

# Recent Advances in Our Understanding of Insulin Action and Insulin Resistance

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Insulin signaling at the target tissue results in a large array of biological outcomes. These events are essential for normal growth and development and for normal homeostasis of glucose, fat, and protein metabolism. Elucidating the intracellular events after activation of the IR has been the primary focus of a large number of investigators for decades, and for excellent reasons. Understanding the signaling pathways involved in insulin action could lead to a better understanding of the pathophysiology of insulin resistance associated with obesity and type 2 diabetes, and identifying key molecules and processes could lead to newer and more effective therapeutic agents for treating these common disorders.

This review summarizes our previous understanding of how insulin acts and outlines some recent developments in our understanding of insulin action and insulin resistance at the cellular level, beginning with a discussion on the discovery of evolutionarily conserved molecules of the insulin signaling pathways. This article will also provide a summary of a few *in vitro* and cellular models of insulin resistance and a description of some new paradigms in the cellular mechanisms of insulin action.

This review will not attempt to be all-inclusive; for a more comprehensive un-

derstanding, readers are referred to more complete reviews on insulin action (1–5).

## INSULIN ACTION AT THE CELLULAR LEVEL

### Our current knowledge

Circulating insulin rapidly reaches the target tissue, where it interacts with its cognate receptor. The IR (IR), which is widely expressed, is a transmembrane tyrosine (Tyr) kinase that is expressed as a tetramer in an  $\alpha 2\beta 2$  configuration (6,7). Insulin binding to specific regions of the  $\alpha$  subunit leads to a rapid configurational change in the receptor that eventuates in autophosphorylation of specific Tyr residues of the intracellular region of the  $\beta$  subunits through a transphosphorylation mechanism.

Autophosphorylation results in activation of the Tyr kinase activity of the receptor (8). In the inactive state, the catalytic site of the Tyr kinase is occluded by the “activation-loop,” preventing access of ATP and various substrates. Autophosphorylation of Tyr residues at positions 1,158, 1,162, and 1,163 in the activation-loop causes a conformational change that allows ATP and substrates to reach the catalytic site (9,10).

The activated IR kinase phosphorylates substrate proteins on Tyr residues,

and these phosphorylated Tyr residues serve as docking sites for downstream effectors (Fig. 1). Molecules such as Shc, IR substrate (IRS) (1,4), and Gab-1 engage the IR directly and provide a docking interface with downstream substrates. IRS proteins contain a conserved pleckstrin homology (PH) domain, located at the NH<sub>2</sub>-terminus, that serves to localize the IRS proteins in close proximity to the receptor (11,12). IRS proteins contain a phosphate-Tyr binding (PTB) domain COOH-terminal to their PH domain. The PTB domain, present in a number of signaling molecules (13), shares 75% sequence identity (14) between IRS-1 and IRS-2 and functions as a binding site to the NPXY motif of the juxtamembrane region of the IR to promote IR/IRS-1 interactions (15,16). The COOH-terminal region of IRS proteins is poorly conserved. It contains multiple Tyr phosphorylation motifs that serve as docking sites for SH2 domain-containing proteins, like the p85 $\alpha$  regulatory subunit of phosphatidylinositol 3-kinase (PI3-K), growth factor receptor binding protein-2 (Grb2), Nck, Crk, Fyn, SHP-2, and others, all of which mediate the metabolic and growth-promoting functions of insulin (2,17).

Insulin-receptor signaling involves two major pathways—the mitogen-activated protein (MAP) kinase and the PI3-K. Although these pathways are described in a linear fashion, it should not be forgotten that each pathway could, under certain circumstances, activate the other. Thus, Akt may activate Raf kinase, and conversely, Ras may activate PI3-K. The MAP kinase pathway is activated by the binding of Grb2 to Tyr-phosphorylated Shc or IRS via its SH2 domain. Grb2 is prebound to mammalian Son of Sevenless (mSOS), a nucleotide exchange protein that catalyzes the exchange of GDP for GTP on Ras (a small GTPase protein); this results in activation of Ras. The prenylated form of Ras binds the inner leaflet of the plasma membrane, and on activation, it binds the NH<sub>2</sub>-terminal region of Raf, recruiting Raf to the plasma membrane. Ras-Raf interaction displaces the 14-3-3 proteins that are bound to Raf and

