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Communication

The Pleckstrin Homology Domain in Insulin Receptor Substrate-1 Sensitizes Insulin Signaling*

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The NH_2 terminus of insulin receptor substrate-1 (IRS-1) contains a pleckstrin homology (PH) domain. We deleted the PH domain in IRS-1 (IRS-$1^{\Delta PH}$) and expressed the mutant in Chinese hamster ovary and 32D cells. During insulin stimulation, IRS-$1^{\Delta PH}$ is poorly tyrosine-phosphorylated in CHO cells, but undergoes serine/threonine phosphorylation. Similarly, IRS-$1^{\Delta PH}$ fails to undergo insulin-stimulated tyrosine phosphorylation in 32D cells, which uncouples the activation of phosphatidylinositol 3′-kinase and $p70^{s6k}$ from the endogenous insulin receptors. Overexpression of the insulin receptor in $32D^{IR}$ cells, however, restores tyrosine phosphorylation of IRS-$1^{\Delta PH}$ and rescues insulin responses including mitogenesis. Thus, while the PH domain is not required for the engagement of downstream signals, it is one of the elements in the NH_2 terminus of IRS-1 that is needed for a sensitive coupling to insulin receptors, especially at ordinary receptor levels found in most cells and tissues.

During insulin stimulation, IRS-1[1] becomes tyrosine-phosphorylated and binds to the Src homology-2 domains in several signaling proteins (SH2 proteins) (1). As a consequence of docking SH2 proteins, IRS-1 mediates multiple downstream signals, including the direct activation of PI 3′-kinase and SH-PTP2, and the stimulation of mitogen-activated protein kinase and $p70^{s6k}$; IRS-1 at least partially regulates mitogenesis, chemotactic signaling, and glucose transport (1–5). Since IRS-signaling proteins are not engaged by most growth factor receptors, specific interactions between IRS-signaling proteins and receptors must mediate productive coupling.

Deletion of the first 500 amino acids of IRS-1 prevents phosphorylation of the COOH-terminal portion of the molecule expressed in COS cells (6). A pleckstrin homology (PH) domain exists between residues 13 and 115, which is absolutely identical among the rat, mouse, and human isoforms (7–9), and is 62% identical in the recently cloned IRS-2.[2] PH domains were originally recognized as a repeat in pleckstrin and later found in various signal transduction proteins (7). Although the amino acid sequence of various PH domains is poorly conserved, the PH domain in spectrin and pleckstrin has a common structure composed of three β-sheets and an α-helix arranged around a hydrophobic core (10, 11). Although the exact function of PH domains is obscure, recent studies suggest that they mediate protein-protein interactions or bind phospholipids (12–14).

In this study we have investigated the role of the PH domain in IRS-1 by deleting it and studying this mutant (IRS-$1^{\Delta PH}$) in CHO^{IR} cells, which contain endogenous IRS-1, and in 32D cells, which lack endogenous IRS-signaling proteins. Our results suggest that the PH domain is not required for the engagement of downstream signals; rather, it is one of the elements that mediates the coupling between the insulin receptor and IRS-signaling proteins.

MATERIALS AND METHODS

Construction of IRS-$1^{\Delta PH}$—The cDNA for rat IRS-1 in pBluescript (15) was digested with *Bsp*EI and *Bsp*MI, and the resulting fragment containing the majority of IRS-1 was religated in the presence of linkers formed by annealing the oligonucleotides 5′-CCG/GAG/GAT/CCC/CTT/AAG-3′ and 5′-CCT/CCT/TAA/GGG/GAT/CCT-3′. The resulting mutant cDNA was subcloned into pCMVhis for expression using *Sac*I and *Hin*dIII (3).

