS proteins have been identified and characterized, including IRS-1, IRS-2, IRS-3 and IRS-4 (2–5). Recently, two more signaling proteins, IRS5/DOK4 and IRS6/DOK5, have been identified as potentially new members of the IRS family (6). IRSs are tyrosine-phosphorylated following insulin or IGF-1 stimulation and form signaling complexes at the receptor with Src homology 2 domain-containing proteins, including the p85 regulatory subunit of phosphatidylinositol 3-kinase, Grb2, Nck, and Shc (7, 8). All IRS proteins share a number of structural and functional characteristics, including an N-terminal pleckstrin homology and a phosphotyrosine binding domain, followed by a large C-terminal region containing multiple sites of tyrosine phosphorylation, which serve as docking sites for Src homology 2 domain-containing signaling proteins. IRS proteins also carry a large number of potential sites for serine and threonine phosphorylation, which may regulate protein-protein interactions (9). Despite the overall similarity, the IRS proteins differ in their subcellular distribution and tissue/developmental expression pattern. Each IRS is phosphorylated on a specific tyrosine residues, leading to its binding to the Src homology 2 domains of distinct signaling proteins and therefore, activation of specific signaling cascades. Disruption of each individual IRS gene causes distinct phenotypes in mice (10–14), further indicating that the four IRS proteins play different roles in the regulation of the pleiotropic effects of insulin, IGF-1, and other growth factors.

IRS-4 was initially detected in human embryonic kidney 293 (HEK293) cells (5, 15). Like other IRSs, IRS-4 enhances insulin- and IGF-1-induced mitogenesis in a variety of cells such as HEK293 cells (16, 17), NIH3T3 cells (18), adipose cells (19), and hematopoietic cells (20). It has also been shown that IRS-4 mediates mitogenic signaling by interleukin-4 in hematopoietic cells (20), by growth hormone in LB cells, a murine T-cell lymphoma devoid of IGF-1 receptor (21), and by hepatocyte growth factor in pancreatic β-cells (22). IRS-4 has been implicated in liver regeneration as indicated by the substantial induction after partial hepatectomy (23). Recently, it was found that IRS-4 expression level is decreased in polycystic ovary syndrome (PCOS) theca cells (24). IRS-4 has a compensatory role for IRS-1 in adipocyte differentiation (25) and for IRS-2 in pancreatic β-cells (26). However, the negative regulation of IRS-1 and IRS-2 by IRS-4 in IGF-1 signaling has also been suggested (27). IRS-4 lacks both putative SHP-2 binding motifs present in IRS-1, IRS-2, and IRS-3 (15). In comparison with other IRSs, IRS-4 exhibits a more limited tissue expression. Besides HEK293 cells, the IRS-4 protein has only been detected in heart and skeletal muscle cells (28), although IRS-4 mRNA is expressed in a variety of human and rodent tissues including pituitary, thyroid, ovary, prostate, hypothalamus, liver, heart, and skeletal muscle (29). Tissue-specific expression of IRS-4 suggests the potential involvement of IRS-4 in the regulation of insulin/IGF-1 signaling in these tissues. Five amino acid polymorphisms have been identified in IRS-4, although their functional relevance remains to be determined (30). To date, only a
few IRS-4-interacting proteins have been identified, including the regulatory and catalytic subunits of phosphatidylinositol 3-kinase (23, 31), the imidazoline receptor antisera selected (31), the suppressor of cytokine signaling-6 (32), Src homology phosphatase (23), and protein kinase C-γ (23). Identification and characterization of novel IRS-4-interacting proteins will help in understanding the cellular functions of IRS-4.

