Since the discovery of insulin and its receptor, the downstream elements responsible for the pleiotropic insulin signal have been difficult to define. The recently discovered insulin receptor substrate, IRS-1, provides an innovative and simple way to think about this problem: IRS-1 may mediate the control of various cellular processes by insulin. Overexpression of IRS-1 enhances insulin-stimulated DNA synthesis in Chinese hamster ovary cells, and microinjection of IRS-1 protein potentiates the maturation of Xenopus oocytes. We suspect that insulin signals are enabled when the activated insulin receptor kinase phosphorylates specific tyrosine residues in IRS-1. These phosphorylated sites associate with high affinity to cellular proteins that contain SH2 (src homology-2) domains. This association is specific and depends on the amino acid sequence surrounding the phosphotyrosine residue and the isoform of the SH2 domain. A growing number of SH2 domain-containing proteins that associate with phosphorylated IRS-1 have been identified, and we suspect that IRS-1 has the potential to simultaneously regulate many of them. We have only begun to identify the specific proteins that associate with phosphorylated IRS-1. One of them, the phosphatidylinositol 3'-kinase, is activated when the SH2 domains in its 85,000-Mr regulatory subunit bind to phosphorylated IRS-1. IRS-1 also interacts with other proteins such as SHPTP2, a novel SH2 domain–containing Tyr phosphatase, and GRB-2/sen5, a protein that is implicated in p21^{ras} signaling. The interaction between phosphorylated IRS-1 and multiple SH2 domain–containing proteins may ultimately explain the pleiotropic effects of insulin.

Diabetes 42:643–50, 1993

Since the discovery of insulin more than 70 years ago, its effect on carbohydrate, fat, and protein metabolism has been well established (1). Insulin treatment of responsive cells generates molecular signals that regulate membrane transport processes (glucose, amino acids, and ions), activate and inactivate enzymes, and regulate gene expression, protein synthesis, and DNA synthesis. However, the nature of these signals has been difficult to determine (2).

When the insulin signal pathways become resistant to hormonal stimulation, impaired glucose tolerance may result. This may lead to NIDDM when too much insulin is needed to trigger an adequate insulin response and the pancreas cannot compensate. The biochemical problems that cause insulin resistance are not well understood. Mutations of the insulin receptor appear to be the culprit in a few cases of severe insulin resistance (3), however, the underlying molecular defects in the majority of NIDDM patients are unknown. Approaches that seek to identify marker genes that segregate with NIDDM have succeeded in some well-defined families with maturity-onset diabetes of the young, where alterations have been identified on chromosome 20 near the adenosine deaminase gene in one family (4), and on chromosome 7 in the glucokinase gene in several other families (5). However, identification of each element responsible for insulin resistance in the majority of NIDDM patients may only arise from a full characterization of the molecular pathways of insulin signal transmission.

**THE INSULIN RECEPTOR**

After its release by the B-cells of the pancreas, insulin binds to the insulin receptor on the surface of cells.
The discovery of IRS-1

Two possible models of insulin signal transmission are outlined in Fig. 1. One model suggests that Tyr autophosphorylation causes a conformational change in the β-subunit of the insulin receptor (17), facilitating the interaction of the insulin receptor with cellular elements responsible for downstream signaling. This model is especially attractive because it is used by the EGF and PDGF receptors to interact with PLC-γ, p21ras-GAP, and GRB-2/srm-5 (18–26,79). However, the direct association of these or other molecules implicated in signaling has not been clearly demonstrated for the insulin receptor. Moreover, in contrast to these receptors, the insulin receptor kinase is activated by autophosphorylation (27,28), suggesting that the intracellular insulin signal may be enabled by tyrosyl phosphorylation of some other molecule.

Distinguishing between autophosphorylation-based signaling and substrate-based signaling has been difficult in the case of the insulin receptor because autophosphorylation is linked to receptor kinase activity. Thus, mutation of important sites of Tyr autophosphorylation abrogates the receptor’s signaling either because phosphotyrosines that serve as binding sites for downstream mediators are eliminated or because receptor kinase activity is reduced (29–31).