Cell Lines—Chinese hamster ovary (CHO) cells expressing the human insulin receptor (CHO^{IR}) or the human insulin receptor and IRS-1 (CHO^{IR}/IRS-1) have been described (4, 15). CHO cell lines were maintained in Ham's F-12 medium containing 10% fetal bovine serum. CHO^{IR} cells were transfected with IRS-$1^{\Delta PH}$ cDNA by the calcium phosphate method and selected in 10 mM histidinol (4, 15). Surviving cells were cloned and maintained in the presence of 10 mM histidinol. 32D cells and cell lines expressing IRS-1 (32D/IRS-1), IR ($32D^{IR}$), or IR and IRS-1 ($32D^{IR}$/IRS-1) have been described previously (2). 32D cell lines were maintained in RPMI 1640 media supplemented with 10% fetal bovine serum and 5% WEHI-3-conditioned media (a source of IL-3). 32D and $32D^{IR}$ cells were electroporated with the cDNA for IRS-$1^{\Delta PH}$ and selected with 5 mM histidinol to obtain cell lines expressing the PH deletion mutant (2, 3). Cell lines were selected for expression by immunoblotting lysates of histidinol-resistant cell lines with αIRS-1 antibodies. Expression of the human insulin receptor was confirmed by αIR immunoblotting cell lines co-expressing the insulin receptor. For experiments, CHO^{IR} cells were made quiescent by incubation in Ham' F-12 medium supplemented with 0.5% bovine serum albumin for 18–24 h; 32D cells were made quiescent by incubation in unsupplemented Dulbecco's modified Eagle's medium for 4 h.

Antibodies and Growth Factors—αIRS-1 antibodies were rabbit polyclonal antisera against a COOH-terminal peptide of IRS-1; they were used at a 1:300 dilution to immunoblot and 1:100 to immunoprecipitate (16). Being directed against the COOH terminus of IRS-1, this antibody

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[1] The abbreviations used are: IRS-1, insulin receptor substrate-1; SH2, Src homology-2; PI, phosphatidylinositol; CHO, Chinese hamster ovary; IL, interleukin; PAGE, polyacrylamide gel electrophoresis; IH, IRS homology region; PH, pleckstrin homology region.

[2] X. J. Sun, L. M. Wang, Y. Zhang, L. Yenush, M. G. Myers, Jr., E. Glasheen, S. Pons, G. Wolf, S. E. Shoelson, W. S. Lane, J. H. Pierce, and M. F. White, submitted for publication.

recognizes IRS-1 and IRS-$1^{\Delta PH}$ equivalently. αIR antibodies were rabbit antisera raised against a glutathione *S*-transferase fusion protein containing the intracellular β-subunit of the human insulin receptor; they were used 1:300 to immunoblot. αPY antibodies were mouse monoclonal 4G10 purified from tissue culture supernatant by chromatography on Protein A-Sepharose or affinity-purified rabbit polyclonal antibodies (17); both antibodies immunoblotted at 1:300 dilution. Rabbit antisera against $p70^{s6k}$ and their use have been described (18). Insulin was from Calbiochem (San Diego, CA).

Immunoblotting—Proteins were denatured by boiling in Laemmli sample buffer containing 100 mM dithiothreitol and resolved by SDS-PAGE. Gels were transferred to nitrocellulose membranes (Schleicher & Schuell) in Towbin buffer containing 0.02% SDS and 20% methanol (19). Membranes were blocked, probed, and developed as described previously, and visualized using ^{125}I-Protein A (Amersham Corp.) or horseradish peroxidase-conjugated goat anti-mouse secondary antibodies (Cappel) and the Renaissance system (DuPont NEN) (3, 18). Blots were exposed to Kodak X-AR film or imaged on a Molecular Dynamics PhosphorImager.

Metabolic Labeling of CHO^{IR} Cells—Quiescent CHO^{IR} cell lines were washed twice with phosphate-free RPMI 1640 medium and incubated for 3 h in phosphate-free RPMI 1640 medium supplemented with 0.2 mCi/ml [^{32}P]orthophosphate (DuPont NEN). Cells were stimulated with 100 nM insulin for 10 min and lysed in ice-cold 100 mM Tris-HCl, pH 7.4, containing 1% Triton X-100, 100 mM NaF, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 10 mg/ml each of leupeptin and aprotinin. Insoluble material was removed by centrifugation at 10,000 × *g* for 10 min, and αIRS-1 antibodies were added for 1 h at 4 °C. Immune complexes were collected on Protein A-Sepharose (Pharmacia Biotech Inc.) and washed three times in ice-cold 50 mM HEPES, pH 7.4, containing 1% Triton X-100, 150 mM NaCl, 100 mM NaF, and 2 mM sodium orthovanadate. Immune complexes were resolved by 7.5% SDS-PAGE and visualized on a Molecular Dynamics PhosphorImager.

