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IRS proteins and the common path to diabetes

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White, Morris F. IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab 283: E413–E422, 2002; 10.1152/ajpendo.00514.2001.—Although a full understanding of insulin/insulin-like growth factor (IGF) action is evolving, the discovery of insulin receptor substrate (IRS) proteins and their role to link cell surface receptors to the intracellular signaling cascades provided an important step forward. Moreover, Insulin/IGF receptors use common signaling pathways to accomplish many tasks, the IRS proteins add a unique layer of specificity and control. Importantly, the IRS-2 branch of the insulin/IGF-signaling pathway is a common element in peripheral insulin response and pancreatic β-cell growth and function. Failure of IRS-2 signaling might explain the eventual loss of compensatory hyperinsulinemia during prolonged periods of peripheral insulin resistance. Moreover, short-term inhibition of IRS protein functions by serine phosphorylation, or sustained inhibition by ubiquitin-targeted proteosome-mediated degradation suggests a common molecular mechanism for insulin resistance during acute injury or infection, or the sensitivity of β-cells to autoimmune destruction. The broad role of IRS-1 and IRS-2 in cell growth and survival reveals a common regulatory pathway linking development, somatic growth, fertility, neuronal proliferation, and aging to the core mechanisms used by vertebrates for nutrient sensing.

insulin receptor substrate
Type 1 diabetes is also poorly defined at the molecular level, because the disease develops slowly and culminates in a characteristic autoimmune destruction of the pancreatic β-cells. Many genetic loci are associated with type 1 diabetes, but two chromosomal regions consistently emerge: the HLA region at 6p21.3, which probably sets up the immune component, and a variable number of tandem repeats (VNTR) markers located 596 bp upstream of the start site of transcription for the INS gene on chromosome 11p15, which is associated with diminished expression of insulin and the adjacent IGFII gene (25, 64). Whereas the genetics of type 1 and type 2 diabetes are complex, maturity onset diabetes of youth (MODY) is linked to mutations in single genes that impair β-cell function, including hepatocyte nuclear factor (HNF)-4α (MODY 4), HNF-1α (MODY 3), Pdx1 (MODY 4), or HNF-1β (MODY 5) (32, 35, 36).

Our approach to understanding diabetes has been based on the hypothesis that common signaling pathways might mediate both peripheral insulin action and pancreatic β-cell function. When elements of these pathways fail, owing to a combination of genetic variation and epigenetic challenge, diabetes might ensue. Evidence supporting this hypothesis has emerged from our work on the insulin receptor substrates (IRS proteins). Disruption of the gene for the IRS-2 protein Irs2 in mice causes diabetes, because peripheral insulin resistance and dysregulated hepatic gluconeogenesis are exacerbated by pancreatic β-cell failure (91). Although all the experimental evidence is not yet available, failure of components that are regulated by the IRS-2 branch of the insulin/IGF-signaling pathway might be an important cause of diabetes.

**INSULIN/IGF SIGNALING**

The insulin and IGF-I receptors, like the receptors for other growth factors and cytokines, are composed of an extracellular ligand-binding domain that controls the activity of an intracellular tyrosine kinase (29, 85). The IGFIR is activated by either IGF-I or IGF-II, whereas the type b insulin receptor that predominates after birth is activated mainly by insulin (Fig. 1). However, during fetal development, the type a insulin receptor predominates, which is activated by either insulin or IGF-II (34). Dysregulation of insulin receptor gene splicing alters fetal growth patterns and contributes to insulin resistance in adults (34, 75).

During ligand binding, insulin/IGF-I receptors become tyrosine phosphorylated through an autophosphorylation reaction, which is an essential step in the activation cascade (89). Cellular scaffold proteins bind to the autophosphorylation sites and are phosphorylated on multiple tyrosine residues by the activated receptor kinase (61). Most intracellular signals are generated through signaling complexes that are assembled around the tyrosine-phosphorylated scaffold proteins, including the IRS proteins, but also around SHC, APS and SH2B, and GAB1/2, DOCK1/2 and CBL (11, 21, 52, 57, 62, 66, 95). Although the roles of each of these substrates merit attention, recent work with transgenic mice suggests that many insulin responses, especially those that are associated with somatic growth and carbohydrate metabolism, are largely me-
mediated through two IRS proteins, called IRS-1 and IRS-2 (Fig. 1).