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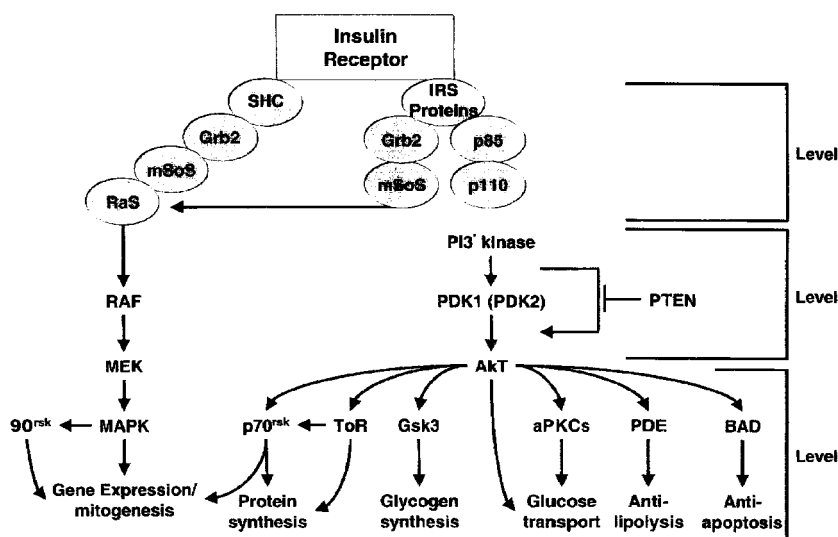
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**Abbreviations:** eIF, eukaryotic initiation factor; FFA, free fatty acid; FH, Forkhead family; FKH, Forkhead transcription factor; Grb2, growth receptor binding protein-2; GSK-3, glucocorticoid synthase kinase-3; IR, insulin receptor; IRS, IR substrate; MAP, mitogen-activated protein; mSOS, mammalian Son of Sevenless; mTOR, mammalian Target of Rapamycin; PDK1, phosphatidylinositol-dependent kinase 1; PEPCK, phosphoenol pyruvate carboxy kinase; PH, pleckstrin homology; PI3-K, phosphatidylinositol 3-kinase; PIP<sub>3</sub>, phosphatidylinositol 3,4,5-phosphate; PKC, protein kinase C; PPAR $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ ; PTB, phosphate-tyrosine binding; RXR, retinoid X receptor; SGK, serum and glucocorticoid-inducible kinase; PtdIns, phospho-inositol; TNF, tumor necrosis factor; TZD, thiazolidinedione.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances



**Figure 1**—IR signaling pathways. The two major pathways are the Ras/Raf/MAP kinase and PI3-K pathways. Initially, the activated IR binds SHC and the IRS molecules, and these interact with downstream substrates. The PI3-K pathway leads to a large variety of biological actions after IR activation.

allows the phosphorylation of Raf by a number of (Ser/Thr) kinases, thus disinhibiting Raf kinase (18). Raf-1 activates a dual-specificity kinase, MEK1, by phosphorylating two regulatory Ser residues. In turn, MEK1 activates extracellular signal-regulated kinase (ERK)-1 and ERK2 by phosphorylating regulatory Tyr and Thr residues (19). Activated ERKs mediate the growth-promoting effects of insulin by phosphorylating transcription factors such as Elk-1, leading to the induction of genes.

The metabolic response to insulin is primarily mediated via the PI3-K pathway. Following the association of the p85/p110 complex of PI3-K with the IRS molecules, PI3-K activity results in production of phosphatidylinositol 3,4,5-phosphate (PIP<sub>3</sub>). PIP<sub>3</sub> binds to the PH domains of PI3-K-dependent kinase (PDK)-1 and Akt (protein Ser/Thr kinase B). This leads to the activation of PDK1, which in turn phosphorylates and activates Akt. Akt has been implicated in regulating the translocation of GLUT4, an insulin-sensitive glucose transporter expressed by muscle and fat cells. Interestingly, Akt may not be the only downstream kinase to regulate GLUT4 translocation to the cell surface. Protein kinase C (PKC) isoforms  $\zeta$  and  $\lambda$  are also activated by PI3-K and PDK1 and regulate GLUT4 translocation (rev. in 20). Indeed,

overexpression of wild-type PKC $\zeta$  increases, whereas overexpression of a dominant-negative PKC $\zeta$  decreases basal and insulin-stimulated glucose transport in adipocytes and muscle cells (21,22).

Stimulation of glycogen synthesis is another key metabolic effect of insulin. Glycogen synthase kinase-3 (GSK-3) mediates, at least in part, the activation of glycogen synthase in response to insulin. Activation of Akt by insulin results in the phosphorylation and inactivation of GSK-3, rendering it incapable of inhibiting glycogen synthase activity (23). GSK-3 also inactivates the protein synthesis eukaryotic initiation factor (eIF)-2B (the guanine nucleotide exchange factor) by phosphorylation. Insulin-mediated activation of Akt reverses these processes, thereby enhancing protein synthesis (24). Insulin can also activate protein synthesis at the translational level by phosphorylation of p70S6 kinase and 4E-BP1 via the kinase mammalian Target of Rapamycin (mTOR). In fact, increased 4E-BP1 phosphorylation is controlled by a parallel signaling pathway that immediately bifurcates upstream of p70s6k, with the two pathways sharing a common rapamycin-sensitive activator. The phosphorylation of 4E-BP causes it to disassociate from the eIF-4E, thus enhancing its ability to initiate protein synthesis (25).

