CHAPTER 50

The Molecular Basis of Insulin Action

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Insulin and insulin-like growth factors (IGF-1 and IGF-2) are essential during all stages of life. They integrate the storage and release of nutrients with somatic growth during development and in adult life. Insulin, IGF-1, and IGF-2 promote tissue and organ maintenance throughout life. Moreover, they interact with other signaling systems during physiologic response to traumatic and chronic stress.

Our classical view of insulin action focuses upon glucose homeostasis, but the insulin signaling system has a much broader implication for heath and disease.[1] Following a meal, pancreatic β cells rapidly secrete insulin, suppressing hepatic gluconeogenesis, and promoting glucose storage in skeletal muscle and lipid storage in adipose tissues[2]; peripheral insulin also circulates to the hypothalamus where it informs the central nervous system that food has been consumed.[3] Dysregulation of insulin signaling causes glucose intolerance that progresses to diabetes when β cells fail to secrete sufficient insulin rapidly enough to maintain normal glucose homeostasis. Moreover, as diabetes ensues, life-threatening systemic disorders develop, including microvascular complications in the retina, renal glomerulus, and peripheral nerves; cardiovascular disease; dyslipidemia and obesity; and degeneration of neurons in the peripheral nervous system.[2]

A coherent explanation for the pathophysiology of common type 2 diabetes is complicated because many genetic polymorphisms appear to contribute to the characteristic pathophysiology. By contrast, maturity-onset diabetes of the young (MODY), which accounts for less than 10% of the cases of type 2 diabetes, is linked to single gene mutations that impair β-cell function: hepatocyte nuclear factor-4α (*MODY1*), glucokinase (*MODY2*), HNF-1α (*MODY 3*), Pdx1 (*MODY4*), or HNF-1β (*MODY 5*).[4–6] Dysregulated expression of these genes might contribute to the progression of common type 2 diabetes, as some of the MODY genes can be regulated in adult β cells through the insulin receptor substrate 2 (IRS2) branch of the insulin/IGF-signaling cascade.[7–10] Thus, dysregulated expression or function of MODY genes could contribute to β-cell failure and the progression toward ordinary type 2 diabetes.

INSULIN AND INSULIN-LIKE GROWTH FACTORS

MEMBERS OF THE INSULIN SIGNALING FAMILY

The mammalian insulin signaling system includes three well-defined ligands: insulin, insulin-like growth factor 1 (IGF-1), and insulin-like growth factor 2 (IGF-2).[11] Worms and fruit flies have a larger array of insulin-like peptides, revealing the utility and flexibility of the system.[12] In mammals, IGF-1 and IGF-2 bind with high affinity (K_d < 1 nM) to the IGF-1 receptors (IGF-1R) (Fig. 50-1). Two insulin receptor isoforms called IRb or IRa bind insulin with high affinity or moderate affinity, respectively. However, during peripheral insulin resistance, circulating insulin concentrations can rise high enough to activate IGF-1 receptors (K_d ~ 50 nM).

The IRa isoform is produced during tissue-specific inclusion of exon-11 in the receptor mRNA. Exon-11 encodes 12 amino acids at the end of the α subunit, which promotes IGF-2 binding at the expense of moderately reduced insulin-binding affinity.[13,14] IRb lacks this extended COOH-terminal tail owing to omission of exon-11, which increases the affinity and specify for insulin while reducing significantly the interaction with IGF-2 (see Fig. 50-1). IRb predominates in classical insulin-sensitive target tissues, including adult liver, muscle, and adipose tissues. IRa predominates in fetal tissues, the adult central nervous system, and hematopoietic cells.[15–18]

Proper regulation of exon-11 splicing is important. Dysregulated splicing alters fetal growth patterns and contributes to rare forms of insulin resistance in adults.[13,19] Severe insulin resistance occurs in patients with myotonic dystrophy