Protein phosphatase 4 (PP4; previously called PPX) (33) is a member of the PP2A subfamily of protein serine/threonine phosphatases, along with PP2A and PP6 (34). PP4, PP6, and PP2A are highly homologous; human PP4 shares 65% identity with PP2A (33, 35). PP4, like PP2A, is a holohexozyme composed of catalytic (C), structural (A), and regulatory (B) subunits. To date, three subunits have been identified for PP4: α4, PP4-R1, and PP4-R2 (36–40). Like PP2A, PP4 contains a putative binding domain for okadaic acid, a potent tumor promoter toxin (41). Okadaic acid inhibits PP4 with a similar range of concentrations (IC50 = 0.1 nM in vitro) as PP2A (42). PP4 is involved in the regulation of microtubule growth or organization at the centrosomes (43) and the centrosome maturation in mitosis and meiosis (44). It has been recently shown that PP4 plays an active role in the regulation of microtubule growth or organization at the centrosomes (43) and the centrosome maturation in mitosis and meiosis (44). It has been recently shown that PP4 plays an active role in the regulation of microtubule growth or organization at the centrosomes (43) and the centrosome maturation in mitosis and meiosis (44). It has been recently shown that PP4 plays an active role in the regulation of microtubule growth or organization at the centrosomes (43) and the centrosome maturation in mitosis and meiosis (44). It has been recently shown that PP4 plays an active role in the regulation of microtubule growth or organization at the centrosomes (43) and the centrosome maturation in mitosis and meiosis (44).

PP4 Down-regulates IRS-4

MATERIALS AND METHODS

Reagents—The SuperSignal chemiluminescence system was purchased from Pierce. TNF-α was purchased from R&D Systems (Minneapolis, MN). The mixture of 1132-methionine-cysteine was purchased from ICN Biomedicals (Irvine, CA). Anti-HA antibody (12CA5) was purchased from Roche Applied Science. Anti-FLAG (M2) and anti-PP4 antibodies (Ab 104 and 6101) were previously described (48). Ab 1640 (anti-IRS-4) and Ab 6101 (anti-PP4) were peptide-purified PP4 polyclonal antibodies (Ab 104 and 6101) were previously described (48). Ab 1640 (anti-IRS-4) and Ab 6101 (anti-PP4) were peptide-purified PP4 polyclonal antibodies (Ab 104 and 6101) were previously described (48).

Phosphatase Assay—HEK293T cells were lysed in buffer containing 50 mM Tris-HCl (pH 8.0), 1% Nonidet P-40, 1 mM dithiothreitol, 0.5 mM p-aminophenylmethanesulfonfyl fluoride, and 2 μg/ml aproton. PP4 was immunoprecipitated with an anti-PP4 (Ab 104) antibody. FLAG-PP4 was immunoprecipitated with anti-FLAG (M2) antibody. The immunoprecipitates were washed three times with buffer containing 50 mM HEPES (pH 7.4), 0.1% Triton X-100, and 500 mM NaCl. Phosphatase assays were performed using Ser/Thr phosphatase assay kit 1, according to the manufacturer’s protocol (Upstate Biotechnology). In brief, the immunoprecipitates were incubated with 4 μM KTPiRR peptide in 40 μl of assay buffer containing 10 mM Tris (pH 7.0) and 0.2 mM CaCl2 and 1 mM EDTA for 30 min. Buffer plus peptide was used as a negative control. The immunoprecipitates were then pelleted, and the assay buffer was transferred to a 96-well, half-volume plate. The assay was terminated by the addition of 100 μl of Malachite Green solution (1 volume of 4.2% (w/v) ammonium molybdate in 4 μl HCl, 3 volumes of 0.045% (w/v) Malachite Green in water, and 1 μl 10% Tween 20 added fresh). After 15 min at room temperature, the assay was read at 650 nm on a PerkinElmer bioassay reader (HTS 7000 plus).