The development of high-affinity antiphosphotyrosine antibodies (α-PY) provided the first evidence for the existence of a direct cellular substrate of the insulin receptor (32,33). Immediately after insulin stimulation, the α-PY immunoprecipitates or immunoblots a tyrosyl-phosphorylated protein that migrates as a broad band of ~185,000 M, during SDS-PAGE (32,33). This protein, originally called pp185, is distinct from the insulin receptor by various criteria (32,33). It is located mainly in the cytoplasm and contains phosphotyrosine, phosphoserine, and phosphothreonine after insulin stimulation (33). Insulin receptors with mutations in the intracellular juxtamembrane region of the β-subunit (Fig. 1) provide indirect evidence that pp185 is important in insulin signaling. Substitution of Tyr or deletion of Tyr and 11 adjacent amino acids from the insulin receptor juxtamembrane region impairs common insulin bioeffects, including glycogen and DNA synthesis (34–37). Moreover, although their kinase is normally activated during in vitro assays, neither of these mutant receptors stimulates the tyrosyl phosphorylation of pp185 in vivo, suggesting that the pp185 may be relevant in insulin signaling (34,36,37).

We purified pp185 from insulin-stimulated rat liver by affinity chromatography on immobilized α-PY (38) and isolated the cDNA for this novel protein, which we call IRS-1 (39,40). An identical protein also was purified from 3T3-L1 cells (41), and the human cDNA was accidentally cloned from liver by others (42).

IRS-1 is a cytosolic protein with a predicted molecular weight of 131,000 M. It migrates between 170,000 and 180,000 M, on SDS-PAGE when expressed in CHO cells or in a baculovirus expression system (39,43). IRS-1 is highly serine phosphorylated and is rapidly Tyr phosphorylated in response to insulin (39,43). Although IRS-1 is highly conserved between rats and humans, it has little extended homology to other known proteins (39,42). One potential nucleotide binding site near the amino terminus of IRS-1 is conserved between the rat and human pro-
we have only begun to identify which sites are phosphorylated in vitro by the purified receptor and whether these Tyr's, with one or two conservative changes in 4 others; this suggests these Tyr's perform some conserved function in insulin action. Fortunately, this interpretation is complicated by the substantial insulin sensitivity of the control cells. However, we conclude that IRS-1 plays a positive role in linking the insulin receptor to serine/threonine phosphorylated of the endogenous IRS-1 is weakly detected with an \( \alpha \)-PY after insulin stimulation (43). However, overexpression by 20-fold of rat IRS-1 in these cells results in an easily detectable IRS-1 band during \( \alpha \)-PY immunoblots. Insulin-stimulated DNA synthesis is monitored in these cells by measuring \( ^3 \)Hthymidine incorporation. The overexpression of IRS-1 alone in the CHO/IRS cells increases by twofold the maximum response of thymidine incorporation to insulin stimulation, with no effect on the sensitivity to insulin (43).

**IRS-1 MEDIATES INSULIN SIGNALS**

One of the most important questions regarding IRS-1 is whether it plays an obligatory role in the biological function of insulin, and whether its dysfunction is important in NIDDM. This is a difficult problem because physiological systems to evaluate the role of specific proteins in insulin action are difficult to manipulate at the molecular level. Initially, we used CHO cells to evaluate the biological potential of IRS-1. Although CHO cells are a poor substitute for fat, muscle, or liver cells, they provide a useful experimental system as they contain less endogenous IRS-1 than insulin-sensitive tissues, are easily manipulated, and display some insulin-sensitive glycogen and DNA synthesis (34).

Before transfection of CHO cells with IRS-1, Tyr phosphorylation of the endogenous IRS-1 is weakly detected with an \( \alpha \)-PY after insulin stimulation (43). However, overexpression by 20-fold of rat IRS-1 in these cells results in an easily detectable IRS-1 band during \( \alpha \)-PY immunoblots. Insulin-stimulated DNA synthesis is monitored in these cells by measuring \( ^3 \)Hthymidine incorporation. The overexpression of IRS-1 alone in the CHO/IRS cells increases by twofold the maximum response of thymidine incorporation to insulin stimulation, with no effect on the sensitivity to insulin (43). Unfortunately, this interpretation is complicated by the substantial insulin sensitivity of the control cells. However, we conclude that IRS-1 plays a positive role in linking the insulin receptor to nuclear events in these cells. A definitive demonstration of the requirement of IRS-1 in insulin signaling awaits the discovery or production of IRS-1 negative cells.