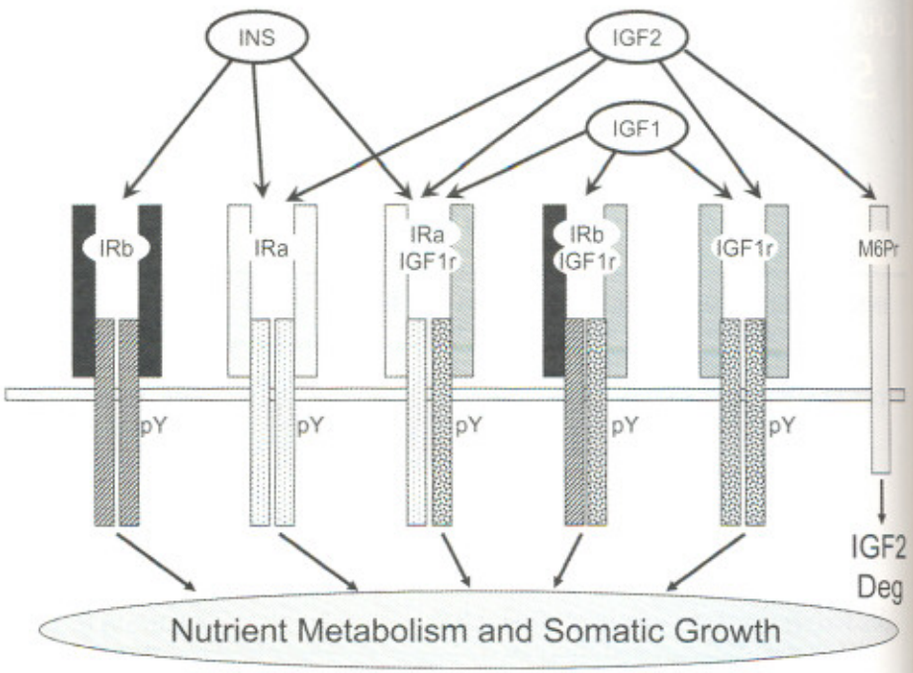

Figure 50-1 The insulin/insulin-like growth factor family. The insulin/IGF family consists of three hormones: insulin, insulin-like growth factor 1 (IGF-1), and insulin-like growth factor 2 (IGF-2). These peptide ligands bind as indicated in the figure to five distinct receptor isoforms that generate cytoplasmic signals: two insulin receptor isoforms, IRa and IRb; the insulin-like growth factor receptor, IGF-1R; and two hybrid receptors, IRa::IGF-1R and IRb::IGF-1R. IGF-2 also binds to the mannose-6-phosphate receptor, which mediates its endocytosis and degradation. The insulin receptor is the primary target for insulin throughout development and life. The IGF-1 receptor is the primary target for IGF-1. IGF-2 binds to the insulin receptor primarily during embryonic development, and binds the IGF-1 receptor throughout life. IGF-2 also binds to the mannose-6-phosphate receptor, which targets the IGF-2 for degradation instead of signaling. Activation of the insulin receptor or the IGF-1 receptor mediate signals primarily via the cytoplasmic proteins IRS1 and IRS2, which mediate somatic cell growth and metabolism.

type 1, because a genetic alteration in RNA splicing causes the accumulation of type A receptors in adult skeletal muscle.[19] Cell-based experiments suggest that IRb displays greater insulin-stimulated tyrosine kinase activity and ability to phosphorylate insulin receptor substrates (IRS)-proteins, and may influence the timing of the insulin signal needed to properly regulate cell growth or differentiation.[14]

The selectivity for insulin and the insulin-like growth factor signaling is further complicated by posttranslational assembly of hybrids between the IGF-1 receptor and the insulin receptor isoforms.[20] Hybrid receptors composed of an αβ-dimer of the IGF-1 receptors and IRa (IGF-1R::IRb) selectively bind IGF-1, whereas IGF-1R::IRa binds all three ligands with similar affinities.[21] The physiologic significance of tissue-specific alternative splicing of insulin receptors and the assembly of receptor hybrids needs to be resolved (see Fig. 50-1).

GROWTH DEVELOPMENT AND SURVIVAL

Studies in humans and experimental animals, *Caenorhabditis elegans* and *Drosophila melanogaster* reveal that insulin and insulin-like growth factor signaling promotes development, growth, function, and survival of central and peripheral tissues.[22,23] Moreover, these signals coordinate nutrient sensing and storage needed to accomplish useful work and balance longevity with reproduction. In vertebrates, homologous IGF-1 receptors are essential during development for about 50% of brain and body growth.[20,24] By contrast, the insulin receptor has its greatest impact on carbohydrate metabolism, as the high-affinity IRb is highly expressed in liver, muscle, and adipose tissues. IGF-1 receptors also influence carbohydrate metabolism especially in skeletal muscle, and through their effects upon islet development and growth.[25,26]

