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# The IRS-Signalling System in Insulin and Cytokine Action [and Discussion]

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# The IRS-signalling system in insulin and cytokine action

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## SUMMARY

IRS-signalling proteins are engaged and phosphorylated on tyrosine residues by the receptors for insulin and IGF-1, and various classes of cytokine receptors, including IL-4, IL-9, and IL-13; IFNα/β and IFNγ; and growth hormone and LIF. IRS-proteins provide an interface between these receptors and signalling proteins which contain Src homology-2 domains (SH2-proteins). The recent identification of IRS-2 provides new insight into the modular structure and function of the IRS-proteins. The IRS-proteins provide a means for signal amplification by eliminating the stoichiometric constraints encountered by most receptors which directly recruit SH2-proteins to their autophosphorylation sites. Moreover, IRS-proteins dissociate the intracellular signalling complex from the endocytic pathways of the activated receptor. The shared use of IRS-proteins by multiple receptors is likely to reveal important connections between various hormones and cytokines that were previously unrecognized, or observed but unexplained. The existence of additional signalling molecules based on the IRS-paradigm is likely.

## 1. INTRODUCTION

In normal individuals, the secretion of insulin from pancreatic β-cells in response to plasma glucose levels tightly controls glucose homeostasis (Polonsky & O'Meara 1995). Insulin stimulates glucose transport into peripheral tissues and inhibits hepatic gluconeogenesis (figure 1). At least two fundamental defects occur in non-insulin-dependent diabetes (NIDDM), including a decrease in the ability of peripheral tissues to respond to insulin (insulin resistance), and impaired insulin secretion from β-cells (DeFronzo *et al.* 1992). It appears that both genetic and environmental factors are responsible for the progression from normal glucose tolerance to NIDDM.

In addition to its primary effects on glucose homeostasis, insulin influences other cellular events including regulation of ion and amino acid transport, lipid metabolism, glycogen synthesis, gene transcription and mRNA turnover, protein synthesis and degradation, and DNA synthesis (figure 1). Thus, insulin plays a key role in the normal storage of ingested fuels and in normal cellular growth and differentiation. The acute importance of insulin is most obvious in the uncontrolled type I diabetic, but its value for long-term health is emphasized by the accumulation of life-threatening complications during the pathogenesis of NIDDM patients (Olefsky 1995). To fully understand the events leading to insulin-resistant states and the pathophysiology of insulin deficiency, it will be helpful to identify the key components in the insulin-signalling pathway.

Initial studies of insulin action focused on hormone binding kinetics, structure of the insulin receptor and hypothetical signalling mechanisms (Kahn *et al.* 1981). Rapid progress was realized after the discovery of the insulin receptor tyrosine kinase (Kasuga *et al.* 1982*a*, *b*). Subsequent studies demonstrated regulation of the kinase by insulin binding and autophosphorylation (Ullrich *et al.* 1985; White & Kahn 1994); the crystal structure of the catalytic domain confirmed these initial ideas (Hubbard *et al.* 1994). Several downstream elements of the insulin-signalling pathway have been identified (figure 2). Most notable are the IRS-proteins (IRS-1 and IRS-2), which are multifunctional interfaces in the insulin signalling cascade (Sun *et al.* 1991, 1995). IRS-proteins were discovered as insulin receptor substrates, but they are also engaged by several cytokine receptors. Other common substrates have also been found, including Shc, a smaller tyrosine phosphorylated protein that links Grb-2/SOS and ultimately MAP kinase to many receptor systems (Pawson 1995). In this section, we discuss some of the known structural and functional features of the insulin and cytokine receptors that engage IRS-proteins, and then focus on IRS-signalling system.

## 2. STRUCTURE AND FUNCTION OF THE INSULIN RECEPTOR KINASE FAMILY

Insulin/IGF-1 receptors are present in virtually all vertebrate tissues at various levels of expression. The receptors are composed of two disulphide-linked α-subunits, which are linked to a transmembrane β-subunit by additional disulphide binds (White & Kahn 1994). The α-subunits are located entirely outside of the cell and contain the ligand binding site(s), whereas the intracellular portions of the β-subunits contain the