Paradoxically, overexpression of IRS-1 causes insulin resistance in CHO cells that contain about one million human insulin receptors (43). The phosphorylation of IRS-1 is 10- to 50-fold higher in cells with high insulin receptors than with endogenous CHO receptors (43).

**TABLE 1**

Potential Tyr phosphorylation sites in IRS-1

<table>
<thead>
<tr>
<th>Position</th>
<th>Surrounding sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td>ARLEYY_{E}NEK</td>
</tr>
<tr>
<td>3</td>
<td>ODWSY_{Q}ALL</td>
</tr>
<tr>
<td>4</td>
<td>EL{D}S_YDTG P</td>
</tr>
<tr>
<td>5</td>
<td>SSD_{E}GYSS P</td>
</tr>
<tr>
<td>6</td>
<td>EL_{S}NY_{L}CMG*</td>
</tr>
<tr>
<td>7</td>
<td>SIE{E}YTEMM*</td>
</tr>
<tr>
<td>8</td>
<td>PTH{S}YPEEG</td>
</tr>
<tr>
<td>9</td>
<td>TDDGY_{M}PMS_{P}^{*}</td>
</tr>
<tr>
<td>10</td>
<td>NGDGY_{M}PMS_{P}^{*}</td>
</tr>
<tr>
<td>11</td>
<td>DPNGY_{M}MMPS_{P}^{+}</td>
</tr>
<tr>
<td>12</td>
<td>CTGDY_{M}NMPS_{P}^{+}</td>
</tr>
<tr>
<td>13,14</td>
<td>745,746</td>
</tr>
<tr>
<td>15</td>
<td>SPGEY_{N}NIE</td>
</tr>
<tr>
<td>16</td>
<td>GSE{E}YMND*</td>
</tr>
<tr>
<td>17</td>
<td>SRG_{D}YMT_{M}Q*</td>
</tr>
<tr>
<td>18</td>
<td>APV{S}ADMR*</td>
</tr>
<tr>
<td>19</td>
<td>KSLNY_{I}DLD</td>
</tr>
<tr>
<td>20</td>
<td>DLS{T}YASIN</td>
</tr>
</tbody>
</table>

Potential Tyr phosphorylation sites are underlined and shown with surrounding acidic amino acids. Nearby acidic amino acids (D/E), thought to be important for Tyr kinase recognition, are in bold.

* Four of the YXXM motifs are followed by the amino acids SP.

**DISCUSSION**

IRS-1 contains over 30 potential serine/threonine phosphorylation sites with homologies to casein kinase II, protein kinase C, the MAP kinases, cdc 2, and cAMP- and cGMP-dependent protein kinase consensus phosphorylation sites (39). Before insulin stimulation of cells, IRS-1 is strongly serine phosphorylated and weakly threonine phosphorylated. After insulin stimulation, Tyr and serine phosphorylation of IRS-1 is increased (43).

The 20 potential Tyr phosphorylation sites on IRS-1 (Table 1) are conserved between rat and human IRS-1, and the surrounding 3-4 amino acids are identical for 16 of these Tyr's, with one or two conservative changes in 4 others; these suggest these Tyr's perform some conserved function (39,42). Interestingly, 6 of these Tyr's lie in the amino acid sequence motif YXXM (Tyr-Met-Xaa-Met) and 3 others lie in related YXXM motifs (Table 1) (39).

Multiple sites on IRS-1 are Tyr phosphorylated both in vivo and in vitro, as phosphopeptide mapping of IRS-1 from insulin-stimulated cells or from in vitro reactions with purified insulin receptor and recombinant IRS-1 demonstrates the presence of many tyrosyl phosphopeptides (43,44). Owing to the complexity of the phosphorylation, we have only begun to identify which sites are phosphorylated in vitro by the purified receptor and whether these correspond to the sites used in vivo.