PI 3′-Kinase, $p70^{s6k}$, and Mitogenesis Assays—*In vitro* phosphorylation of phosphatidylinositol was carried out in immune complexes as described previously and quantitated on a Molecular Dynamics PhosphorImager (3, 20). *In vitro* kinase assays for $p70^{s6k}$ were carried out as described previously and quantitated on a PhosphorImager (5, 21). Mitogenesis was measured by thymidine incorporation into DNA as described previously (2, 3, 5).

RESULTS AND DISCUSSION

Alignment of the NH_2 terminus of IRS-1 with various signaling proteins reveals a PH domain between residues 13 and 115 (7, 12). The function of this PH domain was investigated by deleting it (in-frame) from the IRS-1 cDNA (IRS-$1^{\Delta PH}$) (Fig. 1*A*). IRS-1 and IRS-$1^{\Delta PH}$ were expressed to equivalent levels in 32D cells and in CHO^{IR} and $32D^{IR}$ cells overexpressing the insulin receptor. The 32D cells were used because they do not contain endogenous IRS-signaling proteins. IRS-$1^{\Delta PH}$ migrated at the expected molecular mass in all of the cell lines during SDS-PAGE, suggesting that it was stably expressed and Ser/Thr phosphorylated in a manner similar to wild-type IRS-1 (Fig. 1*B*).

Insulin stimulated the expected tyrosyl phosphorylation of the insulin receptor β-subunit in CHO^{IR}, CHO^{IR}/IRS-1, and CHO^{IR}/IRS-$1^{\Delta PH}$ cells (Fig. 2*A*). During insulin stimulation, tyrosine phosphorylation of endogenous IRS-1 was clearly observed in CHO^{IR} cells, and overexpression of IRS-1 in these cells resulted in a striking increase, as described previously (4). However, insulin-stimulated tyrosine phosphorylation of IRS-$1^{\Delta PH}$ was greatly reduced (>95%) in CHO^{IR}/IRS-$1^{\Delta PH}$ cells. Normal tyrosine phosphorylation of endogenous IRS-1 was detected in the CHO^{IR}/IRS-$1^{\Delta PH}$ cells during insulin stimulation (Fig. 2*A*). Thus, deletion of the PH domain from IRS-1 abrogates its tyrosine phosphorylation in CHO^{IR} cells but does not interfere with the phosphorylation of the endogenous wild-type IRS-1.

We investigated the Ser/Thr phosphorylation of IRS-1 and IRS-$1^{\Delta PH}$ in CHO^{IR} cells by monitoring levels of [^{32}P]phosphate incorporated during insulin stimulation (Fig. 2*B*). Solely as a control for normal levels of Ser/Thr phosphorylation, we included another IRS-1 mutant, IRS-1^{F18}, which contains an intact PH domain but is not tyrosyl-phosphorylated due to the substitution of phenylalanine for tyrosine in all of its putative tyrosyl phosphorylation sites.[3] Insulin stimulated the phosphorylation of IRS-1, IRS-$1^{\Delta PH}$, and IRS-1^{F18} as monitored by decreased mobility and increased [^{32}P]phosphate content (Fig. 2*B*). IRS-1 is highly serine-phosphorylated in the basal state and undergoes tyrosine and Ser/Thr phosphorylation during insulin stimulation (4, 15). Since IRS-$1^{\Delta PH}$ and IRS-1^{F18} are not tyrosine-phosphorylated in either the basal state or during insulin stimulation in CHO^{IR} cells, the phosphorylation of these species reflects only Ser/Thr phosphorylation. Thus, IRS-$1^{\Delta PH}$ is recognized normally by Ser/Thr kinases, suggesting that its structure is not globally disrupted. Therefore, removal of the PH domain interfered specifically with the tyrosine phosphorylation of IRS-1.

