Perspectives in Diabetes

The New Elements of Insulin Signaling

Insulin Receptor Substrate-1 and Proteins With SH2 Domains

MARTIN G. MYERS, JR. AND MORRIS F. WHITE

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 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-M_r 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/*sem*-5, 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 β-cells of the pancreas, insulin binds to the insulin receptor on the surface of cells

From the Research Division, Joslin Diabetes Center; and the Department of Medicine and the Program in Cell and Developmental Biology, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. Morris F. White, Research Division, Joslin Diabetes Center, 1 Joslin Place, Boston, MA 02215.

Received for publication 23 December 1992 and accepted in revised form 3 February 1993.

IRS, insulin receptor substrate; SH, *src* homology; NIDDM, non-insulin-dependent diabetes mellitus; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; GAP, GTPase activating protein; α-PY, anti-phosphotyrosine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CHO, Chinese hamster ovary; IGF, insulin-like growth factor; CSF, colony-stimulating factor; MPF, maturation-promoting factor; PtdIns phosphatidylinositol; DAG, diacylglycerol; IP_3, inositol trisphosphate; MTAg, middle T antigen; PLC, phospholipase C; PLC-γ, phospholipase C-γ.

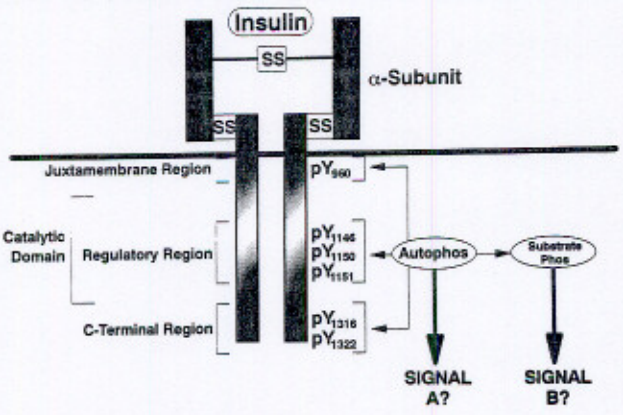

FIG. 1. A model of the insulin receptor and potential receptor signaling mechanisms. Insulin binding to the extracellular α-subunits of the insulin receptor activates the Tyr kinase of the intracellular β-subunits, causing a cascade of autophosphorylation within the β-subunit. Sites in the cytoplasmic juxtamembrane region, the COOH-terminus, and the so-called regulatory region of the kinase domain are autophosphorylated. Tyr phosphorylation of sites in the regulatory region further activates the receptor kinase. Two potential mechanisms of insulin receptor signaling are diagrammed: Downstream signaling may be mediated by some steric change or protein binding secondary to receptor autophosphorylation (signal *A*), as is the case for the PDGF receptor and related receptors. Signaling by the insulin receptor may alternatively be secondary to the Tyr phosphorylation of intracellular substrate proteins (signal *B*). What we now know of IRS-1 suggests that signal *B* is more important in insulin signaling (see text). However, it should be recalled that autophosphorylation also is essential to signal *B*, as it activates the insulin receptor Tyr kinase and allows it to phosphorylate IRS-1 more readily.

throughout the body (6). The receptors are heterotetrameric transmembrane glycoproteins composed of two α- and two β-subunits (Fig. 1). The α-subunits are completely extracellular and contain the insulin-binding domain, whereas the β-subunits are composed of an extracellular domain, a membrane-spanning domain, and a 402 amino acid intracellular tail (7). This intracellular domain contains a Tyr-specific protein kinase similar to that of certain cellular proto-oncogene products (e.g., $p60^{c\text{-}src}$, *c-ros*) and the receptors for certain peptide growth factors (e.g., PDGF and EGF) (7,8).

Insulin binding immediately activates the Tyr kinase in the β-subunit, resulting in autophosphorylation of Tyr residues in three regions, including the juxtamembrane region, the regulatory region, and the COOH-terminal region (9–11) (Fig. 1). The Tyr kinase activity of the receptor is crucial for insulin action; naturally occurring mutations of the insulin receptor that inhibit kinase activity and block autophosphorylation are associated with severe insulin resistance (12,13). Moreover, point mutations of the insulin receptor designed to destroy ATP binding (and thus abolish kinase activity) also abrogate insulin signaling in cultured cells (14–16). Thus, it seems clear that insulin-stimulated autophosphorylation or the subsequent phosphorylation of other proteins by the activated insulin receptor is the key event for signal transmission.