IRS-1 is an in vivo substrate for the IGF-1 receptor and for the insulin receptor (45), but the receptors for PDGF, EGF, and CSF-I do not phosphorylate IRS-1 (M.G.M., M.F.W., unpublished observations). However, we have not exhausted this analysis, and it seems likely that other IRS-1-specific kinases will be identified in the future. Thus, IRS-1 appears to be involved in signaling by only a subset of growth factor receptors and may confer unique attributes to the signaling pathways initiated by certain kinases.

**DISCUSSION**

One of the most important questions regarding IRS-1 is whether it plays an obligatory role in the biological function of insulin, and whether its dysfunction is important in NIDDM. This is a difficult problem because physiological systems to evaluate the role of specific proteins in insulin action are difficult to manipulate at the molecular level. Initially, we used CHO cells to evaluate the biological potential of IRS-1. Although CHO cells are a poor substitute for fat, muscle, or liver cells, they provide a useful experimental system as they contain less endogenous IRS-1 than insulin-sensitive tissues, are easily manipulated, and display some insulin-sensitive glycogen and DNA synthesis (34).

Before transfection of CHO cells with IRS-1, Tyr phosphorylation of the endogenous IRS-1 is weakly detected with an \( \alpha \)-PY after insulin stimulation (43). However, overexpression by 20-fold of rat IRS-1 in these cells results in an easily detectable IRS-1 band during \( \alpha \)-PY immunoblots. Insulin-stimulated DNA synthesis is monitored in these cells by measuring \( ^3 \)Hthymidine incorporation. The overexpression of IRS-1 alone in the CHO/IRS cells increases by twofold the maximum response of thymidine incorporation to insulin stimulation, with no effect on the sensitivity to insulin (43). Unfortunately, this interpretation is complicated by the substantial insulin sensitivity of the control cells. However, we conclude that IRS-1 plays a positive role in linking the insulin receptor to nuclear events in these cells. A definitive demonstration of the requirement of IRS-1 in insulin signaling awaits the discovery or production of IRS-1 negative cells.

Paradoxically, overexpression of IRS-1 causes insulin resistance in CHO cells that contain about one million human insulin receptors (43). The phosphorylation of IRS-1 is 10- to 50-fold higher in cells with high insulin receptors than with endogenous CHO receptors (43). By using multiple clones of CHO cells expressing one million insulin receptors and various levels of IRS-1, we found an inverse relationship between levels of IRS-1 expression and insulin-stimulated thymidine incorporation (43). This counterintuitive result has several potential explanations. One possibility is that as the concentrations of both insulin receptor and IRS-1 increase in the cell, hyperphosphorylation of IRS-1 causes poorly timed or inappropriate signals. This complex relationship between IRS-1 and the insulin receptor may be partially responsible for the insulin resistance observed in two experimental models of diabetes: fasting and insulinopenic (streptozocin) diabetes. These animals display insulin resistance in the context of elevated levels of insulin receptor and IRS-1 (46).
A positive role of IRS-1 in insulin signaling also has been demonstrated recently in Xenopus oocytes (83). Recombinant IRS-1 protein injected into oocytes is Tyr phosphorylated after insulin or IGF-1 stimulation. Interestingly, the majority of oocytes that have been injected with IRS-1 protein mature (complete the first meiotic division) in response to insulin or IGF-1, whereas un.injected oocytes fail to mature in response to insulin or IGF-1. This finding not only confirms the role of IRS-1 in insulin and IGF-1 signaling but implicates IRS-1 in cell cycle control. The maturation of Xenopus (and other, e.g., clam) oocytes has been induced previously by microinjection of MPF, a complex of cyclin and ~34'~' kinase (47-51). The activity of MPF varies across the cell cycle because of its regulation by numerous phosphorylation/ dephosphorylation events (47,48). This activity is crucial for progression across the cell cycle in systems as disparate as yeast and mammalian cells (47). Thus, IRS-1 could be on the pathway between the insulin/IGF-1 receptor and these important regulators of cell growth.