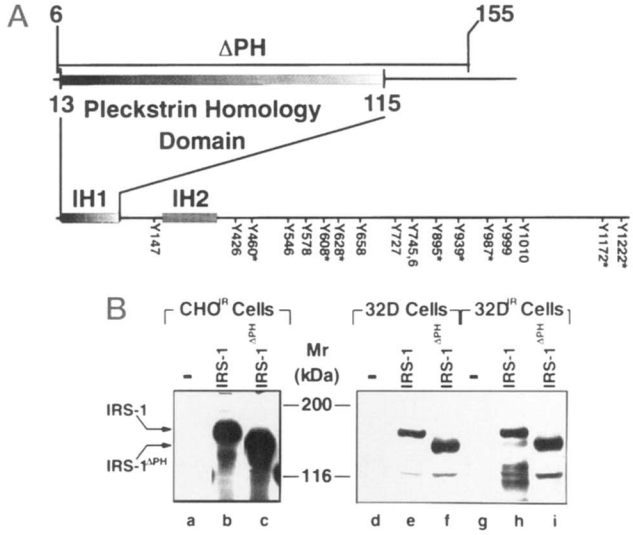

FIG. 1. **PH domain deletion IRS-$1^{\Delta PH}$.** *A*, a linear model of IRS-1 with the location of interesting domains and potential tyrosine phosphorylation sites is shown. The PH domain of IRS-1 extends from amino acids 13–115 (1, 7). Amino acids 6–155 are removed from IRS-$1^{\Delta PH}$. The PH domain (IRS homology region (IH)-1) and another region (IH2) share high identity between IRS-1 and IRS-2 (see footnote 2). The location of potential tyrosine phosphorylation sites is shown; *asterisks* indicate known sites of tyrosine phosphorylation (25). *B*, parental CHO^{IR}, 32D, or $32D^{IR}$ cells and CHO^{IR}, 32D, and $32D^{IR}$ cell lines expressing IRS-1 or IRS-$1^{\Delta PH}$ were lysed, resolved by SDS-PAGE, and immunoblotted with αIRS-1 antibodies. Note, although endogenous IRS-1 was detectable in CHO^{IR} cells on long exposure, it is not seen in the figure as a consequence of the short exposure needed to prevent overexposing the exogenous IRS-1 isoforms. No endogenous IRS-1 was detectable in 32D cell lines, as reported previously (2, 3). Migration of molecular size standards, IRS-1, and IRS-$1^{\Delta PH}$ is indicated.

32D cells are an ideal system for the examination of IRS-1 function, since they contain no endogenous IRS proteins to interfere with the analysis of signaling by exogenous expressed species (2, 3, 5). Activation of endogenous insulin receptors stimulated tyrosyl phosphorylation of IRS-1 in 32D/IRS-1 cells, whereas disruption of the PH domain reduced tyrosine phosphorylation by more than 95% (Fig. 3*A*); no tyrosyl phosphorylation of IRS-1 was observed in untransfected 32D cells, since these cells do not contain IRS-1 (2, 3, 5). Insulin stimulated the association of PI 3′-kinase with IRS-1 in 32D/IRS-1 cells. Moreover, IRS-1 was essential for the activation of $p70^{s6k}$ by insulin (Fig. 3*C*); however, disruption of the PH domain of IRS-1 completely inhibited these events in 32D cells (Fig. 2*C*).

[3] M. G. Myers, Jr., Y. Zhang, E. Glasheen, T. Grammer, L. Yenush, L. M. Wang, X. J. Sun, J. Blenis, J. H. Pierce, and M. F. White, manuscript in preparation.