Work with genetically altered mice reveals a more complicated relation between the metabolic and growth actions of insulin and IGF-1 than originally thought.[10,27–29] Although both receptors mediate growth in vitro, mice without insulin receptors are nearly normal size at birth; however, they develop hyperinsulinemia and ketoacidosis immediately after birth and die within 3 to 7 days.[30,31] By comparison, mice without IGF-1R develop slowly, growing to 45% of normal size at birth.[32] IGF-2 also promotes body growth during development; however, it does not contribute to growth in the central nervous system.[33] In mice, IGF-2, but not insulin, promotes embryonic growth through its interaction with the IGF-1R and the insulin receptor, most likely IGF-1R::IRa hybrids.[20,27,34] Consistent with this model, the growth deficit caused by dysregulation of IGF-1 or IGF-1R can be corrected by increasing the levels of circulating IGF-2. One way to increase systemic IGF-2 levels is by disrupting the mannose-6-phosphate receptor, which ordinarily ferries IGF-2 across the plasma membrane and targets it for degradation.[20,34] A third homologous receptor, called the insulin receptor-related receptor (IRR), is poorly investigated. The IRR is found in the nervous system, pancreatic β cells, and testes, but its function in these tissues is unclear.[27,35] IRR contributes to male sexual development when the insulin and IGF-1 receptors are dysregulated in testes.[36]

Like mice, human neonates with diminished IGF-1 signaling are developmentally retarded; however, unlike mice, human infants born without insulin receptors display both retarded development in utero together with severe fasting hyperglycemia at birth.[37] This developmental disparity arises apparently because insulin is produced during the last trimester of human pregnancies, whereas in mice it is produced just prior to birth.[20] Analysis of mice expressing variable systemic levels of insulin receptors—so-called mosaic mice—confirms that insulin receptor signaling has its greatest effect upon postnatal growth, probably through profound effects upon nutrient homeostasis, rather than direct effects upon cell division and size.[38]

Work with *C. elegans* and *Drosophila* suggests that life span is influenced by the insulin/IGF signaling system.[39,40] In worms, significant life span extension occurs by partial loss-of-function mutations in the insulin receptor gene.[41] Interestingly, life span can be normalized in the mutant worms by restoring insulin cascades in various cells, suggesting that a network of tissue interactions and feedback regulation coordinates aging in *C. elegans*.[42] In mice, the contribution of insulin and IGF signaling to longevity is complex. $Igf1r^{+/-}$ mice live on average 26% longer than their wild-type littermates, perhaps owing to greater resistance to oxidative stress.[43] Whereas inhibition of the insulin/IGF-1 signaling cascade in nematodes and flies increases life span convincingly, defects in insulin signaling in rodents and humans increase the risk for age-related diseases and increased mortality. This complexity might arise from the complicated cross-talk between peripheral and central tissues. By contrast,

disruption of the insulin receptor in adipose tissue increases life span; however, fat insulin receptor knockout mice also display reduced adiposity.[44] It is difficult to determine whether reduced insulin action in adipocytes or improved systemic insulin action that accompanies a lean body mass is responsible for the increased life span.

THE INSULIN RECEPTOR

INSULIN SIGNALING: THE BASICS

The receptors for insulin or IGF-1, like the receptors for other growth factors and cytokines, are composed of an extracellular ligand-binding domain that regulates the activity of an intracellular tyrosine kinase.[1,45,46] Most receptor tyrosine kinases are activated by ligand-induced dimerization that promotes tyrosine autophosphorylation of the kinase activation loop (A loop), and other sites that recruit cellular substrates.[47] By contrast, insulin receptors reside in the plasma membrane as inactivated covalent dimers. Insulin binding increases flexibility of the A loop admitting adenosine triphosphate (ATP) to the catalytic site. Subsequent tyrosine phosphorylation of the A loop stabilizes the active conformation and recruits substrates for phosphorylation.[48,49] The principal insulin receptor and IGF-1 receptor substrates, the IRS-proteins, are phosphorylated on multiple tyrosine residues by the activated receptor kinases. Various signaling proteins such as phosphatidylinositol 3-kinase (PI 3-kinase), Grb-2, short heterodimer partner 2 (SHP2), and others bind to the tyrosine phosphorylation sites, generating an array of cell- and tissue-specific responses. The strength and duration of these insulin signals are modulated through protein and phospholipid phosphatases, or direct inhibition of IRS-protein function.[50–53] Insulin resistance develops when the relation between these signaling pathways is disrupted, which progresses to glucose intolerance, diabetes, and other life-threatening metabolic diseases.