### The importance of nuclear transport of signaling molecules

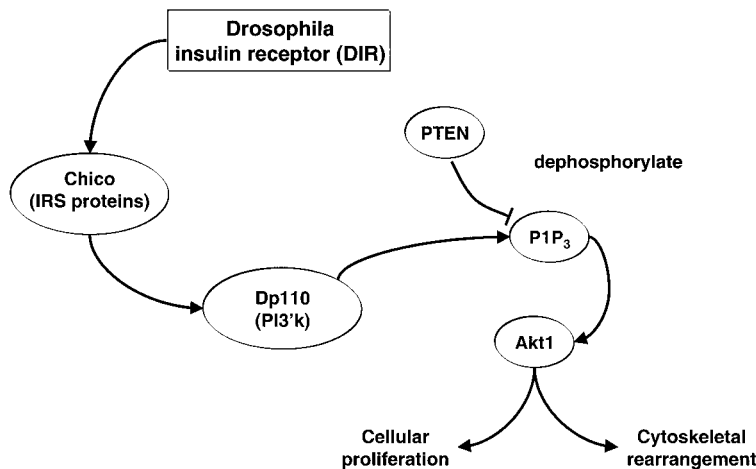
Signaling substrates of the Tyr kinase receptors can be grouped into three levels, depending on their proximity to the receptor. Level I represents proximal substrates such as the IRS proteins and SHC and the proteins that directly interact with them. Level II represents downstream intermediates, including MAP kinases, Akt, and related substrates, and level III molecules affect the final biological responses (Fig. 1). Whereas level I and II molecules function primarily at the plasma membrane or in the cytosol, many of the level III molecules are transported into the nucleus, because their specific function involves the regulation of gene transcription. A prerequisite for the nuclear translocation of these molecules is often phosphorylation by upstream kinases.

For example, overexpression of membrane-bound forms of ERK1 and ERK2 results in their homodimerization with the endogenous ERKs' isoforms, thus preventing them from entering the nucleus after activation of the receptor Tyr kinase. As a result, transcriptional activation of *c-fos* is inhibited; this strongly supports the idea that nuclear translocation is an essential component of the signaling cascade (26).

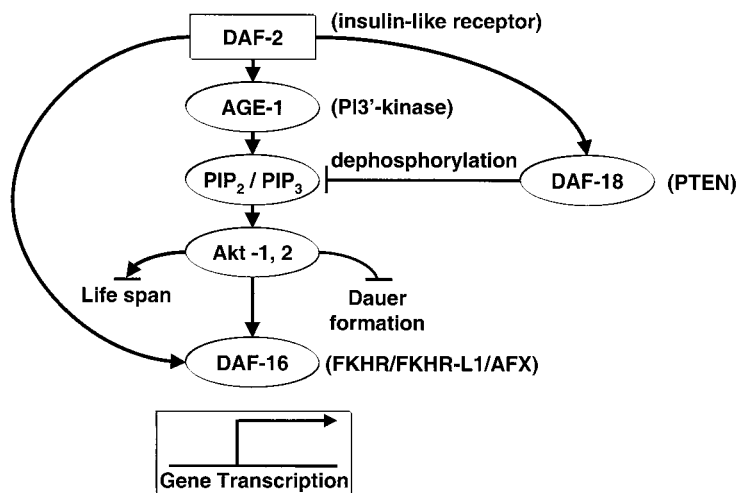
Recently, insulin and IGF-1 have been shown to inhibit nuclear translocation of transcription factors in a process that involves the activation of Akt, a downstream effector of the insulin and IGF-1 receptor Tyr kinases. Akt phosphorylates a family of transcription factors called the Forkhead family (FH), which includes FKHR, FKHL1, and AFX, and represents the mammalian counterparts of DAF16 in the nematodes (27). Phosphorylation of FKHR by Akt, after stimulation by insulin or IGF-1, inhibits the expression of several genes encoding for proteins, such as the IGF binding protein-1 (28). Phosphorylation of other members of the FH family by Akt may similarly inhibit the expression of other insulin-regulated genes, such as phosphoenol pyruvate carboxy kinase (PEPCK).