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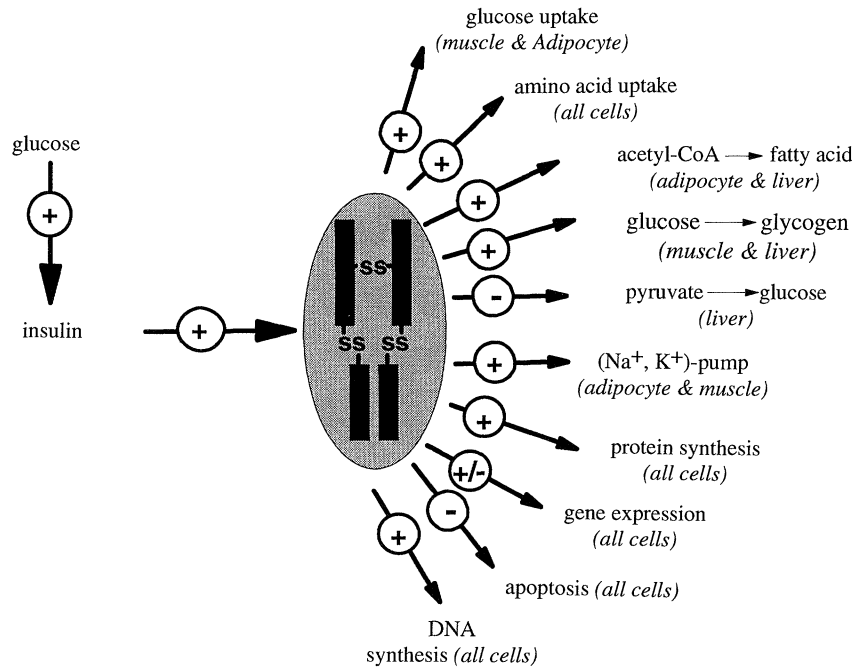


Figure 1. A schematic diagram of summarizing some pleiotropic responses during insulin stimulation.

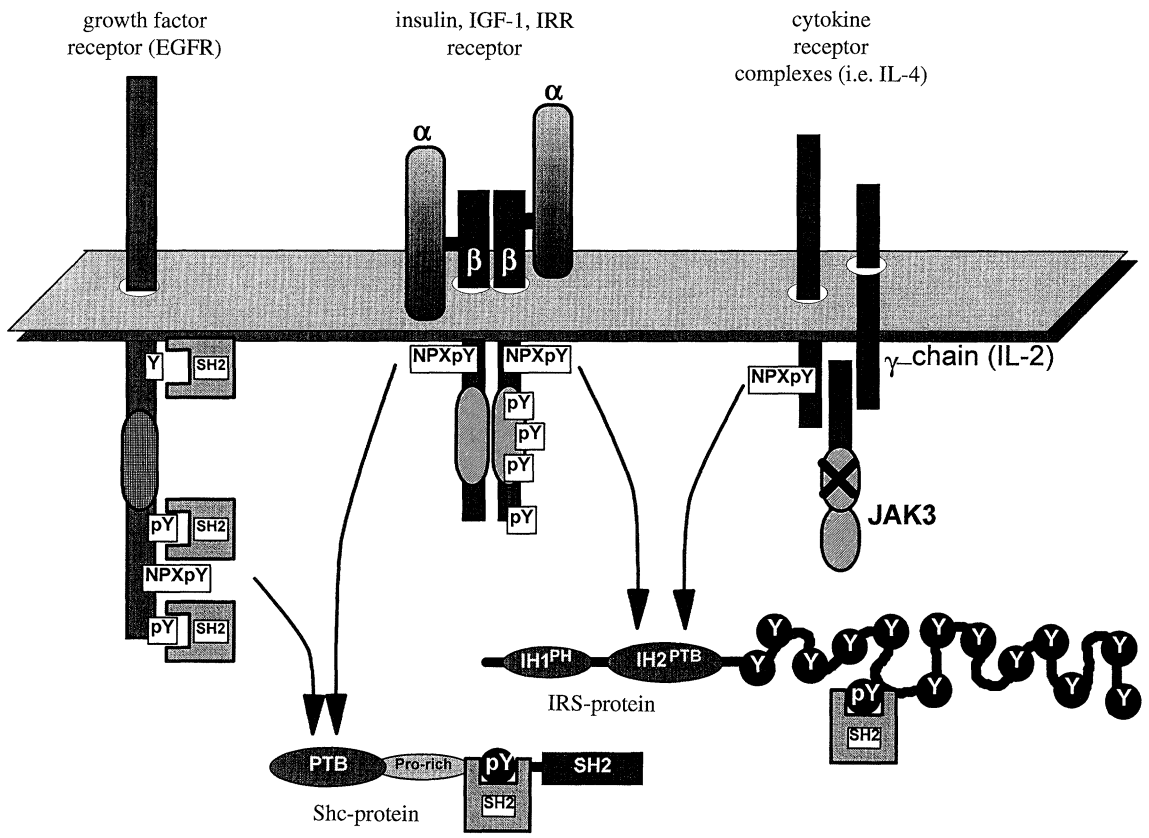


Figure 2. A schematic diagram showing the phosphorylation of Shc and IRS-proteins by receptors in the EGF, insulin and the IL-4 receptor family. Both Shc and IRS-proteins contain a similar domain that recognizes the phosphorylated NPXY-motif, although the exact specificity is different. The pleckstrin homology (PH) domain at the $NH_2$-terminus of IRS-proteins also mediates the receptor recognition. Shc contains a Src homology domain at its COOH-terminus and a single tyrosine phosphorylation site. IRS-1 contains multiple tyrosine phosphorylation sites in its COOH-terminus.

regulated tyrosine protein kinase. The intracellular domains are 80% identical reflecting some common signalling potential (Ullrich *et al.* 1985, 1986).