INSULIN RECEPTOR BIOSYNTHESIS

The insulin proreceptor mRNA is the splice product of 22 exons, including the developmentally regulated exon-11, of a 150-kb gene on human chromosome 19.[54,55] During translation, the proreceptor is stabilized by disulfide bonds, and the α and β subunits are generated by proteolysis (Fig. 50-2*A*). In its native conformation, the mature insulin receptor is a tetramer composed of two extracellular α subunits linked by disulfide bonds to each other and to the extracellular portion of the transmembrane β subunit (Fig. 50-2*B*). The β subunit contains a single transmembrane spanning domain and the intracellular tyrosine kinase.[45,46] During sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the holoreceptor ($\alpha_2\beta_2$) has an apparent molecular mass of 350,000, larger than expected owing to glycosylation of the α and β subunits. Under reducing conditions, the α and β subunits migrate during SDS-PAGE at 135 kilodaltons and 95 kilodaltons, respectively.[56,57]

INSULIN BINDING

The α subunit is too large to analyze by nuclear magnetic resonance, and crystallization is difficult owing in part to extensive glycosylation; however, an approximate molecular model explaining insulin-binding selectivity has emerged from a variety of approaches. Insulin binding has been studied extensively before and after receptor purification from various cells and tissues. These initial efforts revealed complicated binding properties where one insulin molecule binds with high affinity and a second molecule binds with low affinity.[58] The creation of chimeric molecules between the α subunits of the insulin and IGF-1 receptors is especially informative.[59] High-affinity insulin binding is transferred to the IGF-1 receptor by substituting residues 64 to 137 of the insulin receptor α subunit into the homologous positions of the IGF-1 receptor α subunit, revealing a portion of the insulin-binding domain called L1 (residues 1–149). Many other regions in the α subunit also contribute to insulin binding, including the L2 domain (residues x-y), and the COOH terminus (residues Thr704 and Lys718).[60]

The quaternary structure of the isolated complex of biologically active insulin receptor (IR) and insulin is solved approximately by three-dimensional reconstruction using low-dose scanning transmission electron micrographs to guide the assembly of insulin receptor subdomains into an approximate holoreceptor complex.[61,62] The insulin molecule has at least two receptor-binding surfaces, called $S1^{ins}$ and $S2^{ins}$ (Fig. 50-2*C*).[61] $S1^{ins}$ binds to L1 and L2 regions in one α subunit, while $S2^{ins}$ binds to the L1 region in the adjacent α subunit (see Fig. 50-2*B* and *C*). The interaction with both α-subunits result in higher-affinity insulin binding than either one achieves alone. The binding of a second insulin molecule to the complex occurs at a lower affinity because only one contact sites is readily accessible to the second insulin molecule.[63] Structural approximations place the COOH terminus near the L2 region, where it can influence insulin binding.[61] Apparently, extending this sequence by 12 amino acids in the IRa reduces insulin-binding affinity while enhancing IGF-2-binding affinity.

The structural details that emerge from this model are largely consistent with the biochemical and enzymatic data; the effect of naturally occurring and site-directed mutants of insulin and the receptor α subunits; and kinetic and isothermal-binding data.

THE CYTOPLASMIC DOMAIN GENERATES THE INSULIN SIGNAL

Biochemical studies first revealed the tyrosine kinase activity of the insulin receptor[64–66]; however, the cloning of the insulin receptor cDNA greatly expanded the subsequent biochemical and physiologic approaches.[45,46] The identification of naturally occurring mutant insulin receptors in humans and the rational design of kinase-deficient mutants establishes that the tyrosine kinase activity is essential for biologic activity, anticipating the deleterious consequences of its reduction.[67–69]

There are at least seven tyrosine autophosphorylation sites in three distinct regions of the insulin receptor β subunit, including three in the A loop, one or two in the intracellular juxtamembrane region, and two in the COOH terminus (see Fig. 50-2*B*).[49] Autophosphorylation of the A loop stabilizes the open conformation of the catalytic sites, and creates binding sites for other signaling proteins that modulate kinase activity, including Grb10, autoimmune polyglandular syndrome (APS), and SH2B.[65,70–72] Autophosphorylation in the juxtamembrane region is essential for recruitment of IRS-proteins that propagate the insulin signal. Autophosphorylation in the COOH terminus (Tyr1314 and Tyr1328) is poorly understood; however, it has been shown to regulate tyrosine kinase activity and receptor internalization[73–77]; and under certain conditions bind PI 3-kinase.[78,79] The nematode and fruit fly insulin receptor contains an extended COOH terminus that contains several tyrosine phosphorylation sites that bind PI 3-kinase. Consequently, these orthologs activate PI 3-kinase without requiring the expression of IRS-proteins.[80]