IRS proteins lack intrinsic catalytic activities but are composed of multiple interaction domains and phosphorylation motifs. At least three IRS proteins occur in humans and mice, including IRS-1/Irs-1 and IRS-2/Irs-2, which are widely expressed, and IRS-4/Irs-4, which is limited to the thymus, brain, and kidney and possibly β-cells (84). Rodents also express Irs-3, which is largely restricted to adipose tissue and displays activity similar to Irs-1; however, this short ortholog might not occur in humans (70). Phylogenetic analysis reveals a close evolutionary relation between IRS-1/Irs-1 and IRS-2/Irs-2 from humans and mice, which might have diverged from IRS-4/Irs-4 (Fig. 2). The Drosophila IRS protein, called Chico, is weakly related to its mammalian orthologs, as it contains few COOH-terminal tyrosine phosphorylation sites (Fig. 2). Finally, analysis of the human genome sequence reveals at least two putative IRS proteins recognized by adjacent pleckstrin homology (PH) and phosphotyrosine-binding (PTB) domains; however, they contain very short COOH tails with a few tyrosine phosphorylation sites, so their function remains unknown (Fig. 2).

All IRS proteins are characterized by the presence of an NH2-terminal PH domain adjacent to a PTB domain, followed by a variable-length COOH-terminal tail that contains numerous tyrosine and serine phosphorylation sites. The PH and PTB domains mediate specific interactions with the insulin and IGF-I receptor kinases (18, 96). Other cytokine receptors that couple to Janus kinases also engage IRS proteins, including the receptors for growth hormone, interleukin (IL)-4, -9, -13, and -15, and the integrin αvβ3 (95). The PTB domain binds to phosphorylated NPXY motifs in the receptors for insulin, IGF-I, or IL-4; however, other receptors that promote IRS protein tyrosine phosphorylation do not contain NPXY motifs (92). In contrast, the mechanism of PH domain coupling is not known, because physiologically relevant binding partners are undefined; PH-domain binding partners might include phospholipids, acidic peptides, or specific proteins such as PHIP (19, 33).

The COOH-terminal end of each IRS protein contains a set of tyrosine phosphorylation sites that act as on/off switches to recruit and regulate various downstream signaling proteins. IRS-1 and IRS-2 have the longest tails, which contain 20 potential tyrosine phosphorylation sites; however, only a handful have been formally identified. On the basis of primary amino acid sequences, Irs-3 and IRS-4 contain fewer potential sites (Fig. 2). Many of the tyrosine residues cluster into common motifs that bind and possibly activate specific effector proteins, including enzymes [phosphatidylinositol (PI) 3-kinase; the phosphotyrosine phosphatase SHP-2; and the Src-like kinase Fyn] or adapter molecules (GRB-2, NCK, CRK, SHB, and others) (Fig. 2).

**IRS PROTEIN-REGULATED SIGNALING PATHWAYS**

Although we have studied the function of IRS proteins for many years, we understand only the obvious features of these signaling scaffolds. The IRS proteins contribute unique specificity owing to unique regula-
tion and location (74); however, a molecular basis for the subcellular localization and regulation of the IRS protein homologs has so far escaped explanation (46). IRS proteins couple insulin/IGF receptors to the PI 3-kinase and extracellular signal-regulated kinase (ERK) cascades (Fig. 3). Activation of the PI 3-kinase cascade is an important insulin/IGF-regulated pathway. PI 3-kinase is a dimer composed of a 110-kDa catalytic subunit that is associated noncovalently to a 55- or 85-kDa regulatory subunit. PI 3-kinase is activated when the phosphorylated YMXM motifs in IRS proteins occupy both src homology-2 (Sh2) domains in the regulatory subunit (7). Products of PI 3-kinase, including phosphatidylinositol-3,4-bisphosphate and phosphatidylinositol-3,4,5-trisphosphate, attract serine kinases to the plasma membrane, including the phosphoinositide-dependent kinase (PDK1 and PDK2) and at least three protein kinase B (PKB) isoforms (Fig. 3). During co-localization at the plasma membrane, PDK1 or PDK2 phosphorylates and activates PKB1, -2, or -3. The activated protein kinase B (PKB or Akt) phosphorylates many substrates to control various biological signaling cascades, including glucose transport, protein synthesis, glycogen synthesis, cell proliferation, and cell survival, in various cells and tissues (Fig. 3) (4, 14, 95).

