Minireview

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The Insulin Signaling System*

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In mammals, insulin is the principal hormone controlling blood glucose and acts by stimulating glucose influx and metabolism in muscle and adipocytes and inhibiting gluconeogenesis by the liver. In addition, insulin modifies the expression or activity of a variety of enzymes and transport systems in nearly all cells. Insulin action is mediated through the insulin receptor, a transmembrane glycoprotein with intrinsic protein tyrosine kinase activity. The level of tyrosine kinase activity reflects the serum concentration of insulin and appears to mediate the insulin response through tyrosine phosphorylation of the receptor itself and substrates like insulin receptor substrate-1 (IRS-1).[1] Non-insulin-dependent diabetes mellitus is due in large part to insulin resistance, a state when the target cells no longer respond to ordinary levels of circulating insulin. To understand the mechanisms of control of normal metabolism, as well as the pathogenesis of non-insulin-dependent diabetes mellitus, it is critical to understand the signaling pathways used by the insulin receptor.

Many plasma membrane receptors regulate cellular processes through protein tyrosine kinases. The receptor for insulin, like the receptors for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), contains intrinsic tyrosine kinase activity (1). The antigen receptor on T and B cells, as well as receptors for growth hormone, erythropoietin, and several cytokines, do not have intrinsic tyrosine kinase activity but stimulate tyrosine phosphorylation by association with cytoplasmic tyrosine kinases like Fyn, Tyk-2, or Jak-1 and -2 (2, 3). In each case, ligand binding activates the associated tyrosine kinases, and for the receptor tyrosine kinases most evidence indicates that this step is essential for biological activity. The activated receptors frequently undergo autophosphorylation on tyrosine residues in the cytoplasmic domain. To propagate the signal, many autophosphorylated receptors bind directly to proteins containing Src homology 2 domains (SH2 proteins). The binding of SH2 proteins depends on the amino acid sequence surrounding the tyrosine autophosphorylation site in each receptor (4). EGF and PDGF receptors associate to varying degrees with phosphatidylinositol (PI) 3′-kinase, $p21^{ras}$-GAP, phospholipase Cγ, GRB-2 (an adapter protein that links tyrosine kinases to a $p21^{ras}$ guanine nucleotide exchange protein), cytoplasmic tyrosine kinases like c-Fyn and c-Src, and probably other SH2 proteins in various cellular backgrounds. Presumably, the characteristic biological response of these growth factors results from the exact combination of interacting SH2 proteins (5).

An interesting variation of this model is employed by the insulin receptor. Insulin receptor autophosphorylation stimulates kinase activity, but unlike the EGF and PDGF receptors, the insulin receptor does not appear to have direct associations with SH2 proteins. In contrast, the activated insulin receptor phosphorylates IRS-1, a principle substrate of the insulin receptor, on multiple tyrosine residues, which in turn recognize and bind to the SH2-domains in various signal transduction proteins (Fig. 1). The PI 3′-kinase is activated when phosphorylated IRS-1 binds to the SH2 domains in its p85α regulatory subunit, which establishes a direct molecular connection between circulating insulin and this cellular enzyme (6). Other SH2 proteins including SH-PTP-2 (7), GRB-2 (8), Nck (9), and others associate with IRS-1 to mediate the pleiotropic insulin response (Fig. 1). Interestingly, other upstream activators such as the interleukin-4 receptor also utilize IRS-1 (or a closely related substrate) as an intermediate in signal transduction (10). This review summarizes some of our current views of insulin receptor function and the evolving role of IRS-1.