A PARADIGM FOR INSULIN SIGNALING

Activation of the Ptdlns 3'-kinase. Insulin treatment of cells results in the appearance of Ptdlns 3'-kinase in α-PY immunoprecipitates and causes the accumulation of the products of Ptdlns 3'-kinase in the cell (52). Ptdlns 3'-kinase phosphorylates the lipid Ptdlns on the 3' position of the o-myo-inositol ring, yielding Ptdlns 3'-phosphate or Ptdlns 3-3 (53). Because the enzyme can use alternatively phosphorylated forms of Ptdlns (Ptdlns 4-P, Ptdlns 4,5-P, Ptdlns 4,5,6-P) as substrates, activation of the enzyme also leads to the formation of Ptdlns 3,4-P and Ptdlns 3,4,5-P (54). This pathway differs from the classical Ptdlns pathway in which Ptdlns 4,5-P is cleaved by an isoform of PLC to DAG and 1,4,5 IP, by a PLC isoform, because the lipid products of Ptdlns 3'kinase are not hydrolyzed by any known phospholipase (55). Thus, the mechanisms by which Ptdlns 3'-kinase controls cellular growth and metabolism are not known (54).

Studies with the PDGF receptor and the polyoma MTag suggest that Ptdlns 3'-kinase is involved in cellular growth control (54). Mutants of the PDGF receptor that do not bind to the Ptdlns 3'-kinase are deficient in mediating cellular proliferation in response to PDGF stimulation (19,56,57), although the Tyr kinase activity of these receptors is similar to wild-type PDGF receptor. Similarly, polyoma viruses expressing mutant MTagS that are deficient in binding to Ptdlns 3'-kinase are defective in cellular transformation (58).

The ability of the insulin receptor to stimulate Ptdlns 3'-kinase activity maps to the juxtamembrane region of the β-subunit, the same part of the receptor that is required for biological activity and phosphorylation of IRS-1 (34-37,59). Insulin stimulates the association of Ptdlns 3'-kinase with the insulin receptor and IRS-1 (52,60), however, much more associates with IRS-1 than with the insulin receptor (39). The amount of insulin receptor-associated Ptdlns 3'-kinase is much smaller than that associated with other Tyr-phosphorylated receptors, such as the PDGF. Furthermore, the association of the insulin receptor with Ptdlns 3'-kinase appears to be indirect and actually reflects association between the Ptdlns 3'-kinase/IRS-1 complex and the insulin receptor: IRS-1 forms a stable complex with the insulin receptor in vivo and in vitro, and overexpression of IRS-1 in CHO cells increases the amount of Ptdlns 3'-kinase associated with the insulin receptor after insulin stimulation (43,61). Thus, signaling by the insulin receptor differs from other receptors (such as the PDGF receptor) by the involvement of IRS-1 in the signaling pathway to Ptdlns 3'-kinase (Fig. 2).

Ptdlns 3'-kinase and SH2 domains. Ptdlns 3'-kinase is composed of two subunits—a 110,000-M, catalytic subunit (62) and an 85,000-M, regulatory protein (p85-α) 

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**FIG. 2.** Comparison of Ptdlns 3'-kinase binding to IRS-1 and the PDGF receptor. A: Insulin receptor pathway. B: PDGF receptor pathway. After insulin stimulation, IRS-1 is phosphorylated on YMXM motifs by the activated insulin receptor, whereas after PDGF stimulation, the PDGF receptor autophosphorylates on YMXM motifs in its kinase insert region. Both in the case of IRS-1 and the PDGF receptor, the Tyr-phosphorylated YMXM motifs create high-affinity binding sites for the SH2 domains on the p85-α subunit of Ptdlns 3'-kinase. (The Ptdlns 3'-kinase holoenzyme is a complex of the p85-α regulatory subunit and the p110 catalytic subunit.) This binding further causes an allosteric change in the conformation of p85-α, activating the p110 catalytic subunit.
(63–65). p85-α has no discernible catalytic activity, but has an SH3 domain, a region with homologies to the noncatalytic region of the BCR kinase and p21^ras-GAP, and two SH2 domains (63–65). SH2 domains mediate protein-protein interactions by binding to Tyr-phosphorylated protein motifs (66). Structural analysis suggests that SH2 domains bind phosphotyrosine alone at low affinity, whereas sequences COOH-terminal to the phosphotyrosine residue contribute important contacts for high-affinity interactions (67 and S.E. Shoelson, unpublished observations). The SH2 domains of p85-α mediate the binding of PtdIns 3'-kinase to regulatory proteins like IRS-1 and the PDGF receptor; the SH2 domains of p85-α, expressed as bacterial fusion proteins, bind to Tyr-phosphorylated IRS-1 and block the binding of PtdIns 3'-kinase to immobilized Tyr-phosphorylated IRS-1 (68).