Establishment of the HEK293 Cell Clone (10F1) Stably Transfected with FLAG-PP4—HEK293 cells were grown in complete Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum supplemented with 12.5 mM HEPES, 50 μg/ml gentamycin, and 100 units/ml penicillin/streptomycin (Invitrogen). We transfected the HEK293 cells with a fusion of pp4 by the Fugene 6 method according to the manufacturer’s protocol (Roche Applied Science). The transfected cells were selected with Geneticin (G418; Invitrogen) at a concentration of 750 μg/ml. The cells were replated at a 1:15 dilution whenever they reached 80% confluence. After 10–14 days, the T-75 flasks were trypsinized, and the drug-resistant cells were replated at a limiting dilution to obtain independent clones. Each clone was tested for FLAG-PP4 expression by Western blotting.

Isolation of FLAG-PP4-interacting Protein Complexes—HEK293 cells stably expressing FLAG-PP4, designated as 10F1 clone, were left unstimulated or stimulated with TNF-α (10 ng/ml) for 10 min. Fifteen confluent T175 flasks of 10F1 or parental HEK293 cells (5.175 × 106 cells) were used per purification. The cells were trypsinized, washed with cold-phosphate-buffered saline, and incubated on ice until needed. The cells were lysed in 5 ml of PIP lysis buffer (20 mM HEPES (pH 7.4), 2 mM EDTA, 1% Triton X-100, 150 mM NaCl) freshly supplemented with 6.6 μg/ml aprotonin, 10 μg/ml leupeptin, and 50 μM p-aminophenylmethanesulfonfyl fluoride. A lysis centrifuged at 20,817 × g (14,000 rpm) for 15 min to remove cellular debris. Chromosomal DNA was sheared with a 1-ml syringe. The whole cell extract (105 mg) was incubated with either anti-FLAG-Sepharose or unconjugated Sepharose at 4 °C for 3 h with continuous rotation, packed into a column, and washed three times with 10 ml of NETN buffer. FLAG-PP4 was eluted four times with 1ml column volume of 100 μg/ml FLAG peptide in TBS (50 mM Tris-HCl (pH 7.4) and 150 mM NaCl). 0.1 μl of each 100-μl fraction was used for anti-FLAG Western blotting to determine which fraction(s) contained FLAG-PP4. The fraction containing the most FLAG-PP4 and the corresponding fractions for the negative controls were separated by 10% SDS-PAGE, and the gel was stained with Coomassie Blue.

Identification of Proteins by Mass Spectrometry—Identification of proteins by mass spectrometry was performed as previously described (52, 53). A MALDI-TOF mass spectrometer with delayed extraction, a high-resolution MALDI ion trap mass spectrometer, and an electrospray ion trap mass spectrometer (LCQ; Finnigan MAT, San Jose, CA) coupled on-line with a capillary high pressure liquid chromatograph (Magic 2002, Michom BioResources, Auburn, CA) were used to acquire tandem mass spectra. A 0.1 × 50-μm Magic MS C18 column (5-μm particle diameter, 200-Å pore size) with mobile phases A (methanol/water/acetic acid, 5:95:1) and B (methanol/water/acetic acid, 85:15:1) was used with a gradient of 2–98% mobile phase B over 2.5 min, followed by 98%
FIG. 1. Isolation and identification of IRS-4 as a TNF-α-inducible PP4-interacting protein. A, isolation of PP4-interacting proteins. Whole cell extracts from 5 × 10⁵ HEK293 cells stably expressing FLAG-PP4 (10F1 clone), unstimulated (lane 3), or stimulated for 10 min with 10 ng/ml TNF-α (lane 4) or the parental HEK293 cells (lane 2) were incubated with anti-FLAG-Sepharose for 2 h at 4 °C. As an additional control, 10F1 cells were incubated with unconjugated Sepharose (lane 1). The Sepharose was then placed in a column and washed three times with NETN buffer. FLAG-PP4 and the PP4-interacting proteins were eluted four times with one-column volume of 100 μg/ml FLAG peptide. Twelve fractions
mobile phase B for 2 min. Specific protein bands from Coomassie Blue-stained SDS-polyacrylamide gels were excised, destained, and digested with trypsin (200 ng/digestion) in 20 μl of 50 mM NH₄HCO₃ buffer for 2 h at 37 °C using a protein/enzyme weight ratio of 1:1. The resulting peptides were extracted, and 20–50% of the sample was used to obtain liquid chromatography/tandem mass spectra. The tandem mass spectra were used to search the compiled NCBI nonredundant protein database and expressed sequence tag data bases with the program PepFrags to identify the proteins.