Multiple autophosphorylation sites exist in the β-subunits of the insulin and IGF-1 receptors (Werner *et al.* 1994). For the insulin receptor, autophosphoryl-

ation occurs in three regions, including the intracellular juxtamembrane region ($Tyr_{960}$), the so-called regulatory region ($Tyr_{1146}$, $Tyr_{1150}$ and $Tyr_{1151}$), and the COOH-terminus ($Tyr_{1316}$ and $Tyr_{1322}$) (White & Kahn 1994). The crystal structure of the catalytic domain of the insulin receptor β-subunit suggests that $Tyr_{1150}$ plays an important role in the activation of the receptor kinase (Hubbard *et al.* 1994): in the dephosphorylated state this residue may inactivate the enzyme by occupying the catalytic site and inhibiting ATP binding. Tris-phosphorylation of the regulatory region fully activates the receptor (White *et al.* 1988*b*), suggesting that phosphorylation of $Tyr_{1150}$ alone is not sufficient to fully activate the kinase.

Outside of the catalytic domain, the juxtamembrane region is essential for signal transmission (White *et al.* 1988*a*). This region contains a tyrosine autophosphorylation site ($Tyr_{960}$ in the insulin receptor), which resides in an NPXY-motif (Feener *et al.* 1993). Mutations at or around $Tyr_{960}$ inhibit insulin-stimulated tyrosine phosphorylation of IRS-1/IRS-2 and Shc, suggesting that a common mechanism may be involved (White *et al.* 1988*a*; White & Kahn 1994). Interestingly, the IL-4 receptor also contains an NPXY-motif that is essential for IRS-1 phosphorylation (Keegan *et al.* 1994). Comparisons between the receptor for insulin, IGF-1 and IL-4 reveals a consensus motif, LxxxxNPxYxSxP, that mediate partially the specific interaction with the IRS-proteins (Keegan *et al.* 1994).

The yeast 'two-hybrid' system suggests that the NPXY-motif of the insulin receptor interacts with the $NH_2$-terminus of IRS-1 and Shc (O'Neill *et al.* 1994). Recently, the $NH_2$-terminus of Shc was shown to be a phosphotyrosine binding (PTB) domain which recognizes phosphorylated NPXY-motifs (Kavanaugh & Williams 1994). Similar domains occur in several other proteins (Bork & Margolis 1995), and alignment of IRS-1 and IRS-2 (see below) reveals a similar domain that is likely to engage the phosphorylated NPXY-motif in the insulin receptor (Sun *et al.* 1995).

## 3. THE IRS-SIGNALLING SYSTEM: THE DISCOVERY OF IRS-1 AND IRS-2

Early theories of tyrosine kinase signalling focused on intracellular substrate proteins as second messengers. While the direct substrate hypothesis was set aside for many receptor systems (Pawson 1995), it held true for the insulin receptor, and is once again finding broader acceptance. Use of antiphosphotyrosine (αPY) antibodies provided the first evidence for a direct cellular substrate of the insulin receptor. Ten years ago, we observed a broad band of tyrosyl phosphorylated protein(s) in αPY immunoprecipitates from insulin-stimulated Fao hepatoma cells; it migrated between 165–190 kDa during SDS-PAGE and was designated pp185 (White *et al.* 1985). Our earliest experiments indicated that pp185 was located mainly in the cytoplasm and contains phosphotyrosine, phosphoserine, and phosphothreonine following insulin stimulation (White *et al.* 1987). Tyrosyl phosphorylation of pp185 in αPY immunoprecipitates peaks within seconds after insulin stimulation and gradually decreases during sustained stimulation (White *et al.* 1987).

Using immobilized antiphosphotyrosine antibodies, a constituent of the pp185 band was purified from insulin-treated rat liver and cloned (Rothenberg *et al.* 1991; Sun *et al.* 1991). The cloned protein was designated IRS-1, the first insulin receptor substrate (Sun *et al.* 1991). It has a molecular mass 131 kDa, although it migrates between 170–180 kDa during SDS-PAGE, owing largely to a high level of serine phosphorylation (Sun *et al.* 1991, 1992). However, an additional substrate, designated $pp187^{HMW}$ remained to be identified (Miralpeix *et al.* 1992). We encountered this protein again with 4PS, so designated because it was originally detected as a substrate for IL-4 receptor in FDC-P2 myeloid cells (Wang *et al.* 1993*a*). 4PS is also tyrosine phosphorylated during insulin and IGF-1 stimulation, and migrates more slowly than IRS-1 during SDS-PAGE (Wang *et al.* 1993*a*, *b*). Like $pp185^{HMW}$ in Fao cells, 4PS reacts poorly or not at all with antibodies against IRS-1 (Wang *et al.* 1993*a*). Together, these observations pointed the way to IRS-2.