REGULATION OF KINASE ACTIVITY

The regulatory role of the A loop of the β subunit is well-supported by biochemical studies, mutational analysis, and the crystal structure of the β subunit.[70,71,81–85] Structural studies predict that the unphosphorylated Tyr1162 of the A loop folds into the catalytic site to prevent substrate binding.[86] This configuration restricts ATP binding, which explains the high apparent K_m for ATP before insulin stimulation.[72]

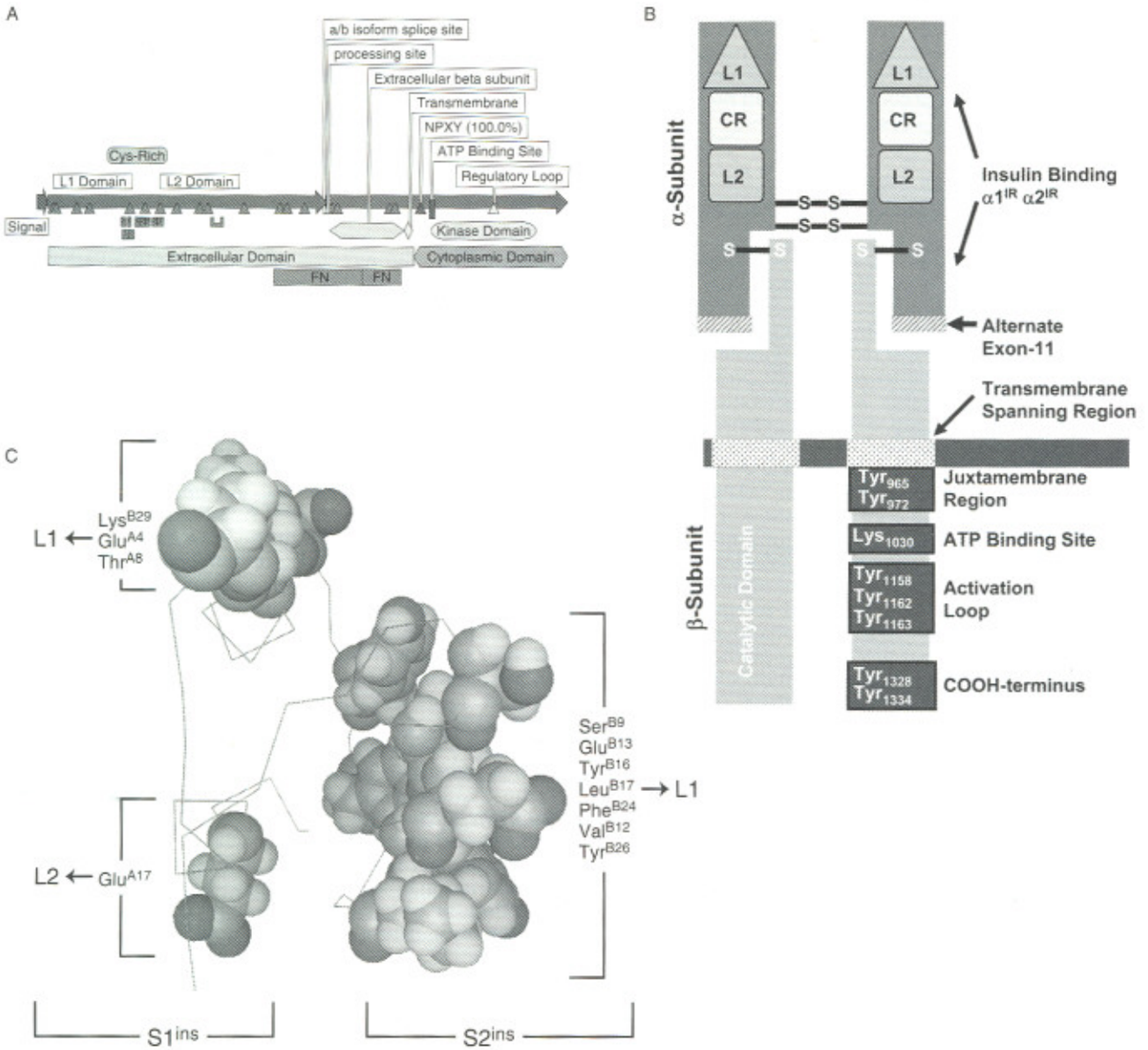

Figure 50-2 Structure of insulin and the insulin receptor. **A,** A linear diagram of the insulin receptor precursor showing the relative position of important landmarks in the α subunit, the ligand contact points L1 and L2, the cysteine-rich region (Cys-rich), disulfide bonds (⊔), glycosylation sites (▲) the IRa/IRb splice site, the processing site between the α subunit and the β subunit, and important landmarks in the β subunit, the extracellular region, the hydrophobic transmembrane region, the PTB recognition motif, the ATP binding site, and the regulatory loop (A loop) in the kinase domain. **B,** A diagram of the insulin receptor extracellular, transmembrane, and intracellular components composed of two extracellular α subunits and two β subunits that contain. The holoreceptor is joined by disulfide bonds between cysteine residues in the extracellular α and β subunits as well as by noncovalent interactions. The α subunit contains several regions that contribute to insulin binding, including the L1 and L2 regions separated by a cysteine-rich region, and a 12-amino acid alternatively spliced region encoded by exon-11. The β subunit contains a tyrosine kinase catalytic domain with an ATP-binding site and a number of tyrosine phosphorylation sites including those in the juxtamembrane, activation