The SH2 domain isoforms in p85-α preferentially recognize phosphotyrosine in YMXM (Tyr-Met-Xaa-Met) and related YVXM motifs (54). As noted above, IRS-1 contains 6 Tyrs in YMXM motifs (Table 1) (39), and these Tyrs represent likely sites for insulin receptor-catalyzed Tyr phosphorylation (69). The PDGF and CSF-1 receptors and MTAg also contain Tyr phosphorylation sites in these motifs and associate strongly with PtdIns 3'-kinase (54).

In the case of growth factor receptors (Fig. 2), these sites lie in the kinase insert region of the receptor and are autophosphorylated on receptor activation (54). This explains the requirement for the kinase insert region of these receptors for association with and activation of PtdIns 3'-kinase (19,56,70). Site-directed mutagenesis of the kinase-insert region of the PDGF receptor has shown that the YMXM and YVXM Tyr's homologous to those of IRS-1 are required for the interaction of PtdIns 3'-kinase with the PDGF receptor (57,71). Similarly, MTAg is phosphorylated on a single YMXM motif by the cellular Tyk kinase pp60^src, and removal of this site abrogates the MTAg/PtdIns 3'-kinase association (54,58). Synthetic peptides containing tyrosyl-phosphorylated YMXM motifs inhibit the binding of PtdIns 3'-kinase to IRS-1, the PDGF receptor, and MTAg; whereas unphosphorylated peptides or peptides containing scrambled sequences do not inhibit the interaction (44,57,68,70,71). These experiments emphasize the requirement for both phosphotyrosine and a specific surrounding sequence motif for successful recognition. As IRS-1 contains 6 YMXM motifs, it may bind multiple PtdIns 3'-kinase molecules, or simultaneously bind several distinct proteins containing a similar SH2 domain isoform.

Moreover, phosphorylation of Tyr residues in other motifs may mediate associations with other distinct SH2 domains.

**Activation of PtdIns 3'-kinase by association with IRS-1.** Based on early findings, which identified p85-α as a Tyr-phosphorylated protein in immunoprecipitates of activated growth factor receptors, it was thought that association of PtdIns 3'-kinase with activated growth factor receptors led to the Tyr phosphorylation of p85-α, and thereby to the activation of PtdIns 3'-kinase (72–74). However, there is disagreement as to whether p85-α is actually Tyr phosphorylated in response to growth factor stimulation in vivo (44,72,75–77). We have been unable to detect Tyr phosphorylation of p85-α during insulin stimulation; however, insulin stimulates PtdIns 3'-kinase activity severalfold (44). Moreover, PtdIns 3'-kinase is activated in vivo during association with Tyr-phosphorylated recombinant IRS-1 (44). Synthetic peptides containing phosphorylated YMXM motifs based on IRS-1 sequences also activate the enzyme (44). In vitro activation by Tyr-phosphorylated IRS-1 proteins can be blocked by incubation with an excess of p85-α SH2 domain fusion protein (68). Thus, the activation of PtdIns 3'-kinase is apparently attributable to the binding of the Tyr-phosphorylated YMXM motifs of IRS-1 to the SH2 domains in p85-α. This finding suggests that SH2 domains are capable of mediating allosteric regulatory functions and associative interactions.

**MULTIPLE DOWNSTREAM ELEMENTS IN IRS-1 SIGNALING**

IRS-1 contains 20 possible Tyr phosphorylation sites, each of which could interact with an SH2 domain-containing protein to mediate a distinct aspect of the insulin signal. The nature of these downstream effectors of IRS-1-mediated signaling is now under intensive study. At least two isoforms of p85 exist (α and β). As described above, the α-isofrom associates with the p110 subunit to form the PtdIns 3'-kinase holoenzyme. The SH2 domains of p85-β possess a high level of sequence identity with those of p85-α (65), and p85-β also associates with IRS-1 (M. Miralpeix, M.F.W., unpublished observations). However, less is known about the function of p85-β, as it does not appear to associate with p110 (78).