After unsuccessful low-stringency cDNA screening and PCR analysis, we purified 4PS by affinity chromatography and isolated its cDNA using an optimized cDNA probe based on limited primary amino acid sequence (Sun *et al.* 1995). Extensive homology between IRS-1 and 4PS suggests that they are members of the same family of signalling proteins (IRS-proteins); and 4PS was renamed IRS-2. Antibodies against IRS-2 recognize 4PS in FDC-P2 cells and $pp185^{HMW}$ in Fao cells; however, the existence of additional family members is not excluded (Sun *et al.* 1995).

## 4. THE STRUCTURE AND FUNCTION OF THE IRS-SIGNALLING PROTEINS

IRS-1 and IRS-2 are 43% identical (Sun *et al.* 1995). At least two regions in the $NH_2$-terminus contain significant identity and are designated IRS-homology domains: IH-1 and IH-2 (figure 3). The boundaries of the IH1 correspond exactly to a pleckstrin homology (PH) domain, thus we suggest the designation, $IH1^{PH}$ (figure 3). PH domains were first recognized as internal repeats in pleckstrin, the major substrate of PKC in platelets (Musacchio *et al.* 1993). The PH domains have been found to interact with proteins and phospholipids (Musacchio *et al.* 1993; Gibson *et al.* 1994). The $IH1^{PH}$ domain is important for the sensitive interaction between the insulin receptor and IRS-1 (Myers *et al.* 1995). Recently, it was found to interact with $G_{\beta\gamma}$-subunits, suggesting potential cross-talk between IRS-proteins and trimeric G-proteins (Luttrell *et al.* 1995; Pitcher *et al.* 1995).

The second IRS-homology (IH2) domain lies immediately downstream of the $IH1^{PH}$ domain (figure 3). This region contains about 160 amino acids and is 75% identical between IRS-1 and IRS-2 (Sun *et al.*




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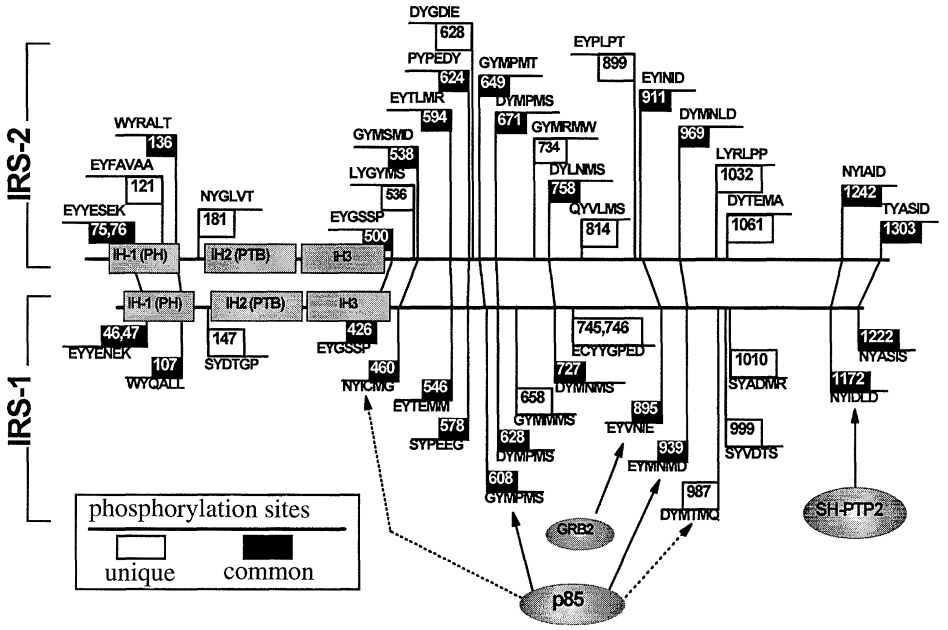


Figure 3. A schematic diagram of IRS-1 and IRS-2. Two conserved modules thought to mediate receptor or other interactions are indicated at the $NH_2$-terminus: $IH1^{PH}$ and $IH2^{PTB}$; a third region, IH3, contains several conserved motifs. Putative tyrosine phosphorylation sites are indicated in the COOH-terminal region. Entirely unique sites are in open boxes, whereas relatively (but not absolutely) conserved motifs are shown in black boxes. IRS-2 is about 100 residues longer than IRS-1.

1995). Interestingly, this region is similar to the phosphotyrosine binding (PTB) domain in Shc (Kavanaugh & Williams 1994; Bork & Margolis 1995; Gustafson *et al.* 1995; Sun *et al.* 1995). Thus, we designated this region as the $IH2^{PTB}$ domain. The $IH2^{PTB}$ domain binds to phosphorylated LxxxxNPxYxSxP, providing a mechanism for receptor engagement that is regulated by phosphorylation of $Tyr_{960}$ in the insulin receptor (see below).