The Insulin Receptor

The insulin receptor is present in virtually all vertebrate tissues, although the concentration varies from as few as 40 receptors on circulating erythrocytes to more than 200,000 receptors on adipocytes and hepatocytes. The receptor gene is located on the short arm of human chromosome 19, is more than 150 kilobases in length and contains 22 exons, which encode a 4.2-kb cDNA (11). The insulin receptor is composed of two α-subunits that are each linked to a β-subunit and to each other by disulfide bonds (Fig. 2). Both subunits are derived from a single proreceptor by proteolytic processing at a cleavage site consisting of four basic amino acids. There is one site of alternative splicing surrounding exon 11, which results in two receptor isoforms differing by 12 amino acids near the COOH terminus of the α-subunit. The mature heterotetramer ($\alpha_2\beta_2$) contains complex *N*-linked carbohydrate side chains capped by terminal sialic acid residues and migrates with a molecular mass of 300–400 kDa by SDS-PAGE. The α-subunits are located entirely outside of the cell and contain the insulin binding site(s), whereas the intracellular portion of the β-subunit contains the insulin-regulated tyrosine protein kinase (Fig. 2). The insulin receptor family contains two other structurally related molecules, the insulin-like growth factor (IGF-1) receptor and the insulin receptor-related receptor, an orphan receptor for which no ligand has been identified (12). This family shares more than 80% amino acid sequence identity in the kinase domain but has low amino acid sequence identity in the extracellular domain.

The stoichiometry of insulin binding lies between 1 and 2 insulin molecules/receptor, and the binding sites display negative cooperative behavior. Affinity labeling, epitope mapping, and construction of chimeric molecules between the insulin and IGF-1 receptors places the ligand binding determinants within distinct regions of the first 500 amino acids of the α-subunits (13). High affinity insulin binding is transferred into the IGF-1 receptor by replacing amino acids 64–137 of the IGF-1 receptor α-subunit with the corresponding residues from the insulin receptor. Further substitution of residues 325–524 from the insulin receptor into this chimera enhances insulin binding; remarkably, IGF-1 binding is retained in this chimera because the cysteine-rich region contributes the principle IGF-1 binding determinants (13–15).

The unoccupied α-subunit of the insulin receptor inhibits the tyrosine kinase activity of the β-subunit. Removal of the α-subunits by proteolytic cleavage or deletion mutagenesis, or certain point mutations in the α-subunit ($Arg^{86} \rightarrow Pro$) relieve this inhibition. Insulin and EGF receptors may use similar mechanisms for ligand regulation since the insulin receptor kinase domain linked to an EGF binding domain is stimulated by EGF (16). The external ligand binding domain of the insulin receptor is linked to the tyrosine kinase by a single transmembrane segment, which has broad tolerance for structural changes or substitutions (17, 18). However, the insulin receptor is constitutively activated by substitution of the transmembrane segment from the oncogene v-*erbB*-2, which contains a $Val^{664} \rightarrow Glu$ mutation (19); an analogous point mutation ($Val^{938} \rightarrow Asp$) in the transmembrane segment of the insulin receptor also partially activates the receptor kinase (20).

Several functional regions have been defined in the β-subunit including the ATP binding domain and autophosphorylation sites

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[1] The abbreviations used are: IRS-1, insulin receptor substrate-1; EGF, epidermal growth factor; CHO, Chinese hamster ovary; IGF, insulin-like growth factor; IL, interleukin; kb, kilobase(s); MAP, mitogen-activated protein; PAGE, polyacrylamide gel electrophoresis; PDGF, platelet-derived growth factor; PI, phosphatidylinositol; SH-2, Src homology-2.