The p85-α and p85-β are most distinct in the so-called BCR-homology region, which may form part of the contact region with downstream mediators (65). Moreover, the SH3 domains of p85-α and p85-β are different enough to be responsible for unique signaling behavior. Thus, p85-β may act as an adapter molecule that links IRS-1 to another signaling enzyme (Fig. 3).

Two newly discovered SH2 domain-containing proteins, SHPTP2 (84) and GRB-2/sem-5 (79,85), have been found to interact with IRS-1 after insulin stimulation of the intact cell. SHPTP2 is a protein Tyr phosphatase that contains two SH2 domains. The association of a Tyr phosphatase with IRS-1 in a tyrosyl phosphate-dependent manner provides an interesting potential mechanism by which the insulin signal may either be mediated or downregulated (Fig. 3).

GRB-2/sem-5 is a small cytoplasmic protein that contains two SH3 domains and one SH2 domain and has no apparent activity of its own; like p85-α and p85-β, it is thought to act as an adapter molecule to link downstream signaling elements to tyrosyl phosphoproteins such as IRS-1 (79). Interestingly, genetic analysis implicates sem-5, the homologue of GRB-2 in C. elegans, as an upstream regulator of p21^ras. Moreover, the microinjection of both GRB-2 and p21^ras into cultured cells results in increased DNA synthesis, whereas microinjection of either GRB-2 or p21^ras individually produces no effect on DNA synthesis (79). By analogy, the regulation of p21^ras by insulin (84) may be mediated by the binding of GRB-2/sem-5 to IRS-1 (Fig. 3). GRB-2 may subsequently
regulate p21<sup>ras</sup> by binding and regulating a GDP/GTP exchange factor.

We have studied other SH2 domain-containing proteins as potential downstream elements of insulin signaling, p21<sup>ras</sup>, GAP, and PLC-γ. Interestingly, we have been unable to detect the association of GAP or PLC-γ with IRS-1 in the intact cell (M.G.M., M.F.W., unpublished observations). The lack of interaction is not surprising because the SH2 domains in GAP and PLC-γ recognize tyrosyl-phosphorylated sequence motifs not found in IRS-1 (39, 57, 82). Thus, IRS-1 differentiates insulin signaling from other growth factor receptors by binding and regulating a different subset of SH2 domain-containing proteins.

As more SH2 domain-containing proteins are discovered and examined, it is likely that more signaling molecules that associate with IRS-1 will be found (Fig. 3). Each IRS-1-associated protein may represent the beginning of a unique cellular signaling pathway that mediates a different subset of cellular insulin actions. Whereas IRS-1 has been linked to the cellular proliferative response (43), it is likely that IRS-1 also will play a major role in insulin-mediated metabolic signaling and glucose transport. The metabolic effects of insulin in various tissues may be determined by unique downstream elements in each cell type, or by a unique pattern of IRS-1 phosphorylation.}

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**REFERENCES**


24. Daidone M, Ariki E, Miralpeix M, Backer JM, White MF: IRS-1 is a common element if insulin and IGF-1 signaling to the phospholipidinositol 3-kinase. Endocrinology in press


41. Totty JV, Hunter T: Isolation of a human cDNA clone that encodes a novel receptor tyrosine kinase. EMBO J. 11:3469–79, 1992


A phosphatidylinositol-3 kinase binds to platelet-derived growth factor receptors through a specific receptor sequence containing phosphatidylinositol kinase activity. Cell 85, 1021-29, 1992


Cohen B, Yoakim M, Piwnica-Worms H, Roberts TM, Schaffhausen BS: Tyrosine phosphorylation is a signal for the trafficking of pp85, as 85,000-M, phosphorylated polypeptide associated with phosphatidylinositol kinase activity. Proc Natl Acad Sci USA 67: 4456-82, 1990