The region in IRS-1 between residues 313 and 462 (designated IH3 in our discussion, figure 3) was shown recently to have similarity to the PTB domain in Shc (Gustafson *et al.* 1995). The IH3 region is the least conserved of the three homologous regions in the $NH_2$-terminus of IRS-1 and IRS-2, and may actually be regarded best as a collection of conserved motifs (Sun *et al.* 1995). The IH3 boundaries in IRS-1 include a known tyrosine phosphorylation site ($Tyr_{460}$), suggesting that this region may mediate downstream signals, rather than upstream receptor recognition as previously suggested (Gustafson *et al.* 1995).

IRS-1 and IRS-2 each contain over 30 potential Serine/Threonine phosphorylation sites with motifs recognized by various kinases (Sun *et al.* 1991, 1995). Many are clustered in the $IH2^{PTB}$ domain and IH3 region. Before insulin stimulation of cells, IRS-1 is strongly serine phosphorylated and weakly threonine phosphorylated, and following insulin stimulation, serine phosphorylation increases significantly. Casein Kinase II, MAP kinase, and PI-3 kinase phosphorylate IRS-1 *in vitro*, although further work is required to fully characterize the serine phosphorylation of IRS-1 (Tranisijevik *et al.* 1993; Lam *et al.* 1994). Serine phosphorylation may stimulate IRS-1 degradation in cells (Jullien *et al.* 1993; Tanti *et al.* 1994). In particular, serine phosphorylation of the $IH2^{PTB}$ domain may inhibit association of IRS-proteins with activated receptors by blocking the binding of phosphorylated NPXY-motifs.

Among the eight confirmed tyrosine phosphorylation motifs within IRS-1, six are well conserved in IRS-2 ($Tyr_{649}$, $Tyr_{671}$, $Tyr_{911}$, $Tyr_{969}$, $Tyr_{1242}$, $Tyr_{1303}$). The SH2 domains in p85 strongly bind to $Y_{608}$MPM and $Y_{939}$MNM in IRS-1 (Sun *et al.* 1993), which correspond to $Y_{649}$MPM and $Y_{969}$MNL in IRS-2 (figure 3); however, the substitution of $Met_{972}$ with Leu may alter the interaction. Nevertheless, there are eight other YXXM motifs in IRS-2 which may bind p85 (figure 3). The SH2 domain of Grb-2 binds to a $Y_{895}$VNI-motif in IRS-1 (Sun *et al.* 1993; Myers *et al.* 1994*b*), which corresponds to $Y_{911}$INI in IRS-2 (figure 3). IRS-1 also binds to SH-PTP2 at the $Y_{1172}$IDL-motif and the $Y_{1222}$ASI-motifs (Sun *et al.* 1993; Sugimoto *et al.* 1993; Pluskey *et al.* 1995), which corresponds to the $Y_{1242}$IAI- and $Y_{1303}$ASI-motifs in IRS-2; however, the spacing between these motifs in IRS-1 and IRS-2 is different, which may alter the regulation of the engaged SH2-proteins (figure 3). Several other potential tyrosine phosphorylation motifs that occur in either IRS-1 or IRS-2 may provide unique signalling potential (figure 3). Moreover, the COOH-terminus contains other conserved motifs which may mediate novel protein–protein interactions independently of tyrosine phosphorylation.

In summary, the identification of IRS-2 provides new insight into the modular structure and function of the IRS-signalling proteins. Presumably, the IRS-proteins provide a means for signal amplification by eliminating the stoichiometric constraints encountered by receptors which directly recruit SH2-proteins to their autophosphorylation sites. Moreover, IRS-proteins dissociate the intracellular signalling complex




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from the endocytic pathways of the activated receptor. The shared use of IRS-proteins by multiple receptors is likely to reveal important connections between various hormones and cytokines that were previously unrecognized, or observed but unexplained. The existence of additional signalling molecules based on the IRS paradigm is likely.

## 5. JAK-KINASE-ASSOCIATED RECEPTORS: EMERGING UPSTREAM ELEMENTS IN THE IRS-SIGNALLING SYSTEM

Although IRS-signalling proteins are important elements in the insulin/IGF-1 signalling cascade, work during the past few years in our laboratory and elsewhere established the importance of the IRS-signalling system for other receptors as well, including several members of the cytokine receptor superfamily (Ihle 1994). Activation of these receptors stimulates tyrosine phosphorylation of several cellular substrates, including the receptor itself and components of the STAT family of transcription factors (Ihle *et al.* 1994). Unlike growth factor receptors, cytokine receptors do not contain intrinsic tyrosine kinase activity. However, they associate specifically with various janus kinase isoforms, including JAK1, JAK2, JAK3, and TYK2 (table 1).

JAK kinases contain two canonical kinase domains located in tandem at COOH-terminal end: The last domain contains all the consensus sequences expected in active kinases, whereas the first domain lacks several residues that are essential for kinase activity (Ihle 1994). Unlike cytoplasmic tyrosine kinases in the Src family, JAK kinases do not contain SH2 or SH3 domains; however, the $NH_2$-terminus contains unique domains which mediate association with various receptors (Frank *et al.* 1995).