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, $p21^{ras}$-GAP, and GRB-2/*sem*-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_r 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^{960} or deletion of Tyr^{960} 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_r. It migrates between 170,000 and 180,000 M_r 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-

TABLE 1
Potential Tyr phosphorylation sites in IRS-1

	Position	Surrounding sequence
1,2	46,47	A R L **E** Y Y **E** N **E** K
3	107	Q **D** S W Y Q A L L
4	147	**E D** L S Y **D** T G P
5	426	S S **D E** Y G S S P
6	460	**E** L S N Y I C M G*
7	546	S I **E E** Y T **E** M M*
8	578	P T H S Y P **E E** G
9	608	T **D D** G Y M P M S P *†
10	628	G N G **D** Y M P M S P *†
11	658	**D** P N G Y M M M S P *†
12	727	C T G **D** Y M N M S P *†
13,14	745,746	P S **E** C Y Y G P **E D**
15	895	S P G **E** Y V N I **E**
16	939	G S **E E** Y M N M **D***
17	987	S R G **D** Y M T M Q*
18	1010	A P V S Y A **D** M R*
19	1172	K S L N Y I **D** L **D**
20	1222	**D** L S T Y A S I N

Potential Tyr phosphorylation sites are underlined and shown with surrounding amino acid sequences. Nearby acidic amino acids (**D**/**E**), thought to be important for Tyr kinase recognition, are in bold.
*Tyr lying in a YXXM or YMXM motif.
†Four of the YMXM motifs are followed by the amino acids SP.

teins; however, other sequences predictive of protein kinases are not present in IRS-1, suggesting that this motif is not part of a kinase catalytic site (8,39,42). Rat IRS-1 also contains a series of 11 glutamine residues homologous to the polyglutamine tracts found in proteins such as the glucocorticoid receptor, but only 6 of these residues are conserved in human IRS-1 (39,42), and no evidence exists that IRS-1 binds DNA.

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 Tyrs, with one or two conservative changes in 4 others; this suggests these Tyrs perform some conserved function (39,42). Interestingly, 6 of these Tyrs lie in the amino acid sequence motif YMXM (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-I 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.

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 α-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 α-PY immunoblots. Insulin-stimulated DNA synthesis is monitored in these cells by measuring [^{3}H]thymidine 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).

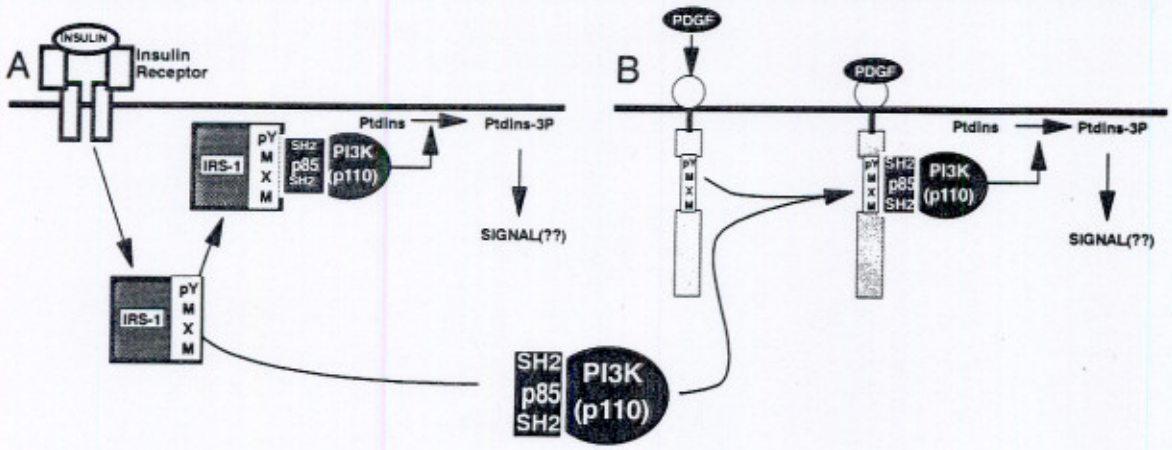

FIG. 2. Comparison of PtdIns 3′-kinase binding to IRS-1 and the PDGF receptor. *A*: Insulin receptor pathway. *B*: PDGF receptor pathway. After insulin stimulation, IRS-1 is phosphorylated on YMXM motifs by the activated insulin receptor, whereas after PDGF stimulation, the PDGF receptor autophosphorylates 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 PtdIns 3′-kinase. (The PtdIns 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.