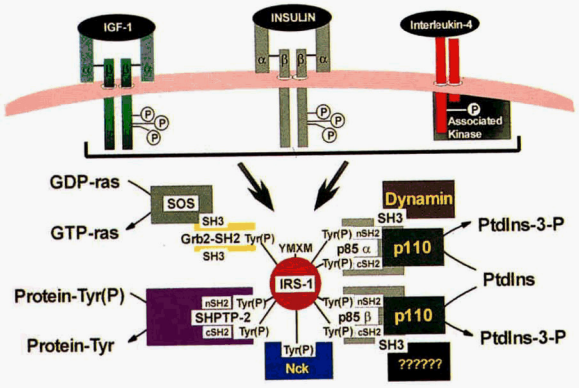

FIG. 1. A schematic model of the IRS-1 signaling pathway. The receptors for insulin, IGF-1 and interleukin-4 (IL-4) are shown stimulating tyrosine phosphorylation of the IRS-1. The insulin and IGF-1 receptor contain an intrinsic kinase activity that undergoes autophosphorylation after ligand binding. The IL-4 receptor is thought to associate with an unknown cytoplasmic tyrosine kinase that mediates IRS-1 tyrosine phosphorylation. The activated IRS-1 signaling complex is shown composed of PI 3′-kinase, GRB-2/SOS ($p21^{ras}$ pathway), and SH-PTP2 (tyrosine phosphatase pathway).

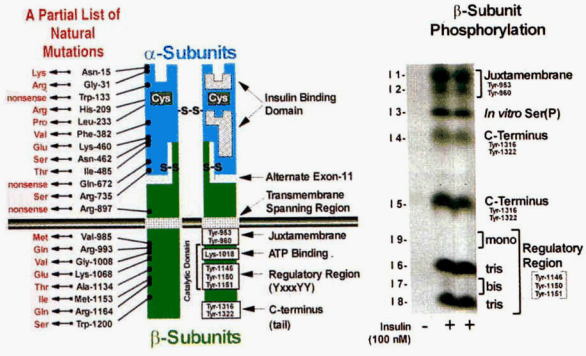

FIG. 2. A model of the insulin receptor is shown to illustrate the relative position of autophosphorylation sites and other functional regions. The insulin receptor α-subunits are linked together and to a β-subunit by disulfide bonds. The relative positions of the insulin binding domain, the cysteine-rich region, the alternate exon-11, and the transmembrane-spanning region are shown. A partial list of naturally occurring mutations in the insulin receptor are shown at the *left* of the figure. The autoradiogram on the *right* illustrates the identification of insulin-stimulated autophosphorylation sites in tryptic digests by separation on Triton PAGE gels (32). Eight peptide bands (*I1–I8*) are shown along with their location in the insulin receptor β subunit.

in the intracellular juxtamembrane region (Tyr^{960}, and possibly Tyr^{953} and Tyr^{972}), a regulatory region (Tyr^{1146}, Tyr^{1150}, and Tyr^{1151}), and the COOH terminus (Tyr^{1316} and Tyr^{1322}) (Fig. 2). Tyrosine autophosphorylation appears to occur through a *trans*-mechanism in which insulin binding to the α-subunit of one αβ-dimer stimulates the phosphorylation of the adjacent covalently linked β-subunit (21). Autophosphorylation of all 3 tyrosine residues in the YXXXYY motif of the regulatory region stimulates kinase activity 10–20-fold (22). Mutation of 1, 2, or 3 tyrosine residues in this region progressively reduces insulin-stimulated kinase activity and results in a parallel loss of biological activity (23, 24). Multisite phosphorylation of the analogous region in the receptors for IGF-1 and the hepatocyte growth factor (c-Met) also activates these kinases. In many cultured cell lines the regulatory region is only bisphosphorylated during insulin stimulation, which may limit the amplitude of the insulin response; however, trisphosphorylation predominates in rat hepatocytes, suggesting a mechanism for tissue-specific up-regulation of the insulin signal (25).

The insulin receptor is phosphorylated on serine and threonine residues in the basal state and in response to stimulation of cells by phorbol esters, cAMP analogues, and insulin itself (26, 27). Sometimes serine phosphorylation decreases insulin-stimulated tyrosine kinase activity. The exact serine phosphorylation sites which are inhibitory are unknown, but might include Ser^{1293}, Ser^{1294}, and Thr^{1336}. The enzyme(s) involved in receptor Ser/Thr phosphorylation *in vivo* remain uncertain. The combination of ligand binding, tyrosine autophosphorylation, and serine/threonine phosphorylation provides three levels of control that are sensitive to extracellular messengers and intracellular events. Yet a fourth mechanism of control may be employed by tumor necrosis factor-α, which causes insulin resistance in adipose tissue, possibly through inhibition of autophosphorylation and kinase activity (28).

