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invited review

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

THE STORAGE AND RELEASE OF ENERGY during feeding and fasting and a large portion of somatic growth are regulated by the insulin/insulin-like growth factor (IGF)-signaling system. Insulin is best known for its role in the regulation of blood glucose, as it suppresses hepatic gluconeogenesis and promotes glycogen synthesis and storage in liver and muscle, triglyceride synthesis in liver and storage in adipose tissue, and amino acid storage in muscle (27). However, the insulin-signaling system has a broader role in mammalian physiology because it is shared with the IGF-I receptor (IGFIR). During development, the insulin/IGF-signaling system promotes somatic growth (8, 56). After birth, it promotes growth and survival of many tissues, including pancreatic β-cells, bone, neurons, and retina, to name a few (28, 42, 58, 69, 91). Except for insulin, which can be replaced by injection as a treatment for diabetes, the complete dysfunction of essential components in the insulin/IGF-signaling system is rare and invariably lethal. In contrast, partial failure of the insulin/IGF-signaling system is associated with many metabolic disorders, including dyslipidemia, hypertension, female infertility, and glucose intolerance that might progress to type 2 diabetes (72).

Diabetes is an epidemic disorder that arises when insulin secretion from pancreatic β-cells fails to maintain blood glucose levels in the normal range, especially when exacerbated by peripheral insulin resistance. The underlying pathophysiology of diabetes is diverse, but pancreatic β-cell failure is the common theme (38). Type 2 diabetes is the most common form, which arises when pancreatic β-cell insulin secretion fails to compensate for peripheral insulin resistance (26). Work over the past decade suggests that type 2 diabetes begins with skeletal muscle insulin resistance (23); however, peripheral insulin resistance might not be enough, as transgenic mice lacking muscle insulin receptors or patients with muscle insulin resistance owing to defective mRNA splicing do not develop dia-

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betes (15, 75). Despite incontrovertible evidence of genetic links for type 2 diabetes, the genes responsible have been difficult to identify, because diabetes is not a Mendelian disorder (17). Consequently, linkage analysis with well defined populations has made slow progress, although a possible role for the serine protease CAPN10 was recently revealed (43, 78).

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 (*MODY1*), glucokinase (*MODY2*), HNF-1α (*MODY 3*), Pdx1 (*MODY4*), 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-

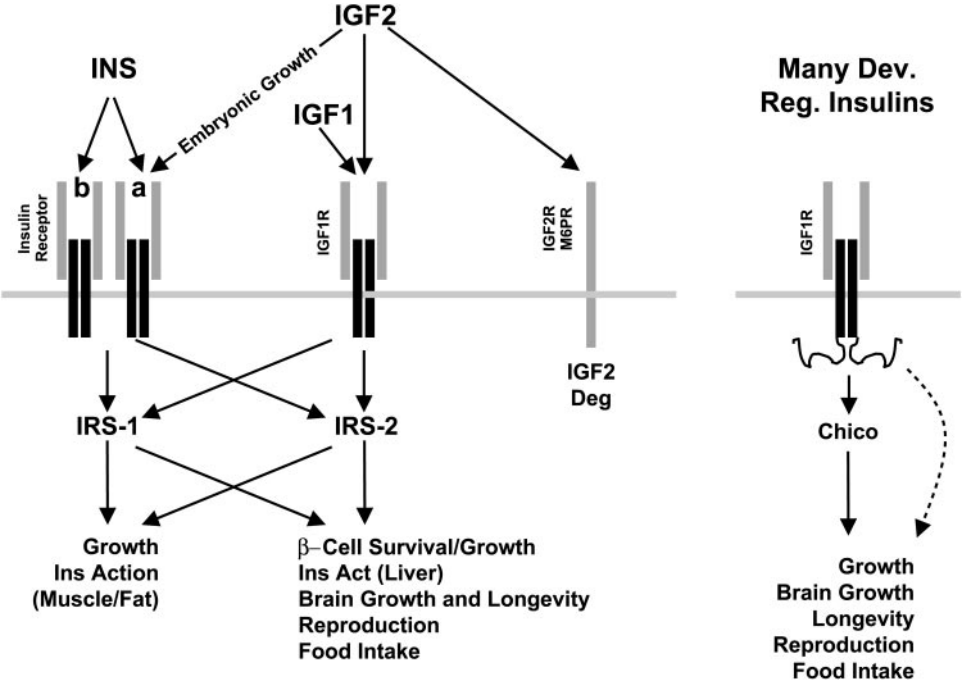

Fig. 1. Diagram summarizing some of the physiological responses regulated by the insulin/insulin-like growth factor (IGF)-signaling pathway. Insulin (INS), IGF-I, and IGF-II bind to the insulin receptors, IGF-I receptors, and IGF-II/mannose 6-phosphate (M6P) receptors, as illustrated. The type a and b isoforms of the insulin receptor are produced by alternative splicing: type a predominates during development, and type b predominates in adults. In vertebrates, insulin receptor substrate (IRS)-1 and IRS-2 function as scaffold proteins to coordinate separate branches of the insulin/IGF-signaling cascades. Transgenic mouse experiments reveal connections between these signaling branches and various physiological responses. Invertebrates, like *Drosophila*, have a single IGF receptor that engages 1 IRS protein, called Chico; however, invertebrates express several insulin-like genes controlled by developmental cues.