**Pulse-chase Analysis—**HEK293T cells were plated at a density of 1.5 × 10⁵ cells per 35-mm plate well and transfected the next day with HA-IRS-4 (2 μg) plus empty vector or PP4 (2 μg) as described above. Thirty-six h after transfection, the medium was replaced with 2 ml of prelabeling medium (Dulbecco’s modified Eagle’s medium lacking cysteine and methionine + 5% fetal calf serum). After 1 h of incubation, the prelabeling medium was replaced with 1 ml of labeling medium (prelabeling medium + 0.1 μCi/ml [³⁵S]methionine and [³⁵S]cysteine). The cells were labeled for 4 h and then chased with fresh, nonradioactive medium for the times indicated. The cells were lysed in lysis buffer (20 mM HEPES (pH 7.4), 2 mM EDTA, 1% Triton X-100, 10% glycerol, and 150 mM NaCl) freshly supplemented with 6.6 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM dithiothreitol, and 50 μM p-aminophenylmethanesulfonfonyl fluoride. 100 μg of cell lysate was immunoprecipitated with an anti-HA antibody (12CA5) and protein A-Sepharose and resolved by SDS-PAGE. The gels were dried and autoradiographed.

**RESULTS**

**IRS-4 Is a TNF-α-inducible, PP4-interacting Protein—**We have previously shown that PP4 is involved in TNF-α signaling (48). In the search for novel cellular functions of PP4, we established a HEK293 cell clone (10F1) stably transfected with FLAG-PP4 and applied a functional proteomic approach to identify TNF-α-inducible, PP4-interacting protein(s) in 10F1 cells. We treated 10F1 cells with TNF-α, and the whole cell extract of 10F1 cells was incubated with anti-FLAG-Sepharose. After extensive washing, FLAG-PP4 and the proteins that complexed with PP4 were eluted with FLAG peptide, subjected to SDS-PAGE, and stained with Coomassie Blue (Fig. 1A). By mass spectrometry, a group of potential PP4-interacting proteins in response to TNF-α stimulation was identified (Fig. 1A). We selected one of these proteins, insulin receptor protein 4 (IRS-4), a docking protein that coordinates cellular responses to insulin and insulin-like growth factor 1 (IGF-1), for further study. A representative spectrum is shown for IRS-4 along with the peptide sequence and its position in the IRS-4 protein (Fig. 1B). To confirm the interaction between PP4 and IRS-4, we took a fraction of the FLAG peptide elution and performed Western blot analysis using an anti-IRS-4 antibody. As shown in Fig. 2, the presence of IRS-4 in FLAG-PP4-interacting protein complex was greatly increased upon TNF-α stimulation (top panel). These data indicate that IRS-4 is a TNF-α-inducible, PP4-interacting protein. We also found that three known PP4-interacting proteins, α4, PP4-R1, and PP4-R2, co-purified with FLAG-PP4 (Fig. 2), further confirming the binding specificity of this interaction was greatly enhanced by TNF-α, IRS-4, PP4-R1, and PP4 (FLAG).