Protein tyrosine kinases invariably contain a COOH-terminal tail that extends beyond the end of the tyrosine kinase homology region (Fig. 2). In the EGF and PDGF receptor, the tail contains autophosphorylation sites that bind to SH2 proteins, but a similar function for the insulin receptor tail has not been established *in vivo*. The COOH terminus of the insulin receptor contains two autophosphorylation sites at Tyr^{1316} and Tyr^{1322}, as well as the threonine and serine phosphorylation sites noted above. Short deletions in the COOH terminus, which remove the tyrosine and threonine residues ($IR_{\Delta 43}$), have no effect on insulin-stimulated autophosphorylation in the other regions, insulin-stimulated receptor kinase activity, or biological activity (29, 30). Deletion of 82 amino acids from the COOH terminus ($IR_{\Delta 82}$) decreases insulin-stimulated autophosphorylation significantly, but again has little effect on substrate phosphorylation or downstream biological effects (31). Thus, the COOH terminus may play a regulatory role but is not essential for signaling.

The intracellular juxtamembrane region of the insulin receptor β-subunit is essential for signal transmission (Fig. 2). This region is encoded by exon 16 of the receptor gene and contains several serine residues and at least one autophosphorylation site (Tyr^{960}), which resides in an $NPXY^{960}$ motif (32). A similar motif exists in the IGF-1 and insulin receptor-related receptors. Replacement of Tyr^{960} with phenylalanine or alanine impairs receptor signal transmission even though autophosphorylation in the other regions is normal and the kinase activates fully *in vitro* (33, 34). This appears to be due to an inability of these mutant receptors to mediate the phosphorylation of endogenous receptor substrates, including IRS-1, in the intact cell. Overexpression of IRS-1 can rescue certain biological responses from the effects of mutations in the juxtamembrane region.[2] Tyrosine phosphorylation in the $NPXY^{960}$ motif may be important for substrate selection, as EGF and PDGF receptors, which lack an NPXY motif, do not phosphorylate IRS-1. Asn^{957} in the juxtamembrane region may also be required for IRS-1 phosphorylation.[3]

In addition to signal transduction, the insulin receptor mediates internalization of insulin. Endocytosis of the insulin-receptor complex leads to insulin degradation, while most of the unoccupied receptors recycle to the plasma membrane. After prolonged insulin stimulation, the receptor itself is degraded, resulting in receptor down-regulation and attenuation of the insulin signal (35, 36). Internalized insulin receptors are active catalytically as kinases, suggesting that insulin-stimulated internalization is important for signal transduction (37). At least two pathways for endocytosis exist to varying degrees in all cells. The coated pit-mediated pathway requires a functional insulin receptor kinase, trisphosphorylation in the regulatory region, and two tyrosine-containing β-turns ($GPLY^{953}$ and $NYEY^{960}$) in the juxtamembrane region (38, 39). Autophosphorylation of Tyr^{960} and residues in the COOH terminus, however, are not necessary for internalization (40). Insulin-stimulated internalization appears to require specific and saturable interactions between the receptor and components of the endocytic system. Some cells contain a constitutive and nonsaturable internalization pathway, which does not require receptor autophosphorylation or an intact juxtamembrane region (38). The relative contribution by this latter pathway seems to vary between cell types but is quite significant in Chinese hamster ovary (CHO) cells.

The Principal Insulin Receptor Substrate, IRS-1

IRS-1 was initially detected in insulin-stimulated Fao hepatoma cells by immunoprecipitation with high affinity anti-phosphotyrosine antibodies and was originally called pp185 based on migration during SDS-PAGE (41). It was purified and cloned from several sources (42–47). The IRS-1 gene is located on chromosome 2q36–37 in humans and contains the entire 5′-untranslated region and protein coding region in a single exon (45).