diated 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 NH_2-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 $\alpha_v\beta_3$ (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-

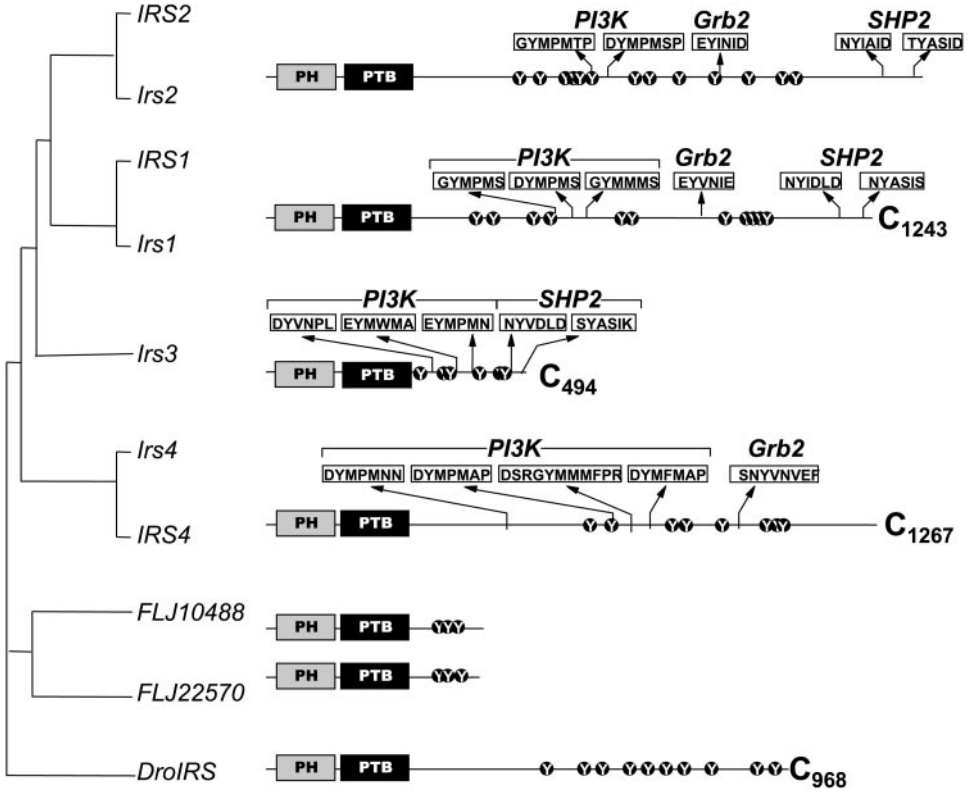

Fig. 2. ClustalW alignment of human (upper-case letters), mouse (mixed case), and *Drosophila* (Chico) IRS proteins from insulin/IGF-signaling cascades. The relative positions of the pleckstrin homology (PH) and phosphotyrosine-binding (PTB) domains are indicated. Potential tyrosine phosphorylation sites are indicated by Y, and known phosphorylation motifs are enclosed in boxes below potential binding partners, including phosphatidylinositol (PI) 3-kinase (PI3K), Grb-2, and SHP-2.

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 FKHRL1, 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 phospho*enol*pyruvate carboxykinase, IGF-binding protein-1, tyrosine aminotransferase, and the glucose-6-phosphatase catalytic subunit (63). Presumably, these genes are inhibited when AFX/FKHR/FKHRL1 is ex-