loop, and COOH-terminal regions. **C,** The insulin diagram shows the amino acids that compose the two surfaces of the insulin molecule ($S1^{ins}$ and $S2^{ins}$) and the amino acids that interact with the L1 and L2 regions of the insulin receptor. (See Color Plate.)

However, the closed A loop is in equilibrium with an alternate conformation that allows ATP access to mediate a basal level of A loop autophosphorylation.[85] Insulin apparently shifts the equilibrium of the A loop toward an open conformation to facilitate ATP binding (decrease the apparent K_m) and autophosphorylation of Tyr1162 (Fig. 50-3*A*). This model, together with early biochemical studies, suggests that the autophosphorylation cascade proceeds rapidly at Tyr1158, resulting in a bis-phosphorylated regulatory loop.[70] The relatively slow phosphorylation of Tyr1163 to generate the tris-phosphorylated A loop is required to stabilize the open conformation to allow unrestricted access by Mg-ATP and protein substrates (see Fig. 50-3*A*).

This model of kinase regulation is validated by recent structural analysis of the kinase domain obtained upon substitution Asp1161 in the middle of the A loop with Ala1161 ($IRKD^{DA}$).[85] $IRKD^{DA}$ dramatically shifts the A loop equilibrium toward the open configuration before autophosphorylation, which increases Mg-ATP binding affinity by 10-fold. The kinetic properties of $IRKD^{DA}$ after autophosphorylation are indistinguishable from those of the wild-type kinase.[85] Substitution of Tyr1162 with Phe also increases basal autophosphorylation, consistent with its role to stabilize the closed conformation; however, this mutation does not increase basal IRS-protein phosphorylation in cells, probably because phosphorylation of the NPEY motif requires insulin binding.[82]