[2] J. M. Backer and M. F. White, personal communication.
[3] T. Kadowaki, personal communication.

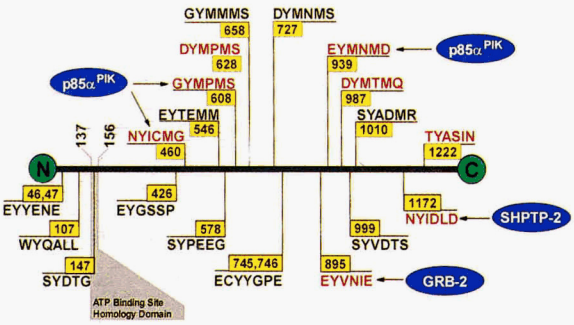

FIG. 3. **Schematic diagram of IRS-1 structure, highlighting the potential tyrosine phosphorylation sites.** Known sites of phosphorylation are in *red*. Preferred sites for the binding of p85α, SH-PTP2, and GRB-2 are indicated (49).

IRS-1 contains 21 potential tyrosine phosphorylation sites, including 6 in YMXM motifs, 3 in YXXM motifs, and 12 in other hydrophobic motifs (Fig. 3). IRS-1 also contains over 30 potential serine/threonine phosphorylation sites in motifs recognized by various kinases. Casein kinase-2 phosphorylates rat IRS-1 at Ser-99 and Thr-502 (48), and preliminary data suggest that purified MAP kinase phosphorylates recombinant IRS-1.[4] There is one potential nucleotide binding site near the amino terminus of IRS-1 but other sequences predictive of protein kinases are not present (Fig. 3).

At least 8 tyrosines in IRS-1 undergo phosphorylation by the activated insulin receptor, including residues 608, 628, 939, and 987 which are in YMXM motifs (49). The methionine residues at the +1 and +3 positions after the tyrosine facilitate phosphorylation by the insulin receptor (50); however, other motifs are phosphorylated including Y^{460}ICM, Y^{895}VNI, Y^{1172}IDL, and Y^{1222}ASI (49). Thirteen additional tyrosine residues may be phosphorylated by the insulin receptor based on the proximity of an aspartate or glutamate residue (Fig. 3).

IRS-1 binds several SH2 proteins through its multiple tyrosine phosphorylation sites. The three-dimensional crystal structure of various SH2 domains suggests that binding specificity is determined by the amino acid sequence motif around the phosphotyrosine residue (51). The PI 3′-kinase was the first SH2 protein found to associate with IRS-1. PI 3′-kinase is composed of two subunits, a 110-kDa catalytic (p110) subunit and an 85-kDa regulatory (p85α) subunit, which possesses two SH2 domains. Both SH2 domains associate specifically with phosphorylated IRS-1 in the intact cell and with recombinant IRS-1 *in vitro*. This association is inhibited by peptides containing a phosphorylated YMXM motif, but not by unphosphorylated YMXM peptides or the phosphorylated peptide containing a scrambled amino acid sequence (6). Tyr^{608} may be the principle site of interaction between IRS-1 and p85; however, IRS-1 contains at least four sites that interact with the SH2 domains of p85: Y^{608}MPM > Y^{939}MNM > Y^{987}MTM and Y^{460}ICM (49).