Akt phosphorylates the FH family of proteins on Ser residues in a consensus site, RXRXXS/T (29). This creates a phosphoserine motif capable of binding members of the 14-3-3 family of proteins (30). The interaction of FKHR with 14-3-3 leads to retention of FKHR in the cyto-

**A. Drosophila**



**B. C. elegans**



**Figure 2**—*Drosophila* and *C. elegans*. Insulin signaling pathways are evolutionarily conserved. A: *Drosophila* express an IR that activates a pathway that involves CHICO (a protein related to the mammalian IRS molecules), Dp110 (PI3-K homologue), Akt, and the phosphatase PTEN. B: Many of the molecules expressed by *C. elegans* include homologues to mammalian counterparts of the insulin signaling pathways. These include DAF-2, an IR-like molecule; AGE-1, a PI3-K-related molecule; DAF-18, a PTEN-related molecule; and DAF-16, which is related to the FKHR in mammalian systems.

plasm and prevents FKHR from translocating to the nucleus. Consequently, the expression of a number of genes is inhibited. Similar effects were observed with IGF-I receptor-induced phosphorylation of FKHL1, which normally induces FAS, partially explaining IGF's antiapoptotic effects (30).

Serum and glucocorticoid-inducible kinase (SGK) is another recently described target of the insulin and IGF-I receptor signaling cascades involving PI3-K. SGK is apparently involved in amplifying the mitogenic signal, because it

demonstrates cytoplasmic-nuclear shuttling dependent on the phase of the cell cycle (31). SGK is hyperphosphorylated in serum-stimulated cells and localizes to the nucleus, and inhibition of PI3-K results in inhibition of this hyperphosphorylated state of the protein and inhibition of the nuclear localization of SGK (32).

Thus, an important paradigm has recently been described in the signaling pathways of the Tyr kinase receptors that involves phosphorylation of signaling molecules, which in turn determines their cytoplasmic or nuclear

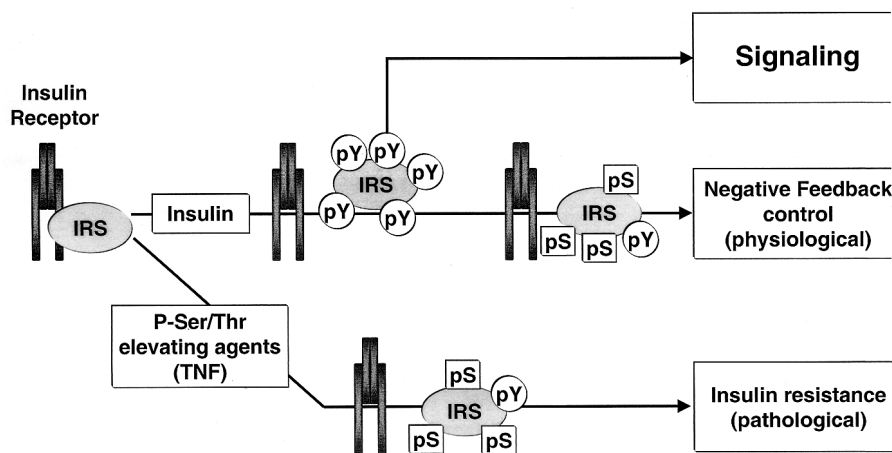
localization and their ability to perform their function(s).

**EVOLUTIONARY ASPECTS OF THE INSULIN SIGNALING CASCADES**

The IR and its postreceptor signaling pathways are highly conserved evolutionarily (Fig. 2A and B). Many of the signaling molecules that mediate the effects of insulin were first discovered and characterized in either *Drosophila* (fly) and/or *Caenorhabditis* (worm) species.

An IR homologue was isolated and characterized in *Drosophila* species (33), and an insulin-like peptide was also found in *Drosophila* extracts (34). The IR of *Drosophila* species is highly conserved, and like the mammalian IR, it is expressed as a tetramer of two  $\alpha$  and two  $\beta$  subunits. Whereas the  $\beta$  subunit of the *Drosophila* IR has a COOH-terminal extension with motifs that are known to bind SH2 and PTB domain-containing proteins, it is apparent from recent studies that when expressed in the absence of IRS-1, the *Drosophila* IR is not active. When coexpressed in cells containing IRS proteins, the *Drosophila* IR is capable of promoting cell survival and interacting with IRS-I via its COOH-terminal extension (35). Recently, a *Drosophila* homologue (CHICO) of vertebrate IRS 1–4 proteins was characterized as showing 41% amino acid identity to the NH<sub>2</sub>-terminal PH domains and 38% identity to the PTB domains of IRS 1–4 (Fig. 2). In the COOH-terminal region, which is less homologous, there are several putative SH2-binding motifs for PI3-K (YXXM) and for Grb2/DRK binding (36). CHICO plays an important role in cell proliferation and overall body growth in *Drosophila*. Furthermore, a *Drosophila* homologue of PTEN (a human tumor suppressor gene and phospholipid phosphatase that inhibits PI3-K and Akt pathways) (Fig. 2A) suppresses hyperplastic growth in flies by inhibiting *Drosophila* CHICO and PI3-K (37).