To determine the unifying mechanism by which PP4 down-regulates IRS-4, we transiently transfected 10F1 cells with HA-IRS-4, immunoprecipitated FLAG-PP4, and performed Western blotting against HA-IRS-4. We found that FLAG-PP4 interacts with HA-IRS-4 and that this interaction was greatly enhanced by TNF-α stimulation (Fig. 3A). To exclude the possibility of an artificial interaction due to the overexpression system and to demonstrate the physiologic relevance of this TNF-α-inducible interaction, we performed immunoprecipitation/Western blot analysis to determine whether endogenous PP4 and IRS-4 interact with each other in HEK293 cells. We treated HEK293 cells with TNF-α, immunoprecipitated endogenous PP4, and Western blotted against endogenous IRS-4. As with the overexpressed proteins, endogenous PP4 and IRS-4 interacted with each other, and this interaction was enhanced after TNF-α stimulation (Fig. 3B). Taken together, our data indicate that IRS-4 is a PP4-interacting protein and that the PP4-IRS-4 interaction is subject to regulation by TNF-α stimulation.

**PP4 Down-regulates IRS-4—**To determine the functional relevance of the PP4-IRS-4 interaction, we examined the effect of PP4 on the protein level of IRS-4. We co-transfected HA-IRS-4 into HEK293 cells with increasing amounts of PP4 or PP2A, the most closely related serine/threonine phosphatase. Western blot analysis showed that co-transfection of PP4 resulted in down-regulation of IRS-4 in a dose-dependent manner, whereas PP2A had no such an effect (Fig. 4A). Our phosphatase assays demonstrated that overexpressed PP4 and PP2A had comparable phosphatase activity under these experimental conditions (Fig. 4B). Thus, these data indicate that the function of the PP4-IRS-4 interaction is to down-regulate IRS-4 expression. In order to corroborate the role of PP4 in regulating IRS-4 protein levels, we examined the effect of PP4-RL, a phosphatase-dead mutant of PP4 in which arginine 236 is replaced with leucine (46, 48), on IRS-4. We found that IRS-4 was degraded in the presence of wild-type PP4 but not phosphatase-dead PP4-RL (Fig. 4C). These results indicate that PP4 phosphatase activity is required for its effect on IRS-4.

**PP4 Decreases the Half-life of IRS-4—**To determine the underlying mechanism by which PP4 down-regulates IRS-4, we determined the half-life of IRS-4 in the presence or absence of PP4. HEK293T cells were transfected with HA-IRS-4 plus empty vector (Fig. 5A) or PP4 (Fig. 5B), pulsed with a mixture of [³⁵S]methionine and [³⁵S]cysteine, and then chased with nonradioactive medium for various periods of time. The half-life of IRS-4 decreased from 4 h (Fig. 5A) to 1 h in the presence of PP4 (Fig. 5B). Thus, our data indicate that overexpression of PP4 leads to the enhanced degradation of IRS-4 protein.

**PP4 Mediates TNF-α-induced Degradation of IRS-4—**We have previously shown that TNF-α stimulates PP4 phosphatase activity (48). Given the observation that TNF-α enhanced

---

Note: The image includes a figure (Fig. 2) that is not described in the text. Additionally, the text contains references to Figures 3 and 4, which are not detailed here but are likely related to the results and discussion presented.
the PP4-IRS-4 interaction (Figs. 2 and 3), we wondered whether TNF-α could stimulate the degradation of IRS-4. To this end, we stimulated HEK293 cells with varying amounts of TNF-α and assayed the lysate for the protein levels of IRS-4. We found that TNF-α treatment led to the degradation of IRS-4 in a dose-dependent manner (Fig. 6A). To determine whether PP4 was involved in the TNF-α-induced degradation of IRS-4, we pretreated HEK293 cells with okadaic acid, a potent PP4 and PP2A inhibitor (41), and then treated the cells with TNF-α. We found that pretreatment with okadaic acid blocked TNF-α-induced degradation of IRS-4 (Fig. 6B). Okadaic acid inhibits both PP4 and PP2A at nearly the same concentration (42). Therefore, to confirm the specific involvement of PP4 in TNF-α-induced degradation of IRS-4, we transfected HEK293T cells with HA-IRS-4 and increasing amounts of PP4 or PP2A (10 ng/ml) for 2.5 min. The lysates were immunoprecipitated with an anti-PP4 antibody. The PP4-IRS-4 interaction was detected by immunoblotting with a peptide-purified anti-IRS-4 antibody (top). Equal immunoprecipitation of PP4 was monitored by immunoblotting with anti-FLAG (bottom) antibodies, respectively.