Insulin stimulates PI 3′-kinase activity *in vivo* via the interaction between the p85 subunit and IRS-1 (52, 53), but it does not stimulate tyrosine phosphorylation of p85 under ordinary circumstances. Recombinant phosphorylated IRS-1 activates PI 3′-kinase with an ED_{50} of 10 nM in immuncomplexes from quiescent CHO or NIH-3T3 cells (6). Synthetic peptides containing a single phosphorylated YMXM motif also activate PI 3′-kinase but are less potent than IRS-1, whereas longer peptides containing two phosphorylated YMXM motifs are more sensitive. These data suggest that activation of the PI 3′-kinase occurs when both SH2 domains are occupied with a phosphotyrosine in a YXXM motif, which is easily accomplished during association with IRS-1. SH-PTP2 (Syp), a protein tyrosine phosphatase that contains two SH2 domains, preferentially binds to Tyr^{1172} in IRS-1 (49). The association of SH-PTP2 with phosphopeptides containing a consensus binding motif (YVNI) activates the phosphatase (54, 55), suggesting a role for IRS-1 in the activation of this signal transduction pathway.[5]

GRB-2 is a small cytoplasmic protein that contains two SH3 domains and one SH2 domain that binds to Tyr^{895} in IRS-1 (Fig. 1). GRB-2 is thought to act as an "adapter molecule" that links the guanine nucleotide exchange factor for $p21^{ras}$ termed mSOS (homologous to the *Drosophila* protein, son-of-sevenless (Sos)) to tyrosyl phosphoproteins such as the EGF receptor, IRS-1, and BCR-abl (56, 57). The GRB-2/mSOS complex thus may activate $p21^{ras}$ by stimulating GTP binding. Microinjection of GRB-2 or $p21^{ras}$ alone into fibroblasts has no effect on DNA synthesis, whereas co-injection of GRB-2 and $p21^{ras}$ stimulates cell proliferation (58). By analogy, the binding of GBR-2/mSOS to IRS-1 may mediate the insulin stimulation of $p21^{ras}$. Ras has been shown to bind directly Raf-1 serine/threonine kinase, which in turn activates MAP kinase by phosphorylation and activation of the MAP kinase kinase (59). Overexpression of GRB-2 in L6 cells increases the sensitivity of MAP kinase to insulin stimulation suggesting that GRB-2 is involved (8). Thus the binding of IRS-1 to GRB-2 might be one pathway used by insulin to regulate Ras. However, alternate pathways for insulin regulation of Ras may exist, including insulin-stimulated tyrosine phosphorylation of Shc (60) or the direct binding of $p21^{ras}$ to the insulin receptor (61).

IRS-1 is essential for some, if not all, of insulin's biological responses. Overexpression of IRS-1 in CHO cells increases insulin stimulation of thymidine incorporation 2-fold (62). Conversely, reduction of IRS-1 levels in CHO cells by expressing antisense cDNA reduces the response and sensitivity of mitogenesis to insulin (63). Microinjection of recombinant IRS-1 into *Xenopus* oocytes reconstitutes insulin stimulation of PI 3′-kinase, MAP kinase, S6 kinase, and oocyte maturation (64, 65). Coinjection of glutathione *S*-transferase fusion proteins containing the SH2 domains of p85 (GST-$SH2^{p85}$) inhibits activation of PI 3′-kinase and oocyte maturation by competing for association with phosphorylated IRS-1. Thus, the pleotropic effects of insulin could be mediated by the interaction of IRS-1 with SH2-containing enzymes and adapter molecules as summarized in Fig. 4.

The fortuitous absence of IRS-1 from the 32D myeloid progenitor cells also demonstrates that IRS-1 is essential for insulin- and interleukin-4 (IL-4)-stimulated mitogenesis (10). In several myeloid cell lines, IL-4 and insulin stimulate the phosphorylation of a 185-kDa molecule termed 4PS and this is associated with an increase in mitogenesis. The 32D line does not respond to insulin or IL-4 and also lacks IL-4/insulin-stimulated phosphorylation of 4PS. 4PS has many characteristics in common with IRS-1, but reacts poorly with antibodies against IRS-1. Following expression of IRS-1, both insulin and IL-4 strongly stimulate IRS-1 phosphorylation and DNA synthesis in 32D cells (10). Since the IL-4 receptor does not contain an intrinsic tyrosine kinase domain, the IL-4