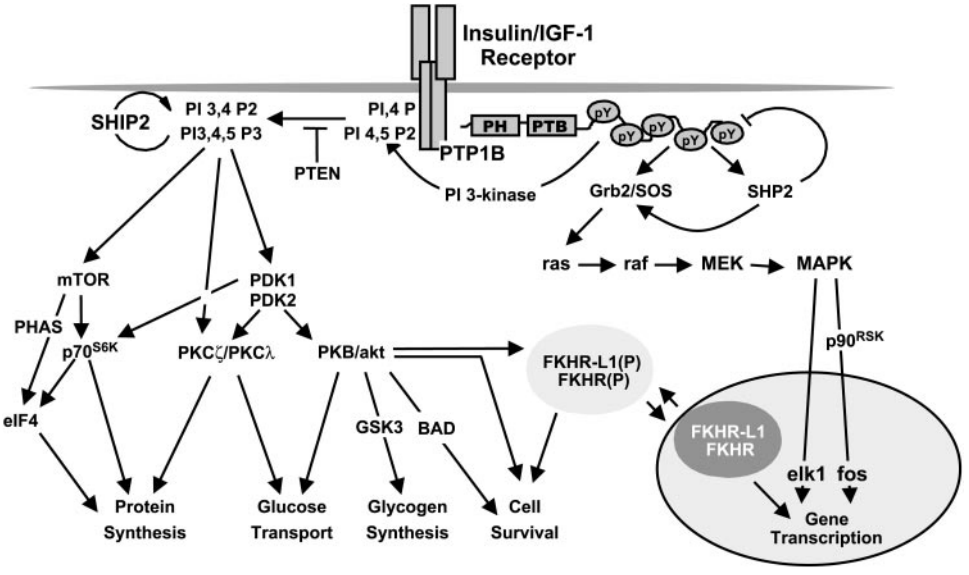

Fig. 3. IRS protein-dependent insulin/IGF-I-signaling cascade. Activation of the receptors for insulin and IGF-I results in tyrosine phosphorylation of the IRS proteins. The IRS proteins thereby bind PI 3-kinase, Grb2/son of sevenless (SOS), and SHP-2. The Grb2/SOS complex mediates the activation of p21ras, thereby activating the ras/raf/mitogen-activated protein (MAP) kinase kinase (MEK)/MAP kinase cascade. SHP-2 feeds back to inhibit IRS protein phosphorylation by directly dephosphorylating the IRS protein and may transmit an independent signal to activate MAP kinase. The activated MAP kinase phosphorylates $p90^{RSK}$, which itself phosphorylates c-fos, increasing its transcriptional activity. MAP kinase also phosphorylates Elk1, increasing its transcriptional activity. The activation of PI 3-kinase by IRS protein recruitment results in the generation of PI-3,4-diphosphate ($PI3,4P_2$) and PI-3,4,5-triphosphate ($PI3,4,5P_3$) (antagonized by the action of PTEN). Insulin also activates the SH2 domain-containing inositol 5-phosphatase (SHIP2), which converts $PI3,4,5P_3$ to $PI3,4P_2$. In aggregate, $PI3,4P_2$ and $PI3,4,5P_3$ activate a variety of downstream signaling kinases, including the mammalian target of rapamycin (mTOR), which regulates protein synthesis via PHAS/p70 S6 kinase ($p70^{S6k}$)/eukaryotic initiation factor 4 (eIF4). These lipids also activate alternate protein kinase C (PKC) isoforms and phosphoinositide-dependent kinase (PDK) isoforms. The PDKs (PDK1, PDK2) activate protein kinase B (PKB), which appears to mediate glucose transport in concert with the atypical PKC isoforms. PKB also regulates glycogen synthase kinase 3 (GSK-3), which may regulate glycogen synthesis, and a variety of regulators of cell survival. PKB-mediated phosphorylation of the proapoptotic protein BAD inhibits apoptosis, and phosphorylation of the forkhead proteins results in their sequestration in the cytoplasm, in effect inhibiting their transcriptional activity.

cluded from the nucleus by PKB-stimulated phosphorylation; however, evidence suggests that the mechanisms might be more complicated, especially when the regulatory factors are expressed at endogenous levels (39). Moreover, IRS proteins might provide specificity to these common regulatory pathways, resulting in differential gene regulation.

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 $p70^{S6k}$, 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 IKKβ 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 IKKβ, salicylates increased insulin-stimulated phosphorylation of Irs proteins in the liver, suggesting that IKKβ 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 NH_2-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 [Ser^{307} in (murine) Irs-1; Ser^{312} 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,

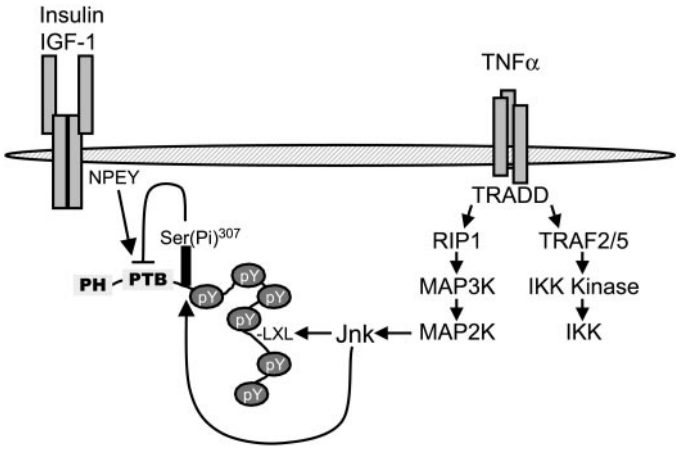

Fig. 4. Schematic mechanism of tumor necrosis factor-α (TNF-α)-induced inhibition of IRS protein signaling. FADD, Fas-associated death domain protein; IKK, IκB kinase; JNK, c-Jun NH_2-terminal kinase; RIP1, receptor-interacting protein 1; TNFR1, TNF receptor type 1; TRAF2, TNF-receptor-associated factor 2. TNF-α binding to TNFR1 results in recruitment of TRAF2/5, RIP1, and FADD through the adaptor protein TRADD. TRAF2/5 and RIP1 appear to lead to activation of the protein kinases JNK and IKK. Activated JNK associates with IRS-1 and the JNK-binding LXL motif and promotes phosphorylation of Ser^{307}. Phosphorylation of Ser^{307} inhibits PTB domain function and inhibits insulin/IGF-stimulated tyrosine phosphorylation and signal transduction.