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 uninjected 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 $p34^{cdc2}$ 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 PtdIns 3′-kinase. Insulin treatment of cells results in the appearance of PtdIns 3′-kinase in α-PY immunoprecipitates and causes the accumulation of the products of PtdIns 3′-kinase in the cell (52). PtdIns 3′-kinase phosphorylates the lipid PtdIns on the 3′ position of the D-*myo*-inositol ring, yielding PtdIns 3′-phosphate or PtdIns 3-P (53). Because the enzyme can use alternatively phosphorylated forms of PtdIns (PtdIns 4-P, PtdIns 4,5-P_2) as substrates, activation of the enzyme also leads to the formation of PtdIns 3,4-P_2 and PtdIns 3,4,5-P_3 (54). This pathway differs from the classical PtdIns pathway in which PtdIns 4,5-P_2 is cleaved by an isoform of PLC to DAG and 1,4,5 IP_3 by a PLC isoform, because the lipid products of PtdIns 3′kinase are not hydrolyzed by any known phospholipase (55). Thus, the mechanisms by which PtdIns 3′-kinase controls cellular growth and metabolism are not known (54)

Studies with the PDGF receptor and the polyoma MTAg suggest that PtdIns 3′-kinase is involved in cellular growth control (54). Mutants of the PDGF receptor that do not bind to the PtdIns 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 PtdIns 3′-kinase are defective in cellular transformation (58).

The ability of the insulin receptor to stimulate PtdIns 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 PtdIns 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 PtdIns 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 PtdIns 3′-kinase appears to be indirect and actually reflects association between the PtdIns 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 PtdIns 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 PtdIns 3′-kinase (Fig. 2).

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

(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 PstIns 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 PstIns 3′-kinase (19,56,70). Site-directed mutagenesis of the kinase-insert region of the PDGF receptor has shown that the YMXM and YVXM Tyrs 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 Tyr kinase pp60$^{c\text{-}src}$, 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 vitro 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-isoform 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

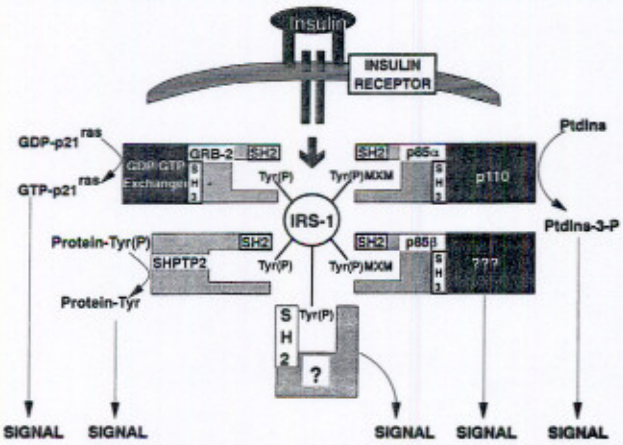

FIG. 3. A revised model of insulin receptor signaling mechanisms. Insulin binding to the extracellular α-subunits of the insulin receptor activates the Tyr kinase activity of the β-subunit. The insulin receptor then phosphorylates the endogenous cellular protein, IRS-1, allowing Ptdins 3′-kinase (a complex of p85-α and p110), GRB-2, p85-β, SHPTP2, and other unidentified SH2 domain–containing proteins to associate with IRS-1. This association is mediated by the binding of SH2 domains to Tyr-phosphorylated amino acid motifs on IRS-1 (see text) and activates Ptdins 3′-kinase. By analogy, this binding may regulate the activity of other associated enzymes.

regulate p21^{ras} 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^{ras}-GAP and PLC-γ contain SH2 domains and associate with the activated PDGF receptor (20,23–26,81). 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 signaling by 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 either by unique downstream factors in each cell type, or by a unique pattern of IRS-1 phosphorylation. Tissue-specific serine/threonine phosphorylation also may affect the interaction of IRS-1 with downstream elements. IRS-1 also may possess other functions in addition to those attributable to a docking protein. For instance, IRS-1 could possess an intrinsic enzymatic activity as it has a conserved nucleotide binding motif. Further study of IRS-1 should continue to reveal new aspects of the mechanism of insulin signal transmission.