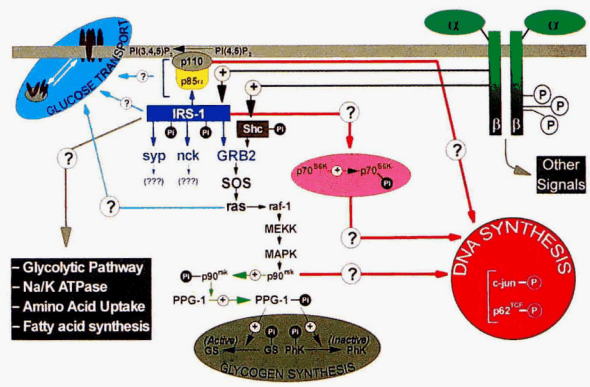

FIG. 4. **A potential network of insulin signaling pathways mediated through activation of the insulin receptor kinase and phosphorylation of IRS-1 or Shc.** Protein phosphorylation is indicated by *Pi*. Several SH2 proteins are illustrated, including Syp (SH-PTP2), Nck, GRB-2, and p85α. GRB-2 links IRS-1 and Shc to the guanine nucleotide exchange factor SOS, which appears to regulate MAP kinase (*MAPK*) through a cascade involving a serine/threonine kinase (Raf-1), a MAP kinase kinase (*MEKK*). Insulin is thought to stimulate $p90^{rsk}$ through the MAP kinase cascade, whereas it stimulates $pp70^{s6k}$ through a distinct cascade involving IRS-1. The glycogen-associated protein phosphatase-1 (*PPG-1*) appears to be activated by $p90^{rsk}$-stimulated phosphorylation, which places the insulin stimulation of glycogen synthase (*GS*) and inhibition of phosphorylase kinase (*PhK*) in the GRB-2/SOS pathway. The possible regulation of glucose carrier translocation and DNA synthesis are shown.

[4] X. J. Jun, R. Davis, and M. F. White, unpublished data.
[5] C. Walsh and B. Neel, personal communication.

receptor probably acts by recruiting and activating a cytosolic tyrosine kinase distinct from, but similar to, Jak-1, Jak-2, Fyn, or Tyk-2 (Fig. 1). The specificity for interaction with IRS-1 may emanate from the cytoplasmic tail of the IL-4 receptor, which, like the insulin receptor, contains an NPXY motif that is essential for IL-4 signaling (66).[6]

Physiologic Significance and Future Directions

The function of the insulin receptor and the structure of IRS-1 begin to provide a rational framework for understanding the pleiotropic effects of insulin on classic target tissues and potential links to non-classical targets such as the immune system (Fig. 4). It is not yet clear exactly what features of these molecules are essential for metabolic and mitogenic responses and whether IRS-1 is essential for all insulin responses. The availability of the *Xenopus* oocyte and 32D cell reconstitution systems provides an opportunity to answer some of these questions. The identification of novel downstream signaling molecules, which interact with the receptor and/or IRS-1, will provide new insights into the mechanism of signal transmission. So far, IRS-1 appears to be an orphan molecule; however, a hint for the existence of "IRS-2" comes from the insulin/IL-4 sensitive myeloid progenitor cells, which contain 4PS. The preparation of genetically engineered mice that lack IRS-1, if they are viable, should provide excitement for the future.

Only recently have data begun to emerge on the physiologic and pathophysiologic significance of the insulin receptor/IRS-1 pathways. Both proteins are regulated in expression at the transcriptional, translational, and post-translational levels (67, 68). Interestingly, IRS-1 protein levels appear to be differentially regulated in different target tissues such as muscle and liver (67). Furthermore, the activity of insulin receptors and IRS-1 is also regulated by their states of tyrosine and serine phosphorylation, as well as potentially by the specific sites phosphorylated. New insights into the pathophysiology and therapy of diabetes and other states of altered insulin action will no doubt result from these emerging concepts of the insulin action pathway.

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