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 $Irs1^{+/-}$ and $Irs2^{+/-}$ crosses that retain one allele of *Irs2* but no *Irs1* ($Irs1^{-/-}$ $Irs2^{+/-}$). The $Irs1^{-/-}$ $Irs2^{+/-}$ mice are extremely small but generally glucose tolerant because they maintain functional β-cells (90). By comparison, $Irs1^{+/-}$ $Irs2^{-/-}$ 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 → Irs-2-signaling pathway for β-cell function (90). Igf-I receptor allelic insufficiency reduces the life span of the $Irs2^{-/-}$ 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 $Irs2^{-/-}$ mice but are normal in $Irs1^{-/-}$ 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

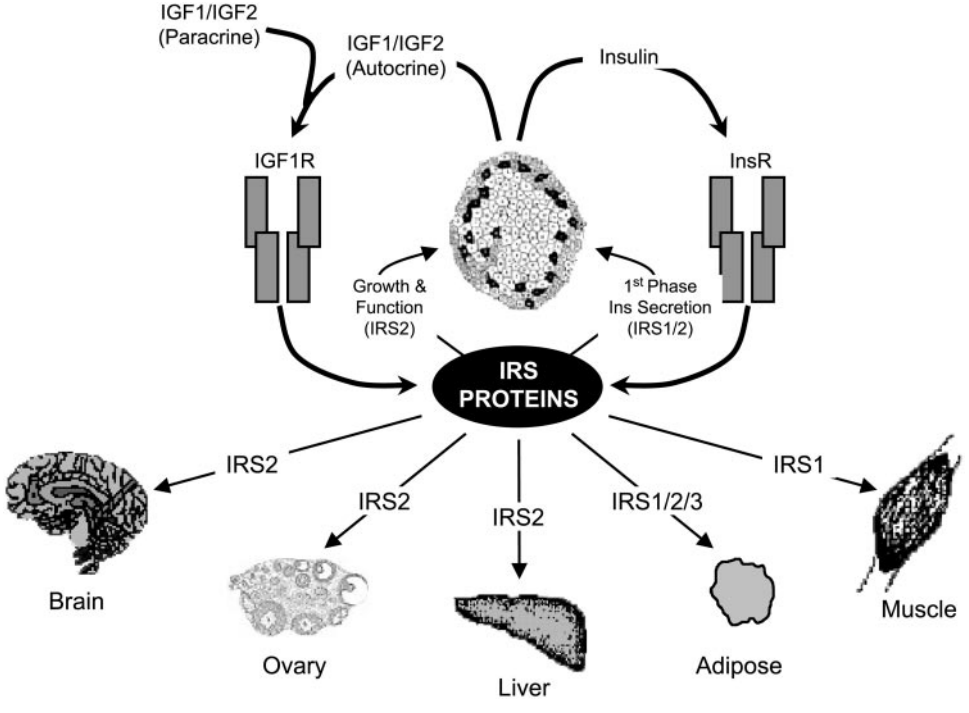

Fig. 5. Schematic diagram summarizing the integration and regulation of energy-consuming processes with nutrient homeostasis through the IRS-1 and IRS-2 branches of the insulin/IGF-signaling system.

glucose levels and secrete insulin. Certainly, many other downstream elements are also in common, but IRS-2 seems to play a pivotal role is 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 neurodegenerative disorders.