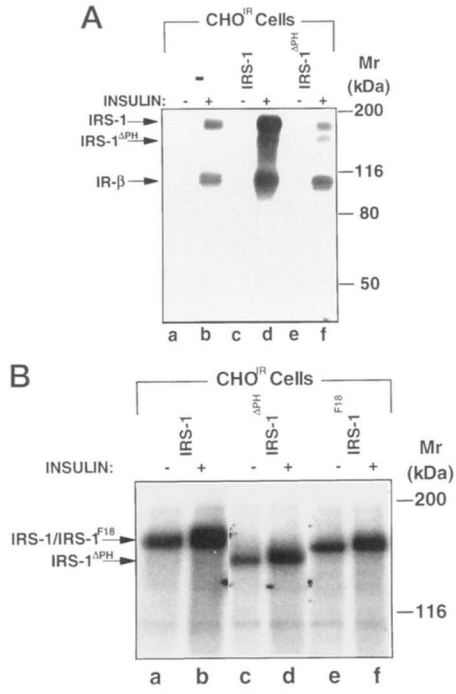

FIG. 2. **Phosphorylation of IRS-1$^{\Delta PH}$ in CHO^{IR} cells.** *A*, quiescent CHO^{IR} cell lines were incubated in the absence or presence of 100 nM insulin for 5 min and lysed. Lysates were resolved by SDS-PAGE, and tyrosyl phosphoproteins were analyzed by immunoblotting with αPY. *B*, quiescent CHO^{IR} cell lines were labeled for 3 h with [^{32}P]orthophosphate and incubated in the absence or presence of 100 nM insulin for 10 min. Cells were lysed and immunoprecipitated with αIRS-1. Immunoprecipitates were resolved by SDS-PAGE and imaged on a Molecular Dynamics PhosphorImager. Phosphoamino acid analysis confirmed the presence of Ser(P) and Thr(P) on all IRS-1 isotypes, but significant amounts of Tyr(P) were found only in wild-type IRS-1 (not shown). The migration of IRS-1, IRS-1$^{\Delta PH}$, IRS-1^{F18}, and molecular size standards is indicated. These experiments are representative of multiple independent assays performed with multiple independently derived clones.

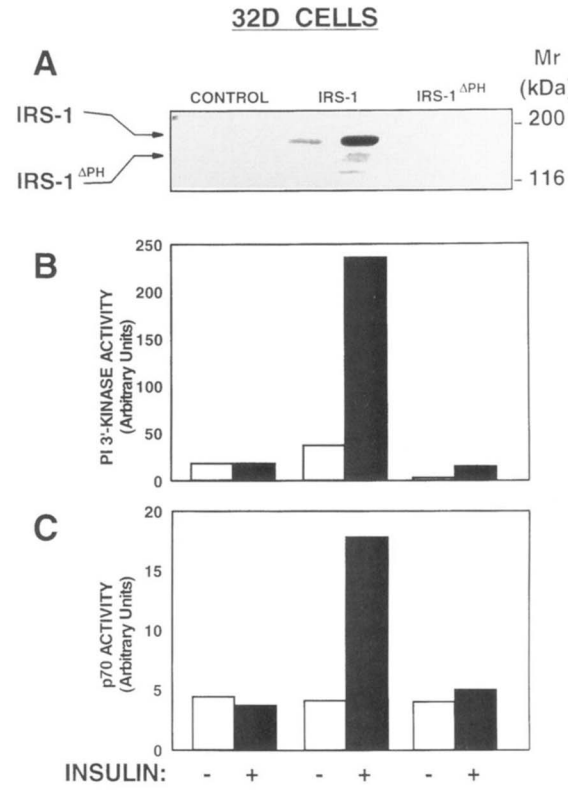

FIG. 3. **Signaling by IRS-1 and IRS-1$^{\Delta PH}$ in 32D cells.** *A*, quiescent 32D cell lines were incubated in the absence or presence of insulin for 5 min and lysed. Lysates were resolved by SDS-PAGE and analyzed by immunoblotting with αPY antibodies. Migration of molecular size standards and IRS-1 is indicated, along with the expected position of IRS-1$^{\Delta PH}$. *B*, quiescent 32D cell lines were stimulated with insulin for 5 min, lysed, and immunoprecipitated with αIRS-1 antibodies. Immunoprecipitates were washed and assayed for associated PI 3′-kinase activity. *C*, quiescent 32D cell lines were stimulated with insulin for 30 min and lysed. p70^{s6k} was immunoprecipitated and its activity assayed in an *in vitro* immune complex assay. Activity in *B* and *C* was quantified on a PhosphorImager and is shown as arbitrary units. Each assay in this figure is representative of at least three independent experiments with multiple independently derived cell lines.