DISCUSSION

PP4 is a highly conserved, PP2A-related serine/threonine phosphatase. To date, the cellular functions of PP4 and the underlying PP4-mediated signal transduction mechanisms are largely unknown. Moreover, okadaic acid, a derivative of a dinoflagellate toxin (41), inhibits PP4 with an IC₅₀ of about 0.1 nM, comparable with that of PP2A (42). It is likely that some cellular functions previously assigned to PP2A may actually belong to PP4. We have previously identified both the c-Jun N-terminal kinase and NF-κB pathways as potential PP4-targeted signaling pathways (46, 48). Here, we provide both biochemical and pharmacological evidence that PP4 is involved in the regulation of IRS-4 and the IRS-4-mediated signaling pathway(s). By functional proteomic analysis, we identified IRS-4 as a TNF-α-inducible, PP4-interacting protein. Moreover, we found that the interaction of PP4 with IRS-4 led to the degradation of IRS-4 through a decrease in the half-life of IRS-4. The physiological relevance of the PP4-IRS-4 interaction was shown by the complete blockage of TNF-α-induced degradation of IRS-4 by okadaic...
Our study identifies the IRS-4-mediated signaling pathway(s) as another novel PP4-targeted pathway. It has been shown that TNF-α plays an inhibitory role in insulin signaling and contributes to the development of insulin resistance (54). Multiple mechanisms, not mutually exclusive, have been proposed to account for TNF-α-induced insulin resistance in obesity, including the elevation of plasma free fatty acids due to its lipolytic action, the down-regulation of the translocation of the insulin-sensitive glucose transporter (GLUT4) to the plasma membrane, the antagonism of the peroxisome proliferator-activated receptor γ pathway, and the activation of c-Jun N-terminal kinase and inhibitor κB kinase (55–58). TNF-α has also been shown to directly interfere with the early steps of insulin signaling. It has been shown that IRS-1 and IRS-2 are prominent TNF-α targets within the insulin signaling cascade and major integration points of the TNF-α and insulin signaling pathways. For example, TNF-α treatment increases the serine/threonine phosphorylation of IRS-1 and IRS-2, which in turn leads to their dissociation from the insulin receptor (55, 59, 60), the down-regulation of IRS proteins (61–63), and thus the inhibition of insulin signaling. We provide evidence here for the first time that IRS-4 is degraded in response to TNF-α stimulation. Since IRS-4 has been shown to be involved in many aspects of insulin signaling, our studies reveal a novel mechanism by which TNF-α regulates IRS-4 and IRS-4-mediated insulin signaling.

TNF-α also inhibits the signaling of another IRS-mediated pathway, the IGF-1 pathway. For example, TNF-α opposes the antiapoptotic function of IGF-1 signaling in neurons and breast cancer cells (64–66). Like other IRS family proteins, IRS-4 has also been shown to mediate signals from IGF-1 as indicated by its tyrosine phosphorylation after IGF-1 stimulation (28). Recently, PP4 has been shown to be a proapoptotic protein (45). Thus, it is likely that PP4 may play a role in TNF-α-induced inhibition of the antiapoptotic function of IGF-1.