In *C. elegans*, the ability of the worm to enter the developmental larval stage “dauer” is modulated by a PI3-K pathway (Fig. 2B). DAF-2 is an insulin/IGF-I receptor-like molecule that activates the PI3-K and Akt pathways and inhibits dauer formation induced by DAF-16, the Forkhead transcription factor (FKH) (38,39). Loss of DAF-2 results in increased longevity and constitutive dauer formation, a stage of developmental arrest



**Figure 3**—Ser/Thr phosphorylation of the IRS molecules induces insulin resistance. Ser/Thr phosphorylation of the IRS proteins may serve as a physiological negative feedback control mechanism or may result in insulin resistance. Modified with permission (59).

and reduced metabolic activity that enhances survival during periods of food deprivation and other environmental stresses. These observations suggest that DAF-16 promotes entry into the dauer phase and enhances longevity and that signaling via the insulin/IGF-1 receptor-PI3-K-PKB pathway may disrupt these effects of DAF-16. Indeed, a *C. elegans* homologue of PTEN has been characterized (DAF-18) and shown to inhibit PI3-K and enhance dauer formation (40,41).

### ROLE OF INSULIN SIGNALING SYSTEMS IN INSULIN RESISTANCE

Insulin resistance is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. It is frequently associated with a number of diseases, including chronic infection, human obesity, and type 2 diabetes (4). At the molecular level, impaired insulin signaling results from mutations or post-translation modifications of the IR itself or any of its downstream effector molecules (rev. in 42). In some cases, insulin resistance could be accounted for by a defect in insulin binding to its receptor (43); however, insulin resistance is most often attributed to a postbinding defect in insulin action. A marked reduction in the receptor kinase activity was observed in several patients (type A) with extreme resistance to insulin, but with normal insulin binding (44,45). Similarly, severe defects in receptor kinase activity are associated with naturally occurring muta-

tions of the IR gene (46). However, these events are rare and do not play an important role in the pathophysiology of typical type 2 diabetes or obesity (47).

Whereas the IR is downregulated in human obesity, there is no further decrease in its activity in liver and muscle of type 2 diabetic patients, suggesting that aggravated insulin resistance of type 2 diabetes is primarily of a postreceptor nature (48). IR Tyr kinase activity in patients with type 2 diabetes is significantly reduced (49). The kinase activity could be inhibited because of elevation in Tyr phosphatase activity (50) or enhanced Ser/Thr phosphorylation of the receptor that impairs its Tyr kinase activity (51,52). Ser/Thr phosphorylation of the IR occurs in response to the treatment of cells with insulin (53), or with activators of PKC or the cAMP-dependent protein kinase (54,55). Accordingly, downstream signaling cascades should be decreased in proportion to the defect in IR Tyr kinase activity; apparently, however, when compared with the reductions in Tyr phosphorylation of the IR and IRS-1, PI3-K is more severely reduced in type 2 diabetic patients (56).

Glycogen synthesis is markedly reduced in the muscle of type 2 diabetic patients. In recent studies using nuclear magnetic resonance technology, Shulman et al. (57) demonstrated that one of the defects resulting in these changes was a reduction in glucose transport. Because intracellular glucose and glucose-6-phosphate levels were reduced, Shulman

et al. hypothesized that glucose transport was defective. Insulin-stimulated signaling pathways studied in isolated muscle preparations from type 2 diabetic patients demonstrated normal IR Tyr phosphorylation, normal MAP kinase (ERK) phosphorylation, and normal glycogen synthase activity. In contrast, insulin-stimulated glucose transport was reduced (58); this was accompanied by a decrease in Tyr phosphorylation of IRS-1 and a reduced association of IRS-1 and PI3-K. Hence, insulin resistance could be attributed to the uncoupling of the IR and IRS proteins, which could be the result of excessive Ser/Thr phosphorylation of the latter (vide infra) (59).

### Ser/Thr phosphorylation of IRS proteins and insulin resistance

Agents that enhance Ser/Thr phosphorylation of IRS proteins or other downstream effectors of the insulin signaling cascade play negative-regulatory roles in insulin action. Ser/Thr phosphorylation impairs insulin-stimulated Tyr phosphorylation of IRS proteins, uncouples insulin signal transduction, and has been implicated in the development of insulin resistance (Fig. 3) (59–61).