Unlike CHO^{IR} cells, overexpression of the insulin receptor in 32D^{IR} cells restored normal insulin signaling by IRS-1$^{\Delta PH}$; both IRS-1 and IRS-1$^{\Delta PH}$ were tyrosine-phosphorylated to equal levels in 32D^{IR} cells during insulin stimulation (Fig. 4*A*). During insulin stimulation, IRS-1 and IRS-1$^{\Delta PH}$ associated normally with PI 3′-kinase (Fig. 4*B*) and both mediated the activation of p70^{s6k} (Fig. 4*C*). Futhermore, insulin stimulated mitogenesis equivalently in 32D^{IR} cells expressing either wild-type IRS-1 or IRS-1$^{\Delta PH}$ (Fig. 4*D*). Thus, overexpression of the insulin receptor in 32D^{IR} cells restored IRS-1$^{\Delta PH}$ phosphorylation and, with it, signaling.

Our results suggest that the PH domain is essential for sensitive coupling between IRS-1 and the insulin receptor. Despite the inability of IRS-1 molecules with PH domain deletions to become tyrosine-phosphorylated and mediate signaling in the low insulin receptor environment of 32D cells, these mutations are rescued by overexpression of the insulin receptor in 32D^{IR} cells. Therefore, disruption of the PH domain does not damage the ability of IRS-1 to mediate downstream signals, as IRS-1$^{\Delta PH}$ is phosphorylated normally and signals effectively in the presence of high levels of insulin receptor in 32D^{IR} cells. Since the PH domain comprises an independent protein module (10, 11), it is not surprising that the remaining portions of IRS-1$^{\Delta PH}$ are properly folded and retain biological function. The observation that a mutant IRS-1 molecule containing a small deletion (30 amino acids) at the beginning of the PH domain also abrogates tyrosyl phosphorylation in 32D cells provides further evidence that these results do not stem from the disruption of sequences downstream of the PH domain.[4]

Why does overexpression of the insulin receptor in CHO^{IR} cells not rescue insulin-stimulated tyrosyl phosphorylation of IRS-1$^{\Delta PH}$? Unlike 32D cells, CHO cells contain endogenous IRS-1, which possesses an intact PH domain. The PH domain must provide a significant advantage during competition for a limited number of insulin receptors, possibly by displacing the low affinity IRS-1$^{\Delta PH}$ away from the receptor.

It remains unclear how the PH domain mediates its function. It could involve direct protein-protein interactions between the receptor and IRS-1; however, our attempts to measure specific interactions between the purified insulin receptor and glutathione *S*-transferase fusion proteins containing the PH domain have been unsuccessful. Recent evidence suggests that PH domains from other signaling molecules bind specific phospholipids (13). Further work should shed light on whether the PH domain acts specifically to target IRS-1 to the insulin receptor or more generally to the membrane compartment where it encounters the tyrosine kinase.

IRS-1 contains a region immediately downstream of the PH domain which has also been implicated in the IR/IRS-1 interaction (IH2 region in Fig. 1*A*) (6). The IH2 region is similar in IRS-1 and IRS-2, and like the PH domain, contains no tyrosine phosphorylation sites (15).[2] The IH_2 region appears to be a

[4] M. G. Myers, Jr., J. H. Pierce, and M. F. White, unpublished data.