IRS-signalling proteins are phosphorylated during stimulation by several cytokines, including IL-4, IL-9 and IL-13, IFNα/β and IFNγ, growth hormone and LIF (table 1). The IL-4 receptor contains an LxxxxNPxYxSxP-motif resembling that in the insulin receptor, which is essential for IL-4-stimulated IRS-1/IRS-2 phosphorylation (Keegan *et al.* 1994); however, the mechanism of IRS-protein recognition is not obvious for other cytokine receptors. None of the tyrosine residues in the GHR appears necessary for IRS-1 tyrosyl phosphorylation (Argetsinger *et al.* 1995). Moreover, NPXY-motifs are not found in the receptors for IFNα/β, IFNγ, LIF, nor in their accessory elements gpl30, JAK1, JAK2, or JAK3 (Argetsinger *et al.* 1995). It is possible that the $IH2^{PTB}$ domain in IRS-protein may recognize phosphotyrosine in other sequence contexts. Alternatively, other subunits may exist in these receptor complexes, or the $IH1^{PH}$ domains in the IRS-proteins may mediate exclusively the interactions with these receptors.

## 6. SIGNAL TRANSDUCTION BY IRS-PROTEINS

Together, IRS-1 and IRS-2 have an extensive potential to interact with downstream signalling molecules through approximately 30 unique phosphorylation motifs, including $p85\alpha/\beta$, $p55^{PIK}$, Grb-2, SH-PTP2, c-fyn, and nck (figure 4). The PI-3 kinase and SH-PTP2 are activated during association with phosphorylated IRS-1, which illustrates one way that IRS-proteins mediate receptor signals (Backer *et al.* 1992; Myers *et al.* 1992). The regulatory subunit, p85, contains two SH2-domains, and the kinase is fully activated when both are occupied (Rordorf-Nikolic *et al.* 1995). Pairs of phosphorylated YMXM-motifs in IRS-proteins apparently provide an ideal system for the regulation of PI-3 kinase activity. Moreover, IRS-1 is absolutely required for insulin-stimulated $p70^{s6k}$, which may be related to the activation of PI-3 kinase by a phosphorylated IRS-protein (Myers *et al.* 1994*a*).

Grb-2 regulates a $p21^{ras}$ guanine nucleotide exchange factor called Sos, one of the upstream elements in MAP kinase regulation (Egan *et al.* 1993; Li *et al.* 1993; Rozakis-Adcock *et al.* 1993). Grb-2/Sos binds to the $Y_{895}$VNI-motif in IRS-1, which contributes to the stimulation of MAP kinase in 32D cells, presumably through the activation of $p21^{ras}$ (Lowenstein *et al.* 1992; Skolnik *et al.* 1993). However, Grb-2/Sos also binds to phosphorylated Shc, an alternate pathway shared with other growth factor receptors (Myers *et al.* 1994*b*).

The IRS-signalling proteins mediate mitogenesis in cells responsive to insulin, IGF-1, and IL-4. Overexpression of IRS-1 in Chinese hamster ovary (CHO) cells doubles the maximal response of thymidine incorporation during insulin stimulation, whereas

Table 1. *A list of cytokine receptors that engage a member of the JAK kinase family to mediate downstream signals*

(Not all receptors in a particular family have been found to mediate IRS-protein tyrosine phosphorylation.)

| Receptor | JAK kinase member | IRS-1/IRS-2 phosphorylation |
|---|---|---|
| erythropoietin | JAK-2 | no |
| GH | JAK-2 | yes |
| prolactin | JAK-2 | not known |
| G-CSF | JAK-2 | no |
| IL-3, MG-CSF, IL-5 | JAK-2 | no |
| IL-6, OSM, LIF, CNTF | JAK-1, JAK-2, TYK-2 | yes (LIF), others no! |
| IFNα/β | JAK-1, TYK-2 | yes |
| IFNγ | JAK-1, JAK-2 | yes (in 3T3-F442A) |
| IL-2, IL-4, IL-13 | JAK-3 | yes (IL-4, IL-13 > IL-2) |