Increased Ser phosphorylation of IRS-1 has been observed after treatment of cells with activators of PKC, Ser/Thr phosphatase inhibitors such as okadaic acid, platelet-derived growth factor (62), insulin or angiotensin II, and with activation of cellular stress pathways by tumor necrosis factor (TNF) (63) and other cytokines (64). Decreased Tyr phosphorylation of IRS proteins and a reduction in their associated PI3-K activity is observed in skeletal muscle and adipocytes both in obesity and type 2 diabetes (56). Similarly, insulin-stimulated Tyr phosphorylation of IRS proteins and the activation of their downstream effectors are decreased in both genetic (65) and induced (66) rodent models of obesity and insulin resistance. Several mechanisms, many of which are discussed further, were proposed to account for the effects of the enhanced Ser/Thr phosphorylation of IRS-1 on its Tyr phosphorylation state; however, it is clear that a decrease in Tyr phosphorylation of IRS-1 is not necessarily secondary to a decline in insulin-receptor kinase activity (1).

### **TNF- $\alpha$ as an inducer of insulin resistance**

TNF- $\alpha$  expression is increased in abdominal fat and muscle tissue of obese individuals and in many animal models of obesity. The degree of TNF- $\alpha$  expression is positively correlated with the degree of obesity and the levels of plasma insulin and decreases with the improvement of insulin sensitivity (67–69). Circulating levels of TNF- $\alpha$  are elevated in obese subjects and decrease with weight reduction (70). Further support for its role in affecting insulin sensitivity resulted from studies using a soluble TNF-receptor IgG fusion protein, which neutralized TNF- $\alpha$  when administered to animals with insulin resistance; this neutralization was associated with improvement in insulin action (71). Whereas similar studies in humans were unsuccessful (72), the possibility remains that TNF- $\alpha$  causes insulin resistance in a paracrine fashion and that circulating levels of TNF- $\alpha$  represent leakage into the circulation after increased tissue expression. Whether the effects of TNF- $\alpha$  are direct or indirect has not yet been determined, because it has been shown that TNF- $\alpha$  stimulates leptin secretion from adipocytes, and free fatty acid (FFA) levels are correlated with TNF- $\alpha$  levels. Both leptin and FFAs play a role in insulin resistance (73–75).

TNF- $\alpha$  has direct effects on the insulin signaling cascade in cultured cells. TNF- $\alpha$  increases the Ser phosphorylation of IRS-1 and IRS-2 (Fig. 3). Serine phosphorylation of these substrates results in a reduction in both insulin-receptor Tyr autophosphorylation and Tyr kinase activity of the receptor and markedly reduces the ability of the IRS molecules to dock with the receptor and interact with downstream pathways, such as PI3-K (59,76) and glucose transport (77). The mechanisms by which TNF- $\alpha$  enhances IRS Ser phosphorylation may include sphingolipid metabolism with the generation of ceramide (78), which in turn regulates Ser/Thr kinases (ceramide-activated kinases, PKC $\zeta$ , and Raf 1) or PKC $\epsilon$ , which directly enhances TNF- $\alpha$  effects (79,80). Interestingly, activation of the peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) by thiazolidinediones (TZDs) reduces the expression of TNF- $\alpha$  and hinders TNF- $\alpha$ 's inhibition of insulin action (81).

Although the role of TNF- $\alpha$  in inducing or perpetuating insulin resistance has

yet to be confirmed, it represents a paradigm from which investigators have slowly accumulated a large amount of information that has enabled them to formulate more exactly the mechanisms involved in the insulin resistance seen in obesity and type 2 diabetes.

### **The role of PPAR $\gamma$ in insulin action and insulin resistance**

The PPAR family includes PPAR $\alpha$ , - $\delta$ , and - $\gamma$ . These nuclear receptors heterodimerize with the retinoid X receptor (RXR) and regulate transcription of a number of genes. PPAR $\gamma$ , in particular, has been shown to be involved in regulating genes involved in adipogenesis and, by implication, insulin action. Adipogenesis involves a number of transcription factors, including PPAR $\gamma$ , C/EBPs, and ADD-1/SREBP-1 (82). For instance, the activation of PPAR $\gamma$  by TZDs enhances adipocyte differentiation and induces gene expression of genes involved in insulin action. These genes include aP2, PEPCCK, acyl CoA synthase, and lipoprotein lipase (83–85). In addition, PPAR $\gamma$  activation inhibits leptin gene expression, as well as the expression of TNF- $\alpha$ , which, in turn, is an inhibitor of PPAR $\gamma$  gene expression (86). Growth factors that stimulate cellular proliferation block fat-cell differentiation. This effect is mediated by the activation of MAP kinase, which phosphorylates PPAR $\gamma$  on Ser and thereby reduces its activity. Thus, it is very apparent that PPAR $\gamma$  may play a significant role in the pathophysiology of obesity and type 2 diabetes. This notion is supported by the recent description of two families whose mutations in PPAR $\gamma$  caused severe insulin resistance and diabetes (87).