IRS proteins regulate gene transcription through at least two pathways, including the PKB-mediated forkhead transcription factors, and the ras/ERK/Rsk-regulated factors Elk and fos (Fig. 3). The forkhead transcription factors play a central role in the regulation of metabolic enzymes, whereas the ERK/Rsk-regulated factors appear to control growth (51); however, overlap and cross talk between the regulated gene products is expected. Gene regulation by ERK and PKB generally works in opposite directions, because phosphorylation of forkhead transcription factors inhibits its activity, whereas phosphorylation of Elk and fos promotes transcriptional activity. Three forkhead orthologs, AFX, FKHR, and FKHR1, are located in the nucleus under basal conditions, where they bind to the consensus sequence T(G/A)TTT(T/G)(G/T). This element occurs in several genes that are known to be active in the absence of insulin and inhibited by insulin, including phosphoenolpyruvate carboxykinase, IGF-binding protein-1, tyrosine aminotransferase, and the glucose-6-phosphatase catalytic subunit (63). Presumably, these genes are inhibited when AFX/FKHR/FKHL1 is ex-
INVESTIGATION OF THE ROLE OF IRS PROTEINS IN INSULIN RESISTANCE

Insulin resistance is a serious medical problem that leads to type 2 diabetes when pancreatic β-cells fail to compensate by increasing the amount of secreted insulin (26). At the physiological level, obesity, inactivity, and aging are common causes of insulin resistance. Although moderate compensatory hyperinsulinemia might be well tolerated in the short term, chronic hyperinsulinemia exacerbates insulin resistance and contributes directly to β-cell failure and diabetes (26, 68, 77). Importantly, the β-cell failure probably does not arise from overwork but rather from dysregulated growth and survival signals that accompany insulin-resistant states.

The insulin-signaling system is complex, and a common mechanism explaining the occurrence of acute and chronic insulin resistance in humans is difficult to identify. Recent experiments with transgenic mice teach us that dysregulation at many steps in the signaling cascade, including regulatory interactions, might lead to insulin resistance. However, only a few of these steps can be considered to be specific to the insulin- or IGF-signaling pathways, as most elements are shared with other systems. For example, mutations in the insulin receptor are an obvious source of lifelong insulin resistance, but these are rare and usually not accompanied by β-cell failure (20, 24, 40, 88). In contrast, elevated activity of protein or lipid phosphatases, including PTP1B, SHIP2, or PTEN, might be a clinically relevant cause of insulin resistance. Inhibition of these phosphatases by gene knockout or by chemical inhibitors increases glucose tolerance, suggesting that specific phosphatase inhibitors might be useful treatments for diabetes (22, 30, 47). However, modulation of the activity of shared signaling proteins might result in undesirable phenotypes, including hyperactivation of parallel receptor signals by phosphatase inhibitors. Other drug targets, including Akt or p70S6k, are difficult to work with because they require activation. Moreover, coordination with the IRS proteins might be essential to ensure specificity.