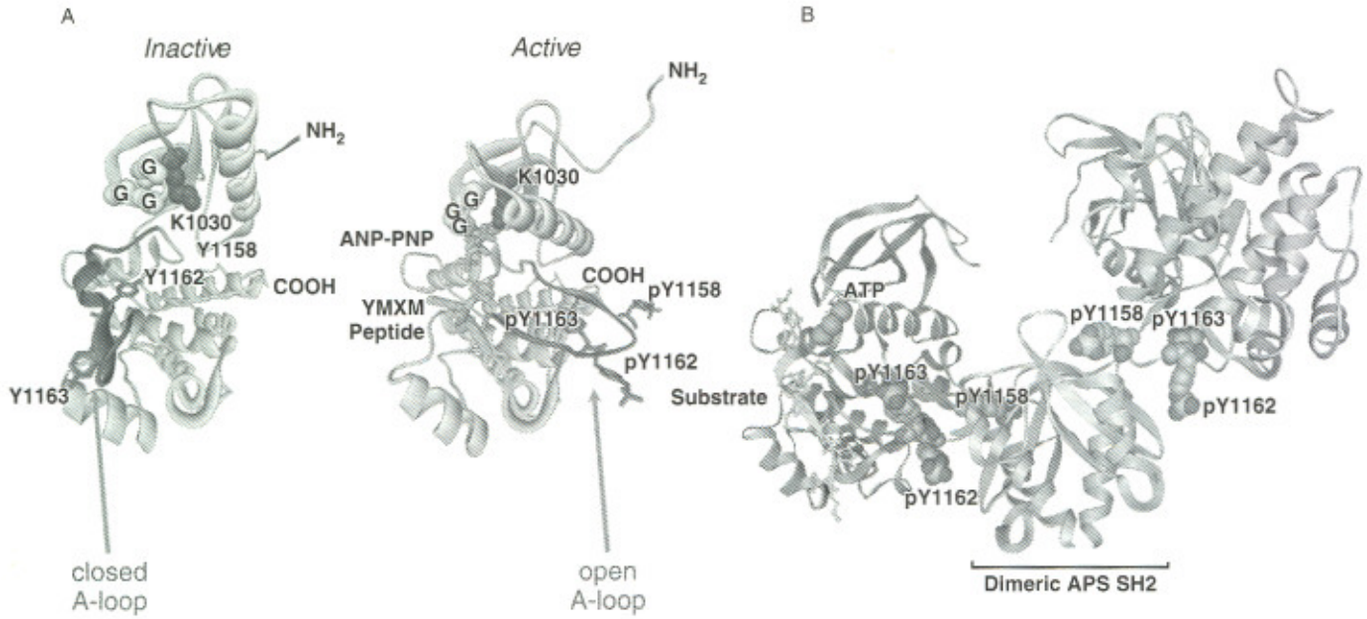

Figure 50-3 The role of insulin receptor autophosphorylation. **A,** Structure of the insulin receptor activation loop shown as ribbon diagrams of the kinase domain of the insulin receptor along with the side chains of important amino acids, including the three glycine residues and K1030 that comprise the ATP-binding site. The activation loop (A loop) is shown in red; the three activation loop tyrosine residues (Y1158, Y1162, and Y1163) are shown with their side chains. In the inactive, unphosphorylated state (*left panel*), the activation loop blocks access by potential substrates. Following phosphorylation (*right panel*), however, the activation loop moves, allowing substrates such as YMXM peptides of the IRS-proteins (shown in green) to access the active site. **B,** A structural representation of the binding of dimeric APS SH2 domains to the phosphorylated A loop of the insulin receptor. These structure were based on published coordinates.[83,86,90] (See Color Plate.)

Upon tris-phosphorylation, at least two phosphotyrosine residues in the A loop are completely solvent-exposed, creating sites for protein interaction (see Fig. 50-3*A*). A region of IRS2, called the kinase regulatory loop binding domain, binds to the phosphorylated A loop on the activated insulin receptor; however, the structural basis of this interaction remains unknown.[87,88] These exposed phosphotyrosine residues also interact with the Src homology-2 (SH2) domain containing proteins that promote or inhibit access to the catalytic site. Grb10 and several related SH2 proteins block the catalytic site while bound to the A loop.[89] By contrast, APS binds to the A loop from behind the catalytic domain to stabilize the active conformation (see Fig. 50-3*B*).[90] However, disruption of APS in mice increases peripheral insulin sensitivity and reduces circulating insulin levels, suggesting that APS might have an inhibitory function, possibly indirectly through elevated leptin and adiponectin levels.[91] Another member of the APS family, SH2B, binds via its SH2 domain to the A loop of IR.[91a–c] In Chinese hamster ovary cells, stable overexpression of SH2B enhances insulin-stimulated activation of both Erk1, Erk2, and Akt.[91d] These observations raise a possibility that SH2B plays a positive regulatory role during insulin receptor activation. Consistent with this model, disruption of the gene for SH2B causes insulin resistance and maturity onset obesity.[92] An explanation for the distinct phenotypes of the $APS^{-/-}$ and the $SH2B^{-/-}$ mice will be informative.

SUBSTRATE RECRUITMENT AND PHOSPHORYLATION SITE SELECTION

Substrate selectivity by protein kinases, including the insulin receptor, is a two-step process. First, a specific interaction between the kinase and the substrate aligns potential phosphorylation sites with the activated catalytic domain. Second, the catalytic domain selects and phosphorylates specific tyrosine residues based on their amino acid contexts. Although phosphorylation of the regulatory loop is important to open the A loop, phosphorylation of the NPEY972 motif in the juxtamembrane region is essential for substrate recruitment.[93] The juxtamembrane region in the insulin receptor, the polypeptide segment that connects the transmembrane helix to the kinase domain, is about 35 residues long and contains two autophosphorylation sites (Tyr965 and Tyr972) (see Fig. 50-2*B*). Unlike other receptor tyrosine kinases, the insulin receptor kinase is not regulated by autophosphorylation in the juxtamembrane region.[94,95] However, pTyr972, which resides in the NPXY motif, is a docking site for the phosphotyrosine-binding (PTB) domains in the IRS-proteins and SHC.[93] In intact cells, phosphorylation of the NPXY motif is among the first sites of insulin-stimulated autophosphorylation, which is essential for substrate recruitment and biologic activity.[93,96] The juxtamembrane region may compete with the A loop for access to the catalytic site, supporting a model in which the juxtamembrane region is phosphorylated before tris-phosphorylation of the A loop.[72]