ACKNOWLEDGMENTS

Portions of this work were generously supported by the Juvenile Diabetes Foundation, the Pew Foundation, and by National Institutes of Health Grants DK-38712 and DK-43808 to M.F.W. M.G.M. is a fellow of the Markey Program in Developmental Biology at the Division of Medical Sciences, Harvard Medical School, Boston, Massachusetts.

We would like to thank Ed Skolnik, Joseph Schlessinger, Bob Lechleider, Ben Neel, Steve Shoelson, Lee-Ming Chuang, Ron Kahn, and Jon Backer for communicating results before publication and for helpful discussions.

REFERENCES

1. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–68, 1992
2. Rosen OM: After insulin binds. *Science* 237:1452–58, 1987
3. Moller DA, Flier JS: Insulin resistance-mechanisms, syndromes, and implications. *N Engl J Med* 325:938–48, 1991
4. Bell GI, Xiang KS, Newman MV, Wu SH, Wright LG, Fajans SS, Spielman RS, Cox NJ: Gene for non-insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism on human chromosome 20q. *Proc Natl Acad Sci USA* 88:1484–88, 1991
5. Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, Lesage S, Velho G, Iris F, Passa P: Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature* (Lond) 356:721–22, 1992
6. White MF, Kahn CR: Structural and functional studies of the insulin receptor kinase. In *Insulin Receptors, Part A: Methods for the Study of Structure and Function.* Kahn CR, Harrison L (Eds.). New York, Liss, 1988, p.125–45
7. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao Y-C, Tsubokawa M, Mason A, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J: Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* (Lond) 313:756–61, 1985
8. Hanks SK, Quinn AM, Hunter T: The protein kinase family: conserved features and deduced phylogeny of the catalytic domain. *Science* 241:42–52, 1990
9. Myers MG Jr, Backer JM, Siddle K, White MF: The insulin receptor functions normally in Chinese hamster ovary cells after truncation of the C-terminus. *J Biol Chem* 266:10616–23, 1991
10. White MF, Kahn CR: The casacade of autophosphorylation in the β-subunit of the insulin receptor. *J Cell Biochem* 39:429–41, 1989
11. Feener EP, Backer JM, King GL, Wilden PA, Sun XJ, Kahn CR, White MF: Insulin stimulates serine and tyrosine phosphorylation in the juxtamembrane region of the insulin receptor. *J Biol Chem.* In press
12. Odawara M, Kadowaki T, Yamamoto R, Shibasaki Y, Tobe K, Accili D, Bevins C, Mikami Y, Matsuura N, Akanuma Y, Takaku F, Taylor SI, Kasuga M: Human diabetes associated with a mutation in the tyrosine kinase domain of the insulin receptor. *Science* 245:66–68, 1989
13. Moller DE, Yokota A, White MF, Pazianos AG, Flier JS: A naturally occurring mutation of insulin receptor alanine 1134 impairs tyrosine kinase function and is associated with dominantly inherited insulin resistance. *J Biol Chem* 265:14979–85, 1990
14. Ebina Y, Araki E, Taira M, Shimada F, Mori M, Craik CS, Siddle K, Pierce SB, Roth RA, Rutter WJ: Replacement of lysine residue 1030 in the putative ATP-binding region of the insulin receptor abolishes insulin- and antibody-stimulated glucose uptake and receptor kinase activity. *Proc Natl Acad Sci USA* 84:704–708, 1987
15. Chou CK, Dull TJ, Russell DS, Gherzi R, Lebwohl D, Ullrich A, Rosen OM: Human insulin receptors mutated at the ATP-binding site lack protein tyrosine kinase activity and fail to mediate postreceptor effects of insulin. *J Biol Chem* 262:1842–47, 1987
16. McClain DA, Maegawa H, Lee J, Dull TJ, Ullrich A, Olefsky JM: A mutant insulin receptor with defective tyrosine kinase displays no biological activity and does not undergo endocytosis. *J Biol Chem* 262:14663–71, 1987
17. Perlman R, Bottaro D, White MF, Kahn CR: Conformational changes in the α-subunits and β-subunits of the insulin receptor identified by anti-peptide antibodies. *J Biol Chem* 264:8946–50, 1989
18. Reedijk M, Liu XQU, Pawson T: Interactions of phosphatidylinositol kinase, GTPase-activating protein (GAP), and GAP-associated proteins with the colony-stimulating factor 1 receptor. *Mol Cell Biol* 10:5601–608, 1990