REFERENCES

1. **Abe H, Yamada N, Kamata K, Kuwaki T, Shimada M, Osuga J, Shionoiri F, Yahagi N, Kadowaki T, Tamemoto H, Ishibashi S, Yazaki Y, and Makuuchi M.** Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. *J Clin Invest* 101: 1784–1788, 1998.
2. **Aguirre V, Uchida T, Yenush L, Davis RJ, and White MF.** The c-Jun NH_2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser307. *J Biol Chem* 275: 9047–9054, 2000.
3. **Ahlgren U, Jonsson J, Jonsson L, Simu K, and Edlund H.** Beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev* 12: 1763–1768, 1998.
4. **Alessi DR and Cohen P.** Mechanism of activation and function of protein kinase B. *Curr Opin Genet Dev* 8: 55–62, 1998.
5. **Araki E, Lipes MA, Patti ME, Bruning JC, Haag B III, Johnson RS, and Kahn CR.** Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 372: 186–190, 1994.
6. **Aspinwall CA, Qian WJ, Roper M, Kulkarni RN, Kahn CR, and Kennedy RT.** Roles of insulin receptor substrate-1, phosphatidylinositol 3-kinase, and release of intracellular Ca^{2+} stores in insulin-stimulated insulin secretion in β-cells. *J Biol Chem* 275: 22331–22338, 2000.
7. **Backer JM, Myers MG Jr, Shoelson SE, Chin DJ, Sun XJ, Miralpeix M, Hu P, Margolis B, Skolnik EY, Schlessinger J, and White MF.** Phosphatidylinositol 3′-kinase is activated by association with IRS-1 during insulin stimulation. *EMBO J* 11: 3469–3479, 1992.
8. **Baker J, Liu JP, Robertson EJ, and Efstratiadis A.** Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 75: 73–82, 1993.
9. **Baron SH.** Salicylates as hypoglycemic agents. *Diabetes Care* 5: 64–71, 1982.
10. **Baud V and Karin M.** Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol* 11: 372–377, 2001.
11. **Baumann CA, Ribon V, Kanzaki M, Thurmond DC, Mora S, Shigematsu S, Bickel PE, Pessin JE, and Saltiel AR.** CAP defines a second signalling pathway required for insulin-stimulated glucose transport. *Nature* 407: 202–207, 2000.
12. **Bohni R, Riesgo-Escovar J, Oldham S, Brogiolo W, Stocker H, Andruss BF, Beckingham K, and Hafen E.** Autonomous control of cell and organ size by CHICO, a drosophila homolog of vertegrate IRS1–4. *Cell* 97: 865–875, 1999.
14. **Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, and Greenberg ME.** Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. *Cell* 96: 857–868, 1999.
15. **Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, and Kahn CR.** A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 2: 559–569, 1998.
17. **Burghes AH, Vaessin HE, and de La Chapelle A.** Genetics. The land between Mendelian and multifactorial inheritance. *Science* 293: 2213–2214, 2001.
18. **Burks DJ, Pons S, Towery H, Smith-Hall J, Myers MG Jr, Yenush L, and White MF.** Heterologous PH domains do not mediate coupling of IRS-1 to the insulin receptor. *J Biol Chem* 272: 27716–27721, 1997.
19. **Burks DJ, Wang J, Towery H, Ishibashi O, Lowe D, Riedel H, and White MF.** IRS pleckstrin homology domains bind to acidic motifs in proteins. *J Biol Chem* 273: 31061–31067, 1998.
20. **Carboni JM, Yan N, Cox AD, Bustelo X, Graham SM, Lynch MJ, Weinmann R, Seizinger BR, Der CJ, Barbacid M, and Manne V.** Farnesyltransferase inhibitors are inhibitors of Ras but not R-Ras2/TC21 transformation. *Oncogene* 10: 1905–1913, 1995.
21. **Chiang SH, Baumann CA, Kanzaki M, Thurmond DC, Watson RT, Neudauer CL, Macara IG, Pessin JE, and Saltiel AR.** Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature* 410: 944–948, 2001.
22. **Clement S, Krause U, Desmedt F, Tanti J-F, Behrends J, Pesesse X, Sasaki T, Penninger J, Doherty M, Malaisse W, Dumont JE, Le Marchand-Brustel Y, Erneux C, Hue L, and Schurmans S.** The lipid phosphatase SHIP2 controls insulin sensitivity. *Nature* 409: 92–97, 2001.
23. **Cline GW, Rothman DL, Magnusson I, Katz LD, and Shulman GI.** ^{13}C-nuclear magnetic resonance spectroscopy studies of hepatic glucose metabolism in normal subjects and subjects with insulin-dependent diabetes mellitus. *J Clin Invest* 94: 2369–2376, 1994.
24. **Comb DG and Roseman S.** Glucosamine metabolism. IV. Glucosamine-6-phosphate deaminase. *J Biol Chem* 232: 807–827, 1958.
25. **Cox NJ, Wapelhorst B, Morrison VA, Johnson L, Pinchuk L, Spielman RS, Todd JA, and Concannon P.** Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am J Hum Genet* 69: 820–830, 2001.
26. **DeFronzo RA.** Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev* 5: 177–269, 1997.
27. **DeFronzo RA and Ferrannini E.** Regulation of intermediary metabolism during fasting and feeding. In: *Endocrinology*, edited by DeGroot LJ and Jameson JL. Philadelphia, PA: Saunders, 2001, p. 737–755.
28. **Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, and Greenberg ME.** Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* 275: 661–665, 1997.
29. **Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, Ou JH, Masiar F, Kan YW, Goldfine ID, Roth RA, and Rutter WJ.** The human insulin receptor cDNA: the structural basis for hormone activated transmembrane signalling. *Cell* 40: 747–758, 1985.
30. **Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, and Kennedy BP.** Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283: 1544–1548, 1999.
31. **Facchini FS, Hua NW, Reaven GM, and Stoohs RA.** Hyperinsulinemia: the missing link among oxidative stress and age-related diseases? *Free Radic Biol Med* 29: 1302–1306, 2000.
32. **Fajans SS, Bell GI, and Polonsky KS.** Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345: 971–980, 2001.
33. **Farhang-Fallah J, Yin X, Trentin G, Cheng AM, and Rozakis-Adcock M.** Cloning and characterization of PHIP, a novel