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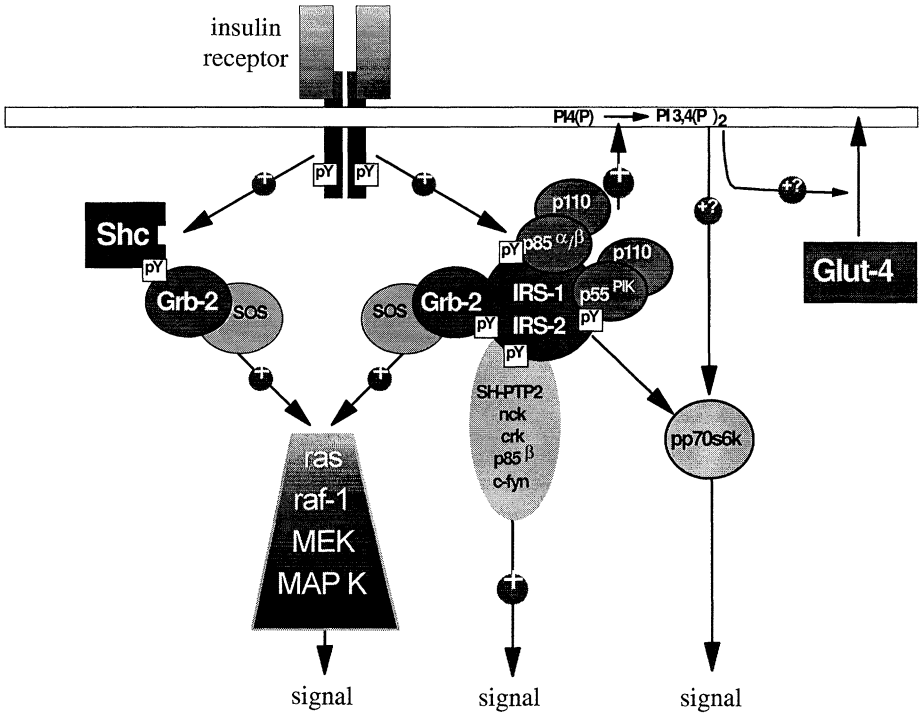


Figure 4. A schematic diagram of the insulin signalling through tyrosine phosphorylation of Shc and IRS-proteins.

reducing its level with antisense oligonucleotides or expression constructs diminishes this response (Sun *et al.* 1992; Waters *et al.* 1993). The complete absence of IRS-proteins from the 32D myeloid progenitor cells provides an ideal system for the analysis of IRS-1/IRS-2 during receptor signalling. 32D cells grow in the presence of IL-3, but are insensitive to insulin, IL-4 and IGF-1; however, expression of IRS-1 or IRS-2 rescues the IGF-1 and IL-4 mitogenic response, and co-expression with the insulin receptor restores the insulin response (Wang *et al.* 1993*b*; Sun *et al.* 1995). Thus, IRS-1/IRS-2 mediates mitogenesis and cell cycle progression in a variety of cell systems.

IRS-proteins may also be involved in cellular transformation. Recently IRS-1 was found to associate specifically with the simian virus 40 (SV40) large T antigen: The association between IRS-1 and T antigen appears to require the first 250 amino acids of the T antigen, but the binding domain on IRS-1 is unknown (Santos & Butel 1995). T antigen does not associate with Shc. These results have physiologic significance since the IGF-1 receptors are required for T-antigen transformation of mouse fibroblasts (Coppola *et al.* 1994). Surprisingly, fibroblasts obtained from IGF-$1r^{(-/-)}$ mice cannot be transformed by SV40 large T: The requirement for the IGF-1 receptor is specific as the insulin receptor cannot substitute. Interestingly, transformation can be accomplished in IGF-$1r^{(-/-)}$ fibroblasts by overexpression of IRS-1. The stable complex between IRS-1 and T antigen may be important for transformation (Zhou-Li *et al.* 1995). It is possible that IRS-1 provides a mechanism to localize the T antigen to a specific region where its efficiently transforms the cell. SV40T antigen is largely nuclear; however, a small amount of T antigen can be detected in cytoplasm, and mutant T antigen proteins that are not translocated into the nucleus are still transforming (Santos & Butel 1995): IRS-1 may be responsible for keeping ordinary T antigen in the cytoplasm where it activates a transforming phenotype.

## 7. IRS-PROTEINS AND INSULIN ACTION

The movement of glucose into cells from the circulatory system is largely accomplished by the action of a family of facilitated glucose transporters (Lienhard *et al.* 1992). GLUT1 and GLUT4 are responsible for removing the bulk of glucose from the bloodstream. These molecules differ importantly in their physiologic role. GLUT1 is present in most cells and tissues and is constitutively distributed at the plasma membrane. Thus, GLUT1 is relatively insensitive to acute insulin treatment. Recent data suggest that activation of the $p21^{ras}$/c-raf-1/MAP kinase pathway increases expression of GLUT1 and stimulates glucose uptake (Fingar & Birnbaum 1994). As major control of this serine kinase cascade occurs independently of IRS-1 in 32D and many other cell types (Myers *et al.* 1994*b*; Ouwens *et al.* 1994; Sasaoka *et al.* 1994), control of GLUT1 expression levels by insulin is likely to be largely independent of IRS-1, as well. However, analysis of IRS-$1^{(-/-)}$ and IRS-$2^{(-/-)}$ mice will provide additional insight into this situation.