19. Kazlauskas A, Cooper JA: Autophosphorylation of the PDGF receptor in the kinase insert region regulates interactions with cell proteins. *Cell* 58:1121–33, 1989
20. Meisenhelder J, Suh PG, Rhee SG, Hunter T: Phospholipase Cγ is a substrate for the PDGF and EGF receptor protein-tyrosine kinases in vivo and in vitro. *Cell* 57:1109–22, 1989
21. Coughlin SR, Escobedo JA, Williams LT: Role of phosphatidylinositol kinase in PDGF receptor signal transduction. *Science* 243:1191–93, 1989
22. Kazlauskas A, Cooper JA: Phosphorylation of the PDGF receptor β-subunit creates a tight binding site for phosphatidylinositol 3 kinase. *EMBO J* 9:3279–86, 1990
23. Molloy CJ, Bottaro DP, Fleming TP, Marshall MS, Gibbs JB, Aaronson SA: PDGF induction of tryosine phosphorylation of GTPase activating protein. *Nature* (Lond) 342:711–14, 1991
24. Morrison DK, Kaplan DR, Rhee SG, Williams LT: Platelet-derived growth factor (PDGF)-receptor-dependent association of phospholipase Cγ with the PDGF receptor signaling complex. *Mol Cell Biol* 10:2359–66, 1990
25. Kazlauskas A, Ellis C, Pawson T, Cooper JA: Binding of GAP to activated PDGF receptors. *Science* 247:1578–81, 1990
26. Kaplan DR, Morrison DK, Wong G, McCormick F, Williams LT: PDGF β-receptor stimulates tyrosine phosphorylation of GAP and association of GAP with a signaling complex. *Cell* 61:125–33, 1990
27. White MF, Shoelson SE, Keutmann H, Kahn CR: A cascade of tyrosine autophosphorylation in the β-subunit activates the insulin receptor. *J Biol Chem* 263:2969–80, 1988
28. Obermaier-Kusser B, White MF, Pongratz DE, Su Z, Ermel B, Muhlbacher C, Haring HU: A defective intramolecular autoactivation cascade may cause the reduced kinase activity of the skeletal muscle insulin receptor from patients with non-insulin-dependent diabetes mellitus. *J Biol Chem* 264:9497–504, 1989
29. Wilden PA, Backer JM, Kahn CR, Cahill DA, Schroeder GJ, White MF: Characterization of the insulin receptor with an in vitro mutation at tyrosine 1146: evidence for separate insulin receptor signals regulating cellular metabolism and growth. *Proc Natl Acad Sci USA* 87:3358–62, 1990
30. Wilden PA, Siddle K, Haring E, Backer JM, White MF, Kahn CR: The role of insulin receptor kinase domain autophosphorylation in receptor-mediated activities. *J Biol Chem* 267:13719–27, 1992
31. Wilden PA, Kahn CR, Siddle K, White MF: Insulin receptor kinase domain autophosphorylation regulates receptor enzymatic function. *J Biol Chem* 267:16660–68, 1992
32. White MF, Maron R, Kahn CR: Insulin rapidly stimulates tyrosine phosphorylation of an *M*, 185,000 protein in intact cells. *Nature* (Lond) 318:183–86, 1985
33. White MF, Stegmann EW, Dull TJ, Ullrich A, Kahn CR: Characterization of an endogenous substrate of the insulin receptor in cultured cells. *J Biol Chem* 262:9769–77, 1987
34. White MF, Livingston JN, Backer JM, Lauris V, Dull TJ, Ullrich A, Kahn CR: Mutation of the insulin receptor at tyrosine 960 inhibits signal transmission but does not affect its tyrosine kinase activity. *Cell* 54:641–49, 1988