Multiple serine phosphorylation sites, targeted by different protein kinases under different conditions, have been identified in IRS-1. Whereas most serine phosphorylation sites in IRS-1 so far inhibits IRS-1-mediated insulin signaling, some serine/threonine phosphorylation of IRS-1 exerts a positive effect on insulin signaling. For example, the phosphorylation of serine residues within the phosphotyrosine binding domain of IRS-1 by insulin-stimulated protein kinase B protects IRS-1 protein from the rapid action of protein-tyrosine phosphatases and enables the Ser-phosphorylated IRS-1 protein to maintain its Tyr-phosphorylated, active conformation (67). Recently, phosphorylation of rat IRS-1 on Ser-302 was found to be required for insulin-stimulated tyrosine phosphorylation of IRS-1 and the IRS-1-phosphatidylinositol 3-kinase interaction (68).
Moreover, some basal serine phosphorylation of IRS-1 is necessary for insulin signaling (69). However, the serine/threonine phosphorylation site(s) responsible for the stabilization of IRS proteins has not been identified yet. Given the high homology between IRS-1 and IRS-4, it is likely that IRS-4 is also subject to PP4 dephosphorylation. A functional serine/threonine residue(s), through decreasing the half-life of IRS-4, we hypothesize that PP4 dephosphorylates a functional serine/threonine residue(s), whose phosphorylation contributes to the stabilization of IRS-4, leading to the enhanced degradation of IRS-4.

Therefore, identification of PP4-targeted phosphorylation site(s) will provide new insight into the understanding of the regulation of IRS proteins in vivo.

Acknowledgments—We thank our colleagues for providing valuable reagents; members of the Tan laboratory for helpful discussions and reagents; and Fujisawa, Inc., for financial support. We thank our colleagues for providing valuable secretarial assistance.

REFERENCES

1. White, M. F. (2002) Am. J. Physiol. 283, E413–E422
2. Sun, X. J., Rothenberg, P., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Lane, W. S., Pierce, J. H., and White, M. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2723–2727
3. Fantin, V. R., Wang, Q., Lienhard, G. E., and Keller, S. R. (2000) Mol. Cell. 5, 393–404
4. Koval, A. P., Karas, M., Zick, Y., and LeRoith, D. (1998) J. Biol. Chem. 273, 14780–14787
5. Karas, M., Koval, A. P., Zick, Y., and LeRoith, D. (2001) J. Biol. Chem. 276, 14780–14787
6. Karas, M., Koval, A. P., Zick, Y., and LeRoith, D. (2001) Endocrinology 142, 1835–1840
7. Qu, B. H., Karas, M., Koval, A., and LeRoith, D. (1999) J. Biol. Chem. 274, 17119–17124
8. Zhou, L., Chen, H., Xu, P., Cong, L. N., Sciacchitano, S., Li, Y., Graham, D., Sbraccia, P., Borboni, P., Lauro, R., and Sesti, G. (2000) J. Biol. Chem. 275, 17070–17078
9. Koval, A. P., Karas, M., Zick, Y., and LeRoith, D. (1998) J. Biol. Chem. 273, 47047–47054
10. Giral, J. D., Lefevre, M., and Wadzinski, B. E. (1999) J. Biol. Chem. 274, 21403–21407
11. Tamemoto, H., Kadowaki, T., Tobe, K., Yagi, T., Sakura, H., Hayakawa, T., Lefevre, M., and Wadzinski, B. E., Okubo, K., and Kurokawa, K. (2001) J. Am. Soc. Nephrol. 12, 2001–2008
12. Sun, X. J., Rothenberg, P., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Lane, W. S., Pierce, J. H., and White, M. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2723–2727
13. Liu, S. C., Wang, Q., Lienhard, G. E., and Keller, S. R. (1999) Mol. Cell 4, 1191–1202
Protein Phosphatase 4 Interacts with and Down-regulates Insulin Receptor Substrate 4 following Tumor Necrosis Factor-α Stimulation
Kathie A. Mihindukulasuriya, Guisheng Zhou, Jun Qin and Tse-Hua Tan

J. Biol. Chem. 2004, 279:46588-46594.
doi: 10.1074/jbc.M408067200 originally published online August 24, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408067200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 69 references, 36 of which can be accessed free at http://www.jbc.org/content/279/45/46588.full.html#ref-list-1