insulin receptor substrate-1 pleckstrin homology domain interacting protein. *J Biol Chem* 275: 40492–40497, 2000.
34. **Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, and Vigneri R.** Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol Cell Biol* 19: 3278–3288, 1999.
35. **Frayling TM, Evans JC, Bulman MP, Pearson E, Allen L, Owen K, Bingham C, Hannemann M, Shepherd M, Ellard S, and Hattersley AT.** Beta-cell genes and diabetes: molecular and clinical characterization of mutations in transcription factors. *Diabetes* 50, *Suppl* 1: S94–S100, 2001.
36. **Froguel P and Velho G.** Molecular genetics of maturity-onset diabetes of the young. *Trends Endocrinol Metab* 10: 142–146, 1999.
37. **Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, and Lodish HF.** Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 98: 2005–2010, 2001.
38. **Halban PA, Kahn SE, Lernmark A, and Rhodes CJ.** Gene and cell-replacement therapy in the treatment of type 1 diabetes: how high must the standards be set? *Diabetes* 50: 2181–2191, 2001.
39. **Hall RK, Yamasaki T, Kucera T, Waltner-Law M, O'Brien R, and Granner DK.** Regulation of phosphoenolpyruvate carboxykinase and insulin-like growth factor-binding protein-1 gene expression by insulin. The role of winged helix/forkhead proteins. *J Biol Chem* 275: 30169–30175, 2000.
40. **Hani EH, Suaud L, Boutin P, Chevre JC, Durand E, Philippi A, Demenais F, Vionnet N, Furuta H, Velho G, Bell GI, Laine B, and Froguel P.** A missense mutation in hepatocyte nuclear factor-4 alpha, resulting in a reduced transactivation activity, in human late-onset non-insulin-dependent diabetes mellitus. *J Clin Invest* 101: 521–526, 1998.
41. **Hart AW, Baeza N, Apelqvist A, and Edlund H.** Attenuation of FGF signalling in mouse beta-cells leads to diabetes. *Nature* 408: 864–868, 2000.
42. **Hellstrom A, Perruzzi C, Ju M, Engstrom E, Hard AL, Liu JL, Albertsson-Wikland K, Carlsson B, Niklasson A, Sjodell L, LeRoith D, Senger DR, and Smith LE.** Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. *Proc Natl Acad Sci USA* 98: 5804–5808, 2001.
43. **Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, and Bell GI.** Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26: 163–175, 2000.
44. **Hotamisligil GS, Peraldi P, Budvari A, Ellis RW, White MF, and Spiegelman BM.** IRS-1 mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α-and obesity-induced insulin resistance. *Science* 271: 665–668, 1996.
45. **Hotamisligil GS and Spiegelman BM.** Adipose expression of TNFα: direct role in obesity-linked insulin resistance. *Science* 259: 87–91, 1999.
46. **Inoue G, Cheatham B, Emkey R, and Kahn CR.** Dynamics of insulin signaling in 3T3-L1 adipocytes: differential compartmentalization and trafficking of insulin receptor substrate (IRS)-1 and IRS-2. *J Biol Chem* 273: 11548–11555, 1998.
47. **Ishihara H, Sasaoka T, Hori H, Wada T, Hirai H, Haruta T, Langlois WJ, and Kobayashi M.** Molecular cloning of rat SH2-containing inositol phosphatase 2 (SHIP2) and its role in the regulation of insulin signaling. *Biochem Biophys Res Commun* 260: 265–272, 1999.
48. **Kadowaki T, Tamemoto H, Tobe K, Terauchi Y, Ueki K, Kaburagi Y, Yamauchi T, Satoh S, Sekihara H, Aizawa S, and Yazaki Y.** Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1 and identification of insulin receptor substrate-2. *Diabet Med* 13: S103–S108, 1996.
49. **Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, and Shulman GI.** Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* 108: 437–446, 2001.
50. **Kitamura T, Nakae J, Biggs J, White MF, Arden KC, and Accili D.** The transcription factor FKHR promotes beta cell survival in IRS-2 knockout mice (Abstract). *8th International Symposium on Insulin Receptors and Insulin Action Geneva, Switzerland*, 2001.
51. **Kops GJ and Burgering BM.** Forkhead transcription factors are targets of signalling by the proto-oncogene PKB (C-AKT). *J Anat* 197: 571–574, 2000.
52. **Kotani K, Wilden P, and Pillay TS.** SH2-Balpha is an insulin-receptor adapter protein and substrate that interacts with the activation loop of the insulin-receptor kinase. *Biochem J* 335: 103–109, 1998.
53. **Kuan CY, Yang DD, Samanta Roy DR, Davis RJ, Rakic P, and Flavell RA.** The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* 22: 667–676, 1999.
54. **Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, and Kahn CR.** Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96: 329–339, 1999.
55. **Kushner JA, Ye J, Schubert M, Burks DJ, Dow M, Flint CL, Dutta S, Wright CVE, Montminy M, and White MF.** Pdx1 restores beta cell function in Irs2 knockout mice. *J Clin Invest* 109: 1193–1201, 2002.
56. **Liu JP, Baker J, Perkins AS, Robertson EJ, and Efstratiadis A.** Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 75: 59–72, 1993.
57. **Lock P, Casagranda F, and Dunn AR.** Independent SH2-binding sites mediate interaction of Dok-related protein with RasGTPase-activating protein and Nck. *J Biol Chem* 274: 22775–22784, 1999.
58. **Lupu F, Terwilliger JD, Lee K, Segre GV, and Efstratiadis A.** Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol* 229: 141–162, 2001.
59. **Merforth S, Osmers A, and Dahlmann B.** Alterations of proteasome activities in skeletal muscle tissue of diabetic rats. *Mol Biol Rep* 26: 83–87, 1999.
60. **Mitch WE, Bailey JL, Wang X, Jurkovitz C, Newby D, and Price SR.** Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting. *Am J Physiol Cell Physiol* 276: C1132–C1138, 1999.
61. **Myers, MG Jr and White MF.** The new elements in insulin signaling. Insulin receptor substrate-1 and proteins with SH2 domains. *Diabetes* 42: 643–650, 1993.
62. **Noguchi T, Matozaki T, Inagaki K, Tsuda M, Fukunaga K, Kitamura Y, Kitamura T, Shii K, Yamanashi Y, and Kasuga M.** Tyrosine phosphorylation of p62(Dok) induced by cell adhesion and insulin: possible role in cell migration. *EMBO J* 18: 1748–1760, 1999.
63. **O'Brien RM, Streeper RS, Ayala JE, Stadelmaier BT, and Hornbuckle LA.** Insulin-regulated gene expression. *Biochem Soc Trans* 29: 552–558, 2001.
64. **Paquette J, Giannoukakis N, Polychronakos C, Vafiadis P, and Deal C.** The INS 5′ variable number of tandem repeats is associated with IGF2 expression in humans. *J Biol Chem* 273: 14158–14164, 1998.
65. **Patti ME, Sun XJ, Bruning JC, Araki E, Lipes MA, White MF, and Kahn CR.** 4PS/IRS-2 is the alternative substrate of the insulin receptor in IRS-1 deficient mice. *J Biol Chem* 270: 24670–24673, 1995.
66. **Pawson T and Scott JD.** Signaling through scaffold, anchoring, and adaptor proteins. *Science* 278: 2075–2080, 1997.
67. **Peraldi P, Hotamisligil GS, Buurman WA, White MF, and Spiegelman BM.** Tumor necrosis factor (TNF)-α inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *J Biol Chem* 271: 13018–13022, 1996.