The structure of the activated β subunit reveals a mechanism by which the catalytic domain selects specific motifs for tyrosine phosphorylation.[83] Substrate peptides bind as short antiparallel β strands to the COOH-terminal end of the activation loop, allowing the hydrophobic residues in the Y+1 and Y+3 positions to occupy two small hydrophobic pockets on the COOH-terminal lobe of the kinase (see Fig. 50-3*A*). Tyrosine residues lying within amino acid motifs that contain charged or bulky side chains at the Y+1 and Y+3 positions fit poorly in the kinase active site.[83] Following substrate recruitment, the activated insulin receptor kinase engages tyrosine residues in the context of specific amino acid motifs, including the YMXM motif, YVNI motif, and YIDL motif.[97–99] Thus, specific recruitment of cellular proteins to the activated insulin receptor followed by the phosphorylation of specific tyrosine-containing motifs establishes an important level of signaling specificity and regulation.

IRS-PROTEINS AND INSULIN SIGNALING

After the discovery of the insulin receptor tyrosine kinase, many groups searched for substrates that might mediate downstream signals.[100,101] At first, the "substrate" hypothesis was difficult to prove for any receptor tyrosine kinase because the only known substrates were abundant proteins of dubious physiologic importance. The first evidence for a cellular substrate of a receptor tyrosine kinase came from phosphotyrosine antibody immunoprecipitates that revealed a 185-kilodalton phosphoprotein (pp185) in insulin-stimulated hepatoma cells.[102] This substrate seemed to be biologically important because it was phosphorylated immediately (within 5 sec) after insulin stimulation; and catalytically inactive insulin receptors failed to phosphorylate pp185. Importantly, a few catalytically active but biologically inactive insulin receptor mutants failed to phosphorylate pp185.[93] Together these data provided the first clue that substrate phosphorylation together with autophosphorylation are important steps in signal transduction.

Purification and molecular cloning of pp185 revealed one of the first signaling scaffolds and the first insulin receptor substrate, called IRS1.[103] IRS1 contains many tyrosine phosphorylation sites that are phosphorylated during insulin and IGF-1 stimulation (Fig. 50-4). Many of these tyrosine phosphorylation motifs are recognized binding sites for the SH2 domains in various signaling proteins.[99,104] For example, the interaction between IRS1 and p85 activates the class 1A PI 3-kinase, revealing the first insulin signaling cascade that could be reconstituted successfully in cells and test tubes.[105]

Many cellular substrates of the insulin receptor have now been identified, suggesting that insulin receptor signaling diversity arises from the assembly of signaling complexes around tyrosyl phosphorylated scaffolds. In addition to IRS-1 and its homologues (IRS2, IRS3, and IRS4), the insulin receptor phosphorylates other proteins in various contexts, including Shc, APS and SH2B, Gab1 and 2, Dock 1 and 2 and Cbl (see Fig. 50-4).[106–113] Although the role of each of these substrates merits attention, work with transgenic mice reveals that many insulin responses, especially those that are associated with somatic growth and carbohydrate metabolism, are mediated largely through IRS1 and IRS2, with minor contributions from IRS3 and IRS4.[1]

IRS-PROTEIN STRUCTURE AND FUNCTION

IRS-proteins are composed of multiple interaction domains and phosphorylation motifs (Fig. 50-5). At least three IRS-proteins occur in mice and people, including IRS1 and IRS2 that are widely expressed, and IRS4 that is limited to the thymus, brain, kidney, and β cells.[114] Rodents also express IRS3, which is largely restricted to adipose tissue where it displays activity similar to IRS1.[115] Mice lacking IRS1 are small and insulin-resistant, but generally fail to develop diabetes owing to persistent compensatory hyperinsulinemia.[116,117] By contrast, mice lacking IRS2 develop diabetes between 6 and 10 weeks of age owing to peripheral insulin resistance and β-cell failure.[116] Additional metabolic or genetic stress can cause diabetes in IRS1$^{-/-}$ mice, including the heterozygous disruption of the insulin receptor or IRS2.[118] Although disruption of IRS3 in mice has