Although the molecular mechanisms that cause insulin resistance in humans are largely unknown, some common themes involving a role for the IRS proteins are emerging. Various cytokines or metabolites promote serine phosphorylation of the IRS proteins that inhibit signal transduction. For example, circulating free fatty acids, diacylglycerol, fatty acyl-CoAs, glucose, or ceramides promote serine phosphorylation of Irs-1/Irs-2 (77). Adipose-derived cytokines, especially tumor necrosis factor (TNF)-α, stimulate serine/threonine phosphorylation of Irs-1/Irs-2, which inhibits signaling; disruption of the TNF receptor (44, 45, 67) reduces this phosphorylation and at least partially restores insulin sensitivity and glucose tolerance (86, 87). Other adipose-derived proteins also influence insulin action and Irs-protein tyrosine phosphorylation, including inhibition by resistin or the release from inhibition by ACRP30 (79). The mechanisms involved in these effects might provide important new strategies for treatment of diabetes (36).

The idea that inflammation is associated with insulin resistance has been known for a long time (9) and is consistent with the finding that stress-induced cytokines like TNF-α cause insulin resistance. The signaling cascades regulated by TNF-α are complex and involve many branch points, including the activation of various serine kinases and transcription factors that promote apoptosis or proliferation (10). Recently, high doses of salicylates were shown to reverse hyperglycemia, hyperinsulinemia, and dyslipidemia in obese rodents by sensitizing the insulin-signaling pathway, including Irs protein tyrosine phosphorylation (37, 97). The effect of salicylates was attributed to inhibition of IκB kinase-β (IKKβ), especially as heterozygous disruption of IκKβ protected against the development of insulin resistance during high-fat feeding and in obese, leptin-deficient (ob/ob) mice (37, 97). Although there is no physical interaction between Irs proteins and IκKβ, salicylates increased insulin-stimulated phosphorylation of Irs proteins in the liver, suggesting that IκKβ might inhibit insulin receptor function or its coupling to the substrates (49).

A second branch of the TNF-α-signaling pathway involves activation of the c-Jun NH2-terminal kinase (JNK) (53, 73, 98). JNK is a prototype stress-induced kinase that is stimulated by many agonists during acute or chronic inflammation. JNK phosphorylates numerous cellular proteins, including IRS-1 and IRS-2, Shc, and Gab1 (2). A role for JNK during insulin action is compelling, as both IRS-1 and IRS-2 contain JNK-binding motifs. This motif mediates the specific association of JNK with IRS-1, which promotes phosphorylation of a specific serine residue that is located on the COOH-terminal side of the PTB domain [Ser307 in (murine) Irs-1; Ser312 in (human) IRS-1]. Phosphorylation of this residue inhibits the function of the PTB domain, which disrupts the association between the insulin receptor and IRS-1 and inhibits tyrosine phosphorylation (2). This mechanism might explain, at least in part, the insulin resistance that occurs during trauma and obesity (Fig. 4).

Whereas serine phosphorylation is usually considered a short-term mechanism, regulated degradation of IRS proteins might also promote long-term insulin resistance. Prolonged insulin stimulation substantially reduces Irs-1 and Irs-2 protein levels in multiple cell lines, which is blocked by specific inhibitors of the 26S proteasome (80). These results suggest that proteasome-mediated degradation of Irs2, rather than inhibition of transcription and/or translation of Irs2, determines protein levels and activity of Irs-2-mediated signaling pathways (74). Consistent with this idea,
insulin stimulates ubiquitination of Irs-2. Reduction of Irs-2 by ubiquitin/proteasome-mediated proteolysis in mouse embryo fibroblasts lacking Irs-1 dramatically inhibits the activation of Akt and ERK1/2 in response to insulin/IGF-I; strikingly, proteasome inhibitors completely reverse this inhibition. The activity of the ubiquitin/proteasome system is elevated in diabetes, which might promote degradation of the Irs proteins and exacerbate insulin resistance (59, 60).