35. Backer JM, Kahn CR, Cahill DA, Ullrich A, White MF: Receptor-mediated internalization of insulin requires a 12-amino acid sequence in the juxtamembrane region of the insulin receptor β-subunit. *J Biol Chem* 265:16450–54, 1990
36. Backer JM, Schroeder GG, Cahill DA, Ullrich A, Siddle K, White MF: The cytoplasmic juxtamembrane region of the insulin receptor: a critical role in ATP binding, endogenous substrate phosphorylation, and insulin-stimulated bioeffects in CHO cells. *Biochemistry* 300: 6366–72, 1991
37. Murakami MS, Rosen OR: The role of insulin receptor autophosphorylation in signal transduction. *J Biol Chem* 266:22653–60, 1991
38. Rothenberg PL, Lane WS, Backer JM, White MF, Kahn CR: Purification and partial sequence analysis of pp185, the major cellular substrate of the insulin receptor tyrosine kinase. *J Biol Chem* 266:8302–11, 1991
39. Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA, Cahill DA, Goldstein BJ, White MF: The structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* (Lond) 352:73–77, 1991
40. Miralpeix M, Sun XJ, Backer JM, Myers MG Jr, Araki E, White MF: Insulin stimulates tyrosine phosphorylation of multiple high molecular weight substrates in FAO hepatoma cells. *Biochemistry* 31:9031–39, 1992
41. Keller SR, Kitagawa K, Aebersold R, Lienhard GE, Garner CW: Isolation and characterization of the 160,000-Da phosphotyrosyl protein, a putative participant in insulin signaling. *J Biol Chem* 266:12817–20, 1991
42. Nishiyama M, Wands JR: Cloning and increased expression of an insulin receptor substrate-1-like gene in human hepatocellular carcinoma. *BBRC* 183:280–85, 1992
43. Sun XJ, Miralpeix M, Myers MG Jr, Glasheen EM, Backer JM, Kahn CR, White MF: The expression and function of IRS-1 in insulin signal transmission. *J Biol Chem* 267:22662–72, 1992
44. Backer JM, Myers MG Jr, Shoelson SE, Chin DJ, Sun XJ, Miralpeix M, Hu P, Margolis B, Skolnik EY, Schlessinger J, White MF: The phosphatidylinositol 3′-kinase is activated by association with IRS-1 during insulin stimulation. *EMBO J* 11:3469–79, 1992
45. Myers MG Jr, Sun XJ, Cheatham B, Jachna BR, Glasheen EM, Backer JM, White MF: IRS-1 is a common element if insulin and IGF-1 signaling to the phosphatidylinositol 3′-kinase. *Endocrinology* In press
46. Saad MJA, Araki E, Miralpeix M, Rothenberg PL, White MF, Kahn CR: Regulation of insulin receptor substrate 1 in liver and muscle of animal models of insulin resistance. *J Clin Invest* 90:1839–49, 1992
47. Pines J, Hunter T: Isolation of a human cyclin cDNA: evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with p34*cdc2*. *Cell* 58:833–46, 1989
48. Murray AW, Kirschner MW: Cyclin syntheses drives the early embryonic cell cycle. *Nature* (Lond) 339:275–80, 1989
49. Lohka MJ, Hayes MK, Maller JL: Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc Natl Acad Sci USA* 85:3009–3013, 1988
50. Gautler J, Minshull J, Lohka M, Glotzer M, Hunt T, Maller JL: Cyclin is a component of maturation-promoting factor from *Xenopus*. *Cell* 60:487–94, 1990