TZDs are a new class of drugs recently introduced for the treatment of type 2 diabetes that function by direct binding and activation of PPAR $\gamma$ . PPAR $\gamma$  is expressed at much higher levels in fat tissue when compared with muscle and liver. However, PPAR $\gamma$  ligands enhance insulin sensitivity in both muscle and liver. The effects of TZDs and PPAR $\gamma$  may be indirect, whereby the primary effects on adipocytes are secondarily transmitted to muscle and liver via other mediators, such as changes in TNF- $\alpha$ , leptin, or FFAs (88). For example, TZDs acting via PPAR $\gamma$  induce lipoprotein lipase, thereby increasing triglyceride uptake into fat, and reduce circulating FFAs, which then reduces insulin resistance at the liver and

muscle. Alternatively, the small amount of PPAR $\gamma$  in muscle and other tissues may be sufficient to enable a response to pharmacological doses of TZDs. Interestingly, TZDs may improve insulin action even in the absence of adipose tissue (89). Finally, enhanced GLUT4 gene expression is an important mechanism that may explain the improvement of insulin action at the target tissue after treatment with TZDs.

### **Fatty acids and insulin resistance (lipotoxicity)**

In both obesity and type 2 diabetes, plasma FFA levels are elevated. Increasingly, there is evidence to support the contention that FFAs affect insulin action at the peripheral target tissues. The exact site of inhibition of insulin action has not yet been well defined; however, FFA-induced defects at the level of glucose uptake into muscle, phosphorylation of glucose by glucose-6-phosphate, and glycogen synthesis have been demonstrated (90,91). It has been further proposed that the mechanisms by which TNF- $\alpha$  and leptin cause insulin resistance, and whereby the TZDs improve insulin sensitivity, may be triggered indirectly, via a reduction in FFAs levels (92,93).

### **Feedback regulation of insulin signaling cascades**

An essential component of cellular signal transduction is regulation of the system. This may be achieved by autoregulation, resulting in inhibition of upstream kinases (homologous desensitization) by downstream enzymes or substrates. Alternatively, signals from other apparently unrelated receptor pathways may cause inhibition of the signal (heterologous desensitization).

Ser/Thr residues of IRS proteins have a dual function and serve either as positive or negative modulators of insulin signal transduction. Phosphorylation of Ser residues within the PTB domain of IRS-1 by insulin-stimulated PKB (76) protects IRS proteins from the rapid action of protein Tyr phosphatases and enables the Ser-phosphorylated IRS proteins to maintain their Tyr-phosphorylated active conformation. These findings implicate PKB alone as a positive regulator of IRS-1 functions. In contrast, Ser/Thr kinase, which is different from PKB (76), has been implicated as the kinase(s) that phosphorylates IRS-1 and acts as the negative feedback

control regulator that turns off insulin signals, either by inducing the dissociation of IRS proteins from IR (59), releasing the IRS proteins from intracellular complexes that maintain them in close proximity to the receptor (94), or turning IRS proteins into inhibitors of the insulin-receptor kinase (60).

Several studies provided evidence that at least some of the kinases that negatively regulate IRS protein function are wortmannin-sensitive Ser/Thr kinases that are downstream effectors of PI3-K. Indeed, only wortmannin, a PI3-K inhibitor, effectively inhibited the enhanced Ser phosphorylation, the dissociation of IRS proteins from the IR, and the subsequent reduction in Tyr phosphorylation of IRS proteins observed after a 60-min insulin treatment (76). Other inhibitors that selectively block the activities of MEK, such as PD 098059, were ineffective in preventing the negative feedback control mechanism induced by insulin. Hence, a wortmannin-sensitive Ser/Thr kinase, different from PKB $\alpha$ , presumably acts as the feedback control regulator that turns off insulin signals. Activation of this kinase is expected to take place subsequent to activation of PKB $\alpha$ , which acts as a positive-regulator of IRS-1 function, as previously described.