ROLE OF IRS PROTEINS IN GROWTH AND SURVIVAL

The insulin/IGF-signaling system plays a central role in somatic growth. In particular, disruption of the Igf-I receptor in people and mice diminishes fetal and postnatal growth significantly. The Irs-1 branch of the pathways plays a significant role to mediate the effects of IGF-I/Igf-I on growth. Deletion of the Irs1 gene in mice reduces embryonic and neonatal growth 40%, whereas deletion of Irs2 barely reduces prenatal and early postnatal growth by 10% (90). Growth is reduced 40% in Irs2−/− mice that are also haploinsufficient for Irs1, whereas growth is reduced 70% in Irs1−/− mice also haploinsufficient for Irs2 (90). Thus Irs2 cannot fully replace Irs1 in this process, confirming the hypothesis that the signaling pathways mediated by Irs1 and Irs2 overlap incompletely. An explanation for the incomplete overlap of function is not immediately clear, but a full understanding of these pathways has certain physiological significance.

Regulation of invertebrate growth and longevity by the insulin/IGF-signaling system was first observed in Caenorhabditis elegans, as partial inhibition of insulin/IGF signaling increases nematode life span (83); insulin/IGF signaling also coordinates longevity in Drosophila. Unlike vertebrates, these organisms contain a single insulin/IGF receptor gene and many developmentally regulated insulin-like genes (93). Nevertheless, the PI 3-kinase → PKB cascade is intact in C. elegans and Drosophila; however, IRS proteins have not been identified in C. elegans, whereas Drosophila expresses a single IRS protein ortholog, called Chico (12). Chico is essential in the control of cell size and growth, as homozygous deletion of Chico extends median life span up to 48% (12). It is difficult to understand how insulin resistance might promote longevity of mammals, because the detrimental effects seem to promote systemic degeneration. However, chronic hyperinsulinemia to compensate for glucose intolerance might differentially stimulate intact IRS-1 and the IRS-2 signals in unaffected tissues and cells, resulting in free radical generation and accelerated aging (31).

IRS-2 AND β-CELL FUNCTION: COMMON PATHWAY TO DIABETES

Peripheral insulin resistance is a well known component of type 2 diabetes, but it is clearly not enough, as clinical experience and many transgenic mice reveal. However, if peripheral insulin resistance directly impairs the capacity of the pancreatic β-cells to compensate, a compelling molecular link to diabetes might emerge. Failure of the IRS-2 branch of insulin/IGF signaling reveals this common pathway to diabetes. Not only do Irs2−/− mice develop peripheral insulin resistance, they also eventually fail to sustain compensatory insulin secretion. The convergence of peripheral and islet defects around the Irs-2 branch of the insulin/IGF-signaling pathway reveals the common pathway to diabetes.

In mice, Irs1 and Irs2 contribute to the peripheral insulin response, as both Irs1−/− and Irs2−/− mice are markedly insulin resistant; there is no reason to suspect different roles in humans (5, 48, 91). Irs1 exerts its greatest effect on metabolism by regulating insulin signals in muscle and adipose tissue, whereas it plays a lesser role in mediating insulin’s effects on the liver metabolism (15, 54, 65, 81, 91, 94). Irs1 might also regulate vascular tone, as Irs1−/− mice are slightly hypertensive (1). In contrast, Irs2−/− mice display dysregulated lipolysis, peripheral glucose uptake, and hepatic gluconeogenesis (71).

Diabetes occurs in the Irs2−/− mice but not in Irs1−/− mice because of the differential role of the Irs proteins in pancreatic islets. Mice lacking Irs1 sustain lifelong compensatory hyperinsulinemia, in part because the β-cell mass increases as the mice age (81, 90). Although Irs2−/− mice are transiently hyperinsulinemic, by 10 wk of age (~25 wk for females), the male Irs2−/− mice develop diabetes, and examination of the islet size in these mice invariably reveals decreased β-cell mass. Moreover, insulin immunostaining shows that insulin content in Irs2−/− islets is reduced compared with wild-type or Irs1−/− tissues (90). Moreover, the expressions of several gene products that promote β-cell function, including normal glucose detection, are reduced.
The unique role played by Irs2 in β-cells is dramatically highlighted by the rare progeny of Irs1<sup>−/−</sup> and Irs2<sup>+/−</sup> crosses that retain one allele of Irs2 but no Irs1 (Irs1<sup>−/−</sup> Irs2<sup>+/−</sup>). The Irs1<sup>−/−</sup> Irs2<sup>+/−</sup> mice are extremely small but generally glucose tolerant because they maintain functional β-cells (90). By comparison, Irs1<sup>+/−</sup> Irs2<sup>−/−</sup> mice are only 50% smaller, glucose intolerant, and die at 30 days of age, without any detectable β-cells. Thus Irs2 is essential for β-cell growth and function.