51. Dunphy WG, Brizuela L, Beach D, Newport J: The *Xenopus cdc2* protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell* 54:423–31, 1988
52. Ruderman N, Kapeller R, White MF, Cantley LC: Activation of phosphatidylinositol-3-kinase by insulin. *Proc Natl Acad Sci USA* 87:1411–15, 1990
53. Whitman M, Downes CP, Keeler M, Keller T, Cantley L: Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* (Lond) 332:644–46, 1988
54. Cantley LC, Auger KR, Carpenter C, Duckworth B, Kapeller R, Soltoff S: Oncogenes and signal transduction. *Cell* 64:281–302, 1991
55. Serunian LA, Haber MT, Fukui T, Kim JW, Rhee SG, Lowenstein JM, Cantley LC: Polyphosphoinositides produced by phosphatidylinositol 3-kinase are poor substrates for phospholipase C from rat liver and bovine brain. *J Biol Chem* 264:17809–15, 1989
56. Escobedo JA, Williams LT: A PDGF receptor domain essential for mitogenesis but not for many other responses to PDGF. *Nature* (Lond) 335:85–87, 1988
57. Fantl WJ, Escobedo JA, Martin GA, Turck CW, del Rosario M, McCormick F, Williams LT: Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signaling pathways. *Cell* 69:413–23, 1992
58. Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM: Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* (Lond) 315:239–42, 1985
59. Kapeller R, Chem KS, Yoakim M, Schaffhausen BS, Backer JM, White MF, Cantley LC, Ruderman NB: Mutations in the juxtamembrane region of the insulin receptor impair activation of phosphatidylinositol 3-kinase by insulin. *Mol Endocrinol* 5:769–77, 1991
60. Backer JM, Schroeder G, Kahn CR, Myers MG Jr, Wilden PA, Cahill DA, White MF: Insulin stimulation of phosphatidylinositol 3-kinase activity maps to insulin receptor regions required for endogenous substrate phosphorylation. *J Biol Chem* 267:1367–74, 1992
61. Backer JM, Myers MG Jr, Sun XJ, Chin DJ, Shoelson SE, Miralpeix M, White MF: Association of IRS-1 with the insulin receptor and the PtdIns 3′-kinase: formation of binary and ternary signaling complexes in vivo and in vitro. *J Biol Chem.* In press
62. Hiles ID, Otsu M, Volinna S, Fry MJ, Gout I, Dhand R, Panayotou G, Ruiz-Larrea F, Thompson A, Totty NF, Hsuan JJ, Courtneidge SA, Parker PJ, Waterfield MD: Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. *Cell* 70:419–29, 1992
63. Escobedo JA, Navankasattusas S, Kavanaugh WM, Milfay D, Fried VA, Williams LT: cDNA cloning of a novel 85 kD protein that has SH2 domains and regulates binding of PI3-kinase to the PDGF β-receptor. *Cell* 65:75–82, 1991
64. Skolnik EY, Margolis B, Mohammadi M, Lowenstein E, Fischer R, Drepps A, Ullrich A, Schlessinger J: Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. *Cell* 65:83–90, 1991
65. Otsu M, Hiles I, Gout I, Fry MJ, Ruis-Larrea F, Panayotou G, Thompson A, Dhand R, Hsuan J, Totty N, Smith AD, Morgan SJ, Courtneidge SA, Parker PJ, Waterfield MD: Characterization of two 85 kD proteins that associate with receptor tyrosine kinases, middle-T/pp60*c-src* complexes and PI3-kinase. *Cell* 65:91–104, 1991