68. **Pessin JE and Saltiel AR.** Signaling pathways in insulin action: molecular targets of insulin resistance. *J Clin Invest* 106: 165–169, 2000.
69. **Pete G, Fuller CR, Oldham JM, Smith DR, D'Ercole AJ, Kahn CR, and Lund PK.** Postnatal growth responses to insulin-like growth factor I in insulin receptor substrate-1-deficient mice. *Endocrinology* 140: 5478–5487, 1999.
71. **Previs SF, Withers DJ, Ren JM, White MF, and Shulman GI.** Contrasting effects of IRS-1 vs IRS-2 gene disruption on carbohydrate and lipid metabolism in vivo. *J Biol Chem* 275: 38990–38994, 2000.
72. **Reaven GM.** Banting Lecture 1988. Role of insulin resistance in human disease. *Nutrition* 13: 64–66, 1997.
73. **Rincon M, Whitmarsh A, Yang DD, Weiss L, Derijard B, Jayaraj P, Davis RJ, and Flavell RA.** The JNK pathway regulates the in vivo deletion of immature CD4(+)CD8(+) thymocytes. *J Exp Med* 188: 1817–1830, 1998.
74. **Rui L, Fisher TL, Thomas J, and White MF.** Regulation of insulin/IGF-1 signaling by proteasome-mediated degradation of IRS-2. *J Biol Chem* 276: 40362–40367, 2001.
75. **Savkur RS, Philips AV, and Cooper TA.** Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet* 29: 40–47, 2001.
77. **Shulman GI.** Cellular mechanisms of insulin resistance. *J Clin Invest* 106: 171–176, 2000.
78. **Sreenan SK, Zhou YP, Otani K, Hansen PA, Currie KP, Pan CY, Lee JP, Ostrega DM, Pugh W, Horikawa Y, Cox NJ, Hanis CL, Burant CF, Fox AP, Bell GI, and Polonsky KS.** Calpains play a role in insulin secretion and action. *Diabetes* 50: 2013–2020, 2001.
79. **Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, and Lazar MA.** The hormone resistin links obesity to diabetes. *Nature* 409: 307–312, 2001.
80. **Sun XJ, Goldberg JL, Qiao LY, and Mitchell JJ.** Insulin-induced insulin receptor substrate-1 degradation is mediated by the proteasome degradation pathway. *Diabetes* 48: 1359–1364, 1999.
81. **Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S, Sekihara H, Yoshioka S, Horikoshi H, Furuta Y, Ikawa Y, Kasuga M, Yazaki Y, and Aizawa S.** Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 372: 182–186, 1994.
82. **Thomas MK, Devon ON, Lee JH, Peter A, Schlosser DA, Tenser MS, and Habener JF.** Development of diabetes mellitus in aging transgenic mice following suppression of pancreatic homeoprotein IDX-1. *J Clin Invest* 108: 319–329, 2001.
83. **Tissenbaum HA and Ruvkun G.** An insulin-like signaling pathway affects both longevity and reproduction in Caenorhabditis elegans. *Genetics* 148: 703–717, 1998.
84. **Uchida T, Myers MG Jr, and White MF.** IRS-4 mediates activation of PKB/Akt during insulin stimulation without inhibition of apoptosis. *Mol Cell Biol* 20: 126–138, 2000.
85. **Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M, Mason A, Seeburg PH, Grunfeld C, Rosen OM, and Ramachandran J.** Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 313: 756–761, 1985.
86. **Uysal KT, Wiesbrock SM, and Hotamisligil GS.** Functional analysis of tumor necrosis factor (TNF) receptors in TNF-alpha-mediated insulin resistance in genetic obesity. *Endocrinology* 139: 4832–4838, 1998.
87. **Uysal KT, Wiesbrock SM, Marino MW, and Hotamisligil GS.** Protection from obesity-induced insulin resistance in mice lacking TNF-α function. *Nature* 389: 610–614, 1997.
88. **Vaxillaire M, Rouard M, Yamagata K, Oda N, Kaisaki PJ, Boriraj VV, Chevre JC, Boccio V, Cox RD, Lathrop GM, Dussoix P, Philippe J, Timsit J, Charpentier G, Velho G, Bell GI, and Froguel P.** Identification of nine novel mutations in the hepatocyte nuclear factor 1 alpha gene associated with maturity-onset diabetes of the young (MODY3). *Hum Mol Genet* 6: 583–586, 1997.
89. **White MF, Shoelson SE, Keutmann H, and Kahn CR.** A cascade of tyrosine autophosphorylation in the β-subunit activates the insulin receptor. *J Biol Chem* 263: 2969–2980, 1988.
90. **Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, and White MF.** Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat Genet* 23: 32–40, 1999.
91. **Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, and White MF.** Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391: 900–904, 1998.
92. **Wolf G, Trub T, Ottinger E, Groninga L, Lynch A, White MF, Miyazaki M, Lee J, and Shoelson SE.** The PTB domains of IRS-1 and Shc have distinct but overlapping specificities. *J Biol Chem* 270: 27407–27410, 1995.
93. **Wolkow CA, Kimura KD, Lee MS, and Ruvkun G.** Regulation of C. elegans life-span by insulinlike signaling in the nervous system. *Science* 290: 147–150, 2000.