Several Ser/Thr kinases located downstream of PI3-K are likely candidates to fulfill this role. These include the mTORs (95) and p70 kDa S6 kinase (96), which are activated by phospho-inositols (PtdIns) (3–5) PDK1 (97) and PKB (98). Indeed, mTOR-mediated phosphorylation of IRS-1 on Ser 632, 662, and 731 of IRS-1 was shown to inhibit insulin-stimulated Tyr phosphorylation of IRS-1 and its ability to bind PI3-K (99). Accordingly, membrane-targeted PI3-K was found to stimulate Ser/Thr phosphorylation of IRS-1 and to inhibit IRS-1-associated PI3-K activity (100). Other potential candidates could be members of the PKC family. Atypical PKCs, exemplified by PKC $\zeta$ , were implicated as downstream effectors of PI3-K (101) and as mediators of insulin-stimulated glucose transport (102). In fact, tetradecanoylphorbol acetate, a potent activator of various PKC isoforms, inhibits both IRS-1 interactions with the juxtamembrane region of the IR as well as insulin's ability to phosphorylate IRS proteins (59), presumably through the activation of MAPK (103).

Other downstream effectors of PI3-K are less likely to act as insulin-induced negative regulators of IRS-1 function. Glycogen synthase kinase-3 (GSK-3) is capable of phosphorylating IRS-1, and this modification converts IRS-1 into an inhibitor of IR Tyr kinase activity in vitro (104); however, it is unlikely that GSK-3 could act as an insulin-stimulated kinase of IRS-1, because GSK-3 activity is inhibited by insulin (23). PtdIns (3,4,5) PDKs are downstream effectors of PI3-K and are stimulated in response to insulin (105). However, being upstream activators of PKB, they are less likely candidates for being negative regulators of IRS-1 function.

The findings described above indicate that Ser/Thr phosphorylation of the IRS protein after insulin stimulation has a dual role—either to enhance or to terminate insulin's signal. Insulin activates a wortmannin-sensitive kinase downstream, or independent from PKB, that phosphorylates yet-unidentified Ser/Thr residues within the IRS protein. Phosphorylation of these sites is part of the negative feedback control mechanism induced by insulin that leads to the dissociation of the IR-IRS complexes, turns IRS proteins into inhibitors of the insulin-receptor kinase, and results in the termination of the insulin signal. Agents that induce insulin resistance, such as TNF, take advantage of this mechanism by stimulating the phosphorylation of IRS proteins on the same or similar Ser/Thr sites, the phosphorylation of which results in the dissociation of IR-IRS complexes (Fig. 3) (59). In contrast, Ser residues within the PTB domain of IRS-1, located within consensus PKB phosphorylation sites, presumably function as positive effectors of insulin signaling (76). Once phosphorylated by PKB $\alpha$ , they serve to protect IRS proteins from the rapid action of protein Tyr phosphatases. In such a way, PKB $\alpha$  acts to propagate and accelerate insulin signaling by phosphorylating downstream effectors and by phosphorylating IRS proteins, thus generating a positive feedback loop for insulin action. Both Ser/Thr kinases that phosphorylate IRS-1, the PKB-positive regulator and the wortmannin-sensitive negative regulator, are downstream effectors of PI3-K. This suggests that their action should be orchestrated in a way that enables sustained activation of IRS-1, as a result of phosphorylation by PKB, before

the activation of the negative regulator whose action is expected to terminate insulin signal transduction.

Other aspects of insulin signaling are also subjected to homologous desensitization. Chronic stimulation with insulin, similar to that seen in hyperinsulinemic type 2 diabetic patients, not only down-regulates the IR number, but also results in persistent phosphorylation of mSOS, which keeps it dissociated from Grb2 and allows Ras to return to its GDP-bound inactive phase (106). This result is apparently mediated by the MEK and MAP kinase that phosphorylate mSOS (107,108). In contrast, intermittent insulin stimulation does not induce such desensitization (109). Hence, the two major insulin-signaling pathways, those mediated by IRS proteins and those mediated by Shc, are subjected to homologous desensitization in the form of insulin-induced Ser/Thr phosphorylation. This conclusion steers us in the direction of potential pharmacological interventions in disease states in which this mechanism can be the underlying cause of insulin resistance.

**CONCLUSIONS**— Our understanding of insulin signal transduction pathways and insulin resistance has evolved extremely rapidly over the past few years. Still, further studies are required to unravel the mechanisms controlling these intricate regulatory processes that presumably mediate, at least in part, the insulin resistance associated with obesity and hyperinsulinemia. These new emerging paradigms and target substrates should facilitate the development of compounds that will activate or inhibit various signaling elements in these cascades, thereby renewing our hope for new families of therapeutic agents to treat obesity and type 2 diabetes. Examples of such targeted agents include a recently described insulin-receptor sensitizer acting on the kinase domain (110), a newly described and future PPAR $\gamma$  agonists, and drugs that inhibit the phosphatases, such as PTP1B.

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