66. Koch CA, Anderson D, Moran MF, Ellis C, Pawson T: SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* 252:668–74, 1991
67. Mayer BJ, Jackson, PK, Van Etten RA, Baltimore D: Point mutations in the *abl* SH2 domain coordinately impair phosphotyrosine binding in vitro and transforming activity in vivo. *Mol Cell Biol* 12:609–18, 1992
68. Myers MG Jr, Backer JM, Sun XJ, Shoelson SE, Hu P, Schlessinger J, Yoakim M, Schaffhausen B, White MF: IRS-1 activates the phosphatidylinositol 3'-kinase by associating with the *src* homology 2 domains of p85. *Proc Natl Acad Sci USA* 89:10350–54, 1992
69. Shoelson SE, Chatterjee S, Chaudhuri M, White MF: YMXM motifs of IRS-1 define the substrate specificity of the insulin receptor kinase. *Proc Natl Acad Sci USA* 89:2027–31, 1992
70. Escobedo JA, Kaplan DR, Kavanaugh WM, Turck CW, Williams LT: A phosphatidylinositol-3 kinase binds to platelet-derived growth factor receptors through a specific receptor sequence containing phosphotyrosine. *Mol Cell Biol* 11:1125–32, 1991
71. Escobedo JA, Kaplan DR, Kavanaugh WM, Turck CW, Williams, LT: A phosphatidylinositol-3 kinase binds to platelet-derived growth factor receptors through a specific receptor sequence containing phosphotyrosine. *Mol Cell Biol* 1125–32, 1991
72. Hayashi H, Miyake N, Kanai F, Shibasaki F, Takenawa T, Ebina Y: Phosphorylation in vitro of the 85,000 *M*, subunit of phosphatidylinositol 3-kinase and its possible activation by insulin receptor tyrosine kinase. *Biochem J* 280:769–75, 1991
73. Kaplan DR, Whitman M, Schaffhausen B, Pallas DC, White MF, Cantley L, Roberts TM: Common elements in growth factor stimulation and oncogenic transformation: 85,000 *M*, phosphoprotein and phosphatidylinositol kinase activity. *Cell* 50:1021–29, 1987
74. 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* 87: 4458–62, 1990
75. Hu P, Margolis B, Skolnik EY, Lammers R, Ullrich A, Schlessinger J: Interactions of PI 3-kinase-associated p85 with EGF and PDGF receptors. *Mol Cell Biol* 12:981–90, 1992
76. Kavanaugh WM, Klippel A, Escobedo JA, Williams LT: Modification of the 85,000 *M*, subunit of phosphatidylinositol-3 kinase in platelet-derived growth factor-stimulated cells. *Mol Cell Biol* 12:3415–24, 1992
77. Yonezawa K, Ueda H, Hara K, Nishida K, Ando A, Chavanieu A, Matsuba H, Shii K, Yokono K, Fukui Y, Calas B, Grigorescu F, Dhand R, Gout I, Otsu M, Waterfield MD, Kasuga M: Insulin-dependent formation of a complex containing an 85,000 *M*, subunit of phosphatidylinositol 3-kinase and tyrosine-phosphorylated insulin receptor substrate 1. *J Biol Chem* 267:25958–66, 1992
78. Augustine JA, Schlager JW, Abraham RT: *Biochim Biophys Acta* 1052:313–22, 1990
79. Lowenstein EJ, Daly RJ, Batzer AG, Li W, Margolis B, Lammers R, Ullrich A, Skolnik EY, Bar-Sagi D, Schlessinger J: The SH2 and SH3 domain-containing proteins GRB2 links receptor tyrosine kinases to *ras* signaling. *Cell* 70:431–42, 1992
80. Medema RH, Wubbolts R, Bos JL: Two dominant inhibitory mutants of p21*ras* interfere with insulin-induced gene expression. *Mol Cell Biol* 11:5963–67, 1991
81. Downing JR, Margolis BL, Zilberstein A, Ashmun RA, Ullrich A, Sherr CJ, Schlessinger J: Phospholipase C-γ, a substrate for PDGF receptor phosphorylated on tyrosine during the mitogenic response. *EMBO J* 8:3345–50, 1989
82. Rotin D, Margolis B, Mohammadi M, Daly RJ, Daum G, Li N, Fischer EH, Burgess WH, Ullrich A, Schlessinger J: SH2 domains prevent tyrosine dephosphorylation of the EGF receptor: identification of Tyr992 as the high-affinity binding site for SH2 domains of phospholipase Cγ. *EMBO J* 11:559–67, 1992
83. Chuang L-M, Myers MG Jr, Seidner GA, Birnbaum MJ, White MF, Kahn CR: IRS-1 (insulin receptor substrate-1) mediates insulin and IGF-1 stimulated maturation of Xenopus oocytes. *Proc Natl Acad Sci USA*. In press
84. Freeman RM Jr, Plutzky J, Neel BG: Identification of a human src homology-2-containing protein-tyrosine-phosphatase: A putative homolog of Drosophila corkscrew. *Proc Natl Acad Sci USA* 89: 11239–43, 1992
85. Skolnik EY, Lee C-H, Batzer A, Vincenti LM, Zhou M, Daly R, Myers MG Jr, Backer JM, Ullrich A, White MF, Schlessinger J: The SH2/SH3 domain containing protein GRB-2 interacts with tyrosine phosphorylated IRS-1 and Shc: Implications for insulin control of ras signaling. *EMBO J*. In press