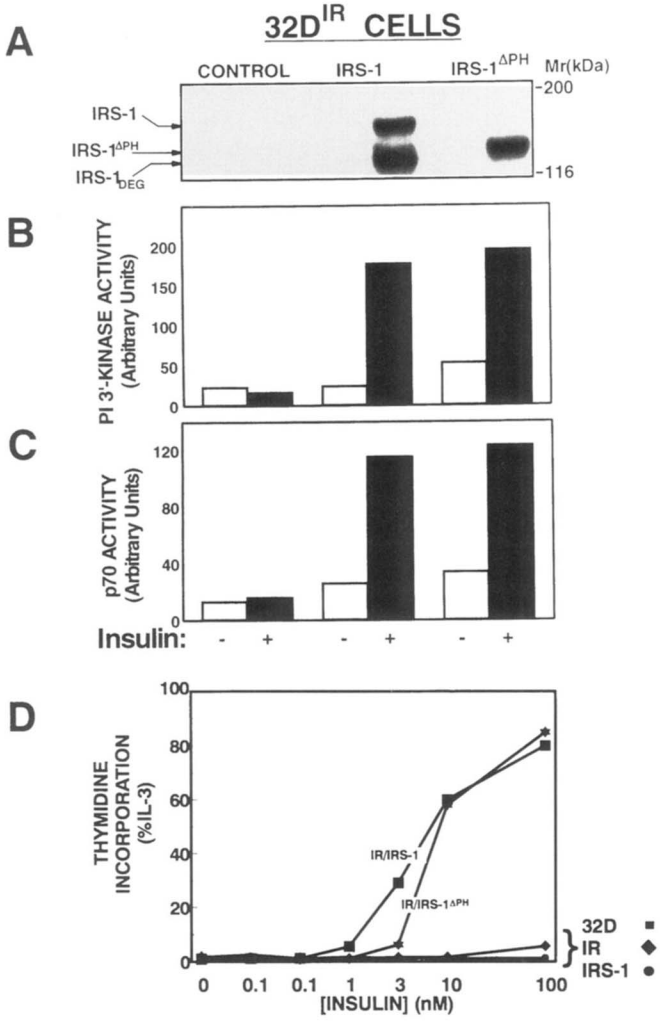

FIG. 4. **Signaling by IRS-1 and IRS-1$^{\Delta PH}$ in 32D^{IR} cells.** *A*, quiescent 32D^{IR} cell lines were incubated in the absence or presence of insulin for 5 min and lysed. Lysates were resolved by SDS-PAGE and analyzed by immunoblotting with αPY antibodies. Migration of molecular size standards, IRS-1, IRS-1$^{\Delta PH}$, and a degradation product of IRS-1 (IRS-1$_{DEG}$) is indicated. *B*, quiescent 32D^{IR} cell lines were stimulated with insulin for 5 min, lysed, and immunoprecipitated with αIRS-1 antibodies. Immunoprecipitates were washed and assayed for associated PI 3′-kinase activity. *C*, quiescent 32D^{IR} cell lines were stimulated with insulin for 30 min and lysed. p70^{s6k} was immunoprecipitated and its activity assayed in an *in vitro* immune complex assay. Activity in *B* and *C* was quantified on a PhosphorImager and is shown as arbitrary units. *D*, cell lines were incubated in various concentrations of insulin for 48 h, followed by the addition of [^{3}H]thymidine for another 3 h. Cells were harvested onto glass filters, and incorporated nucleotide was quantified by scintillation counting. Each assay in this figure is representative of at least three independent experiments with multiple independently derived cell lines.

phosphotyrosine binding domain like the one in Shc (26, 27)[2]; this region binds the NPEY motif in the insulin receptor (6, 22), [2] and its deletion partially inhibits IR-dependent phosphorylation (data not shown). This supports previous results showing that the NPXY$_{960}$ motif is essential for insulin signaling and IRS-1 phosphorylation (15, 23, 24). The role of the PH domain and the IH2 regions in IRS engagement by other systems, such as IL-4 and growth hormone, remains to be determined.

The PH domain is 100% identical in mouse, human, and rat IRS-1, and 62% similar in IRS-1 and IRS-2. This is the highest level of PH domain conservation between distinct proteins yet observed; this conservation goes well beyond the requirement for similar folding recently revealed by structural studies (10, 11) and is consistent with a specific function. The IH2 regions are also conserved between IRS-1 and IRS-2. The COOH terminus, however, is poorly conserved between IRS-1 and IRS-2, however, with the critical exception of short elements surrounding tyrosine phosphorylation sites.[2] Thus, the PH domain and the IH2 regions are likely to cooperate in mediating IR/IRS recognition. Impairment of PH domain or IH2 region interactions is expected to alter the sensitivity of the insulin response, and could contribute to insulin resistance.