Although all of the experiments are not completed, current results point to an important role for the IgfIR<sup>3</sup>Irs-2-signaling pathway for β-cell function (90). Igf-I receptor allelic insufficiency reduces the life span of the Irs2<sup>−/−</sup> mice to only 30 days, owing to the near absence of pancreatic β-cells and extreme hyperglycemia. In contrast, β-cells appear to develop normally without an insulin receptor, although mild glucose intolerance develops owing to reduced first-phase insulin secretion (6, 54). These results suggest provisionally that the Igf-I → Irs-2-signaling pathway might be critical for both the embryonic development and postnatal growth of β-cells and reveals an important interface between the insulin and Igf-signaling pathways.

Downstream of Irs-2, β-cell function is significantly diminished. Activation of Akt by phospholipid products of the PI 3-kinase plays a clear role, at least partially through phosphorylation of a forkhead transcription factor (50); Irs-2 is the likely upstream element in this cascade. Moreover through these elements, the Irs-2 branch of the insulin/Igf-signaling system might be connected to MODY-related transcription factors. Recent work suggests that HNFs and Pdx1 are reduced in Irs2<sup>−/−</sup> mice but are normal in Irs1<sup>−/−</sup> mice (55). Pdx1 is especially important, because it regulates components of the glucose-sensing pathway (3, 41). Genetic mutations in Pdx1 are associated with a form of MODY. Pathological processes that reduce Pdx1 expression cause glucose intolerance, which might lead to diabetes (82). Pdx1 expression and function might be linked to Irs-2 through the forkhead transcription factor Foxo1 (50). Thus regulation of Pdx1 levels through Irs-2 provides a plausible mechanism for the role of insulin resistance in diabetes.

The IRS-2-signaling pathway might also play a role in the pathophysiology of type 1 diabetes. Inflammatory cytokines like TNF-α, IL-1β, and FAS-ligand are well known antagonists of β-cell function, and their ability to inhibit IRS-2 signaling might provide a basis to understand, at least in part, β-cell dysfunction that emerges in type 1 diabetes as well. Moreover, the possibility that IGF-II gene expression is diminished in type 1 diabetes provides a potential explanation for the reduced IgfIR → Irs-2 signaling that might place β-cells at risk. Whether the IRS-2 branch of the insulin/IGF-signaling pathway is a master regulator of β-cell function that fails in both type 1 and type 2 diabetes is a hypothesis that deserves rigorous attention.

**SUMMARY AND PERSPECTIVE**

During the last few years, work with transgenic mice has revealed the broad role played by IRS proteins in mammalian physiology (Fig. 5). At the center of this scheme, IRS-2 is important for IGF receptor-mediated growth and function of pancreatic β-cells. This relation creates a precarious link between tissues that respond to insulin and the pancreatic cells that sense blood glucose.
glucose levels and secrete insulin. Certainly, many other downstream elements are also in common, but IRS-2 seems to play a pivotal role in determining the specificity of the relevant signaling cascades. Future work will establish the extent of its role and its value for therapeutic intervention. IRS-2 also plays an important role in reproduction, as it promotes female fertility owing to its role in the hypothalamic-pituitary-ovarian axis. This might explain the association between certain aspects of polycystic ovarian syndrome and insulin resistance. In addition, IRS-2 signaling, rather than IRS-1 signaling, promotes proliferation of central neurons during development and might play a role in brain longevity (M. Schubert and M. F. White, unpublished observations). Therefore, understanding the IRS-2 branch of the insulin/IGF signaling pathway might provide an avenue for intervention into neuro-degenerative disorders.

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