93a. **Xu P, Jacobs AR, and Taylor SI.** Interaction of insulin receptor substrate 3 with insulin receptor, insulin receptor-related receptor, insulin-like growth factor-1 receptor, and downstream signaling proteins. *J Biochem* 274: 15262–15720, 1999.

94. **Yamauchi T, Tobe K, Tamemoto H, Ueki K, Kaburagi Y, Yamamoto-Handa R, Takahadhi Y, Yoshizawa F, Aizawa S, Akanuma Y, Sonenberg N, Yazaki Y, and Kadowaki T.** Insulin signaling and insulin actions in the muscles and livers of insulin-resistant, insulin receptor substrate 1-deficient mice. *Mol Cell Biol* 16: 3074–3084, 1996.
95. **Yenush L and White MF.** The IRS signaling system during insulin and cytokine action. *Bioessays* 19: 491–500, 1997.
96. **Yenush L, Zanella C, Uchida T, Bernal D, and White MF.** The pleckstrin homology and phosphotyrosine binding domains of insulin receptor substrate 1 mediate inhibition of apoptosis by insulin. *Mol Cell Biol* 18: 6784–6794, 1998.
97. **Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, and Shoelson SE.** Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293: 1673–1677, 2001.
98. **Yuasa T, Ohno S, Kehrl JH, and Kyriakis JM.** Tumor necrosis factor signaling to stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) and p38. Germinal center kinase couples TRAF2 to mitogen-activated protein kinase/ERK kinase kinase 1 and SAPK while receptor interacting protein associates with a mitogen-activated protein kinase kinase kinase upstream of MKK6 and p38. *J Biol Chem* 273: 22681–22692, 1998.