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REFERENCES

1. Myers, M. G., Jr., Sun, X. J., and White, M. F. (1994) *Trends Biochem. Sci.* **19,** 289–294
2. Wang, L. M., Myers, M. G., Jr., Sun, X. J., Aaronson, S. A., White, M. F., and Pierce, J. H. (1993) *Science* **261,** 1591–1594
3. Myers, M. G., Jr., Wang, L. M., Sun, X. J., Zhang, Y., Yenush, L. P., Schlessinger, J., Pierce, J. H., and White, M. F. (1994) *Mol. Cell. Biol.* **14,** 3577–3587
4. Sun, X. J., Miralpeix, M., Myers, M. G., Jr., Glasheen, E. M., Backer, J. M., Kahn, C. R., and White, M. F. (1992) *J. Biol. Chem.* **267,** 22662–22672
5. Myers, M. G., Jr., Grammer, T. C., Wang, L. M., Sun, X. J., Pierce, J. H., Blenis, J., and White, M. F. (1994) *J. Biol. Chem.* **269,** 28783–28789
6. O'Neill, T. J., Craparo, A., and Gustafson, T. A. (1994) *Mol. Cell. Biol.* **14,** 6433–6442
7. Musacchio, A., Gibson, T., Rice, P., Thompson, J., and Saraste, M. (1993) *Trends Biochem. Sci.* **18,** 343–348
8. Keller, S. R., Aebersold, R., Garner, C. W., and Lienhard, G. E. (1993) *Biochim. Biophys. Acta* **1172,** 323–326
9. Araki, E., Sun, X. J., Haag, B. L., Zhang, Y., Chuang, L. M., Yang-Feng, T., White, M. F., and Kahn, C. R. (1993) *Diabetes* **42,** 1041–1054
10. Yoon, H. S., Hajduk, P. J., Petros, A. M., Olejniczak, E. T., Meadows, R. P., and Fesik, S. W. (1994) *Nature* **369,** 672–675
11. Macias, M. J., Musacchio, A., Ponstingl, H., Nilges, M., Saraste, M., and Oschkinat, H. (1994) *Nature* **369,** 675–677
12. Gibson, T. J., Hyvonen, M., Musacchio, A., and Saraste, M. (1994) *Trends Biochem. Sci.* **19,** 349–353
13. Harlan, J. E., Hajduk, P. J., Yoon, H. S., and Fesik, S. W. (1994) *Nature* **371,** 168–170
14. Touhara, K., Inglese, J., Pitcher, J. A., Shaw, G., and Lefkowitz, R. J. (1994) *J. Biol. Chem.* **14,** 10217–10220
15. Sun, X. J., Rothenberg, P., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Cahill, D. A., Goldstein, B. J., and White, M. F. (1991) *Nature* **352,** 73–77
16. Saad, M. J. A., Araki, E., Miralpeix, M., Rothenberg, P. L., White, M. F., and Kahn, C. R. (1992) *J. Clin. Invest.* **90,** 1839–1849
17. White, M. F. and Backer, J. M. (1991) *Methods Enzymol.* **201,** 65–79
18. Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) *Cell* **69,** 1227–1236
19. Towbin, H., Staehelin, T., and Gordon, G. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4354
20. Ruderman, N., Kapeller, R., White, M. F., and Cantley, L. C. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 1411–1415
21. Varticovski, L., Daley, G. Q., Jackson, P., Baltimore, D., and Cantley, L. C. (1991) *Mol. Cell. Biol.* **11,** 1107–1113
22. Gustafson, T. A., He, W., Craparo, A., Schaub, C. D., and O'Neill, T. J. (1995) *Mol. Cell Biol.*, in press
23. White, M. F., Livingston, J. N., Backer, J. M., Lauris, V., Dull, T. J., Ullrich, A., and Kahn, C. R. (1988) *Cell* **54,** 641–649
24. Yonezawa, K., Ando, A., Kaburagi, Y., Yamamotohonda, R., Kitamura, T., Hara, K., Nakafuku, M., Okabayashi, Y., Kadowaki, T., Kaziro, Y., and Kasuga, M. (1994) *J. Biol. Chem.* **269,** 4634–4640
25. Sun, X. J., Crimmins, D. L., Myers, M. G., Jr., Miralpeix, M., and White, M. F. (1993) *Mol. Cell. Biol.* **13,** 7418–7428
26. Bork, P., and Margolis, B. (1995) *Cell* **80,** 693–694
27. Karanaugh, W. M., and Williams, L. T. (1994) *Science* **266,** 1862–1865