In contrast, GLUT4 is constitutively expressed in adipose and muscle, where it is ordinarily sequestered in an intracellular vesicular compartment (Lienhard *et al.* 1992). The bulk of insulin-stimulated glucose uptake is mediated by the translocation of the intracellular GLUT4 to the plasma membrane (Lienhard *et al.* 1992). Two distinct experimental approaches support the conclusion that PI-3 kinase is necessary, although perhaps not sufficient, for insulin-stimulated GLUT4 translocation. First, insulin-stimulated glucose uptake is inhibited in fat cells by wortmannin and




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LY90024, two inhibitors of PI-3 kinase (Cheatham *et al.* 1994; Okada *et al.* 1994*a*, *b*; Ridderstrale & Tornqvist 1994). Alone, these results are questionable because it is impossible to know whether other unknown kinases are also inhibited. However, a p85 mutant ($\Delta$p85) lacking the binding site for p110 inhibits insulin-stimulated glucose uptake (translocation of GLUT1 to the PM in this case) in CHO cells (Hara *et al.* 1994). Together, these results suggest that the activation of PI-3 kinase is necessary for in insulin-stimulated glucose uptake. Since IRS-proteins are upstream regulators of PI-3 kinase during insulin stimulation, they are likely to play an important role during insulin-stimulated GLUT4 translocation (Sun *et al.* 1991; Backer *et al.* 1992; Myers *et al.* 1994*a*); however, direct evidence to support this point is not available.

Disruption of the IRS-1 gene in mice (IRS-1$^{(-/-)}$ has provided new insight into the physiologic role of IRS-1 (Araki *et al.* 1994; Tamemoto *et al.* 1994). Although IRS-1$^{(-/-)}$ mice survive and breed, they demonstrate dramatically altered physiology. These mice display mild hyperinsulinemia and have impaired glucose tolerance compared to controls. Thus, IRS-1 is an important mediator of insulin signalling and glucose homeostasis. Furthermore, insulin-stimulated glucose uptake is reduced by 50% in tissues from these mice, suggesting that IRS-1 is an important mediator of insulin-regulated glucose homeostasis. Although it is surprising at first that IRS-1$^{(-/-)}$ mice survive with only mild insulin resistance, the subsequent discovery of IRS-2 provides a provisional explanation (Araki *et al.* 1994; Sun *et al.* 1995). Thus, additional work is needed to establish a definitive role for IRS-proteins in glucose homeostasis, including disruption of the IRS-2 gene in mice.

During embryonic development, IRS-1$^{(-/-)}$ mice are approximately 50% smaller than wild-type and heterozygous littermates, suggesting an important role for IRS-1 in development (Sun *et al.* 1995; Araki *et al.* 1994). This reduction in size may be due to increased apoptosis during foetal development, suggesting that IRS-1 is a crucial regulator of this important process, as well. These mice may have other defects which have yet to be discovered; the generation of IRS-2$^{(-/-)}$ mice and their interbreeding with IRS-1$^{(-/-)}$ mice will answer many more questions about the role of these important proteins in inulin and cytokine signalling and whole-animal physiology.

Numerous associates over the years have contributed significantly to these ideas, in particular Jon Backer, Xiao Jian Sun, and Martin Myers Jr. This work was supported largely by DK38712 and DK43808. Generous support from the Juvenile Diabetes Foundation International and the American Diabetes Association is gratefully acknowledged.

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### *Discussion*

*Question.* Could Professor White comment on the involvement of the IRS proteins in IL4 control of the Ras pathway?

M. F. White. We do not see activation of the Ras pathway by IL4 in cells that express IRS1. Our provisional interpretation is that we are not getting phosphorylation of the YVNI-motif beginning at position 895 in IRS-1 and are therefore not engaging Grb2; but I should emphasize that we have not specifically looked at the phosphorylation patterns with that question in mind.

*Question.* Does Professor White know what domains of IRS1 are involved in recruiting intracellular low-density microsomes?

M. F. White. We are analysing about 40 IRS1 mutants at present to answer questions of this type in the 3T3-L1 cells, but the answers are not yet available.

P. Cohen (*University of Dundee, U.K.*). Last year our laboratory and Chris Proud both showed that the activation of MAPK can be blocked with phosphoinositide 3-kinase inhibitors (in L6, CHO and 3T3 cells). This involves a complete inhibition of the activation of Raf, without any effect on the GTP loading of Ras. At least in 3T3 cells, this is a specific blockade of the action of insulin, in that EGF or PDGF activation of MAPK is not prevented by these inhibitors. Does this not suggest that phosphoinositide 3-kinase can somehow transmit information to Raf?

M. F. White. In our experiments with 32D cells these pathways seem to be separate. Insulin stimulates MAP kinase in 32D cells without the expression of any IRS-1 or IRS-2. When there is no IRS protein, insulin has no effect on PI-3 kinase. Now, when we express IRS-1 in the 32D cells it does increase the stimulation of MAP kinase by insulin, but this increase can be reversed by mutating the GRB-2 binding site. Thus the stimulation of PI kinase does not seem to be involved in activating MAP kinase in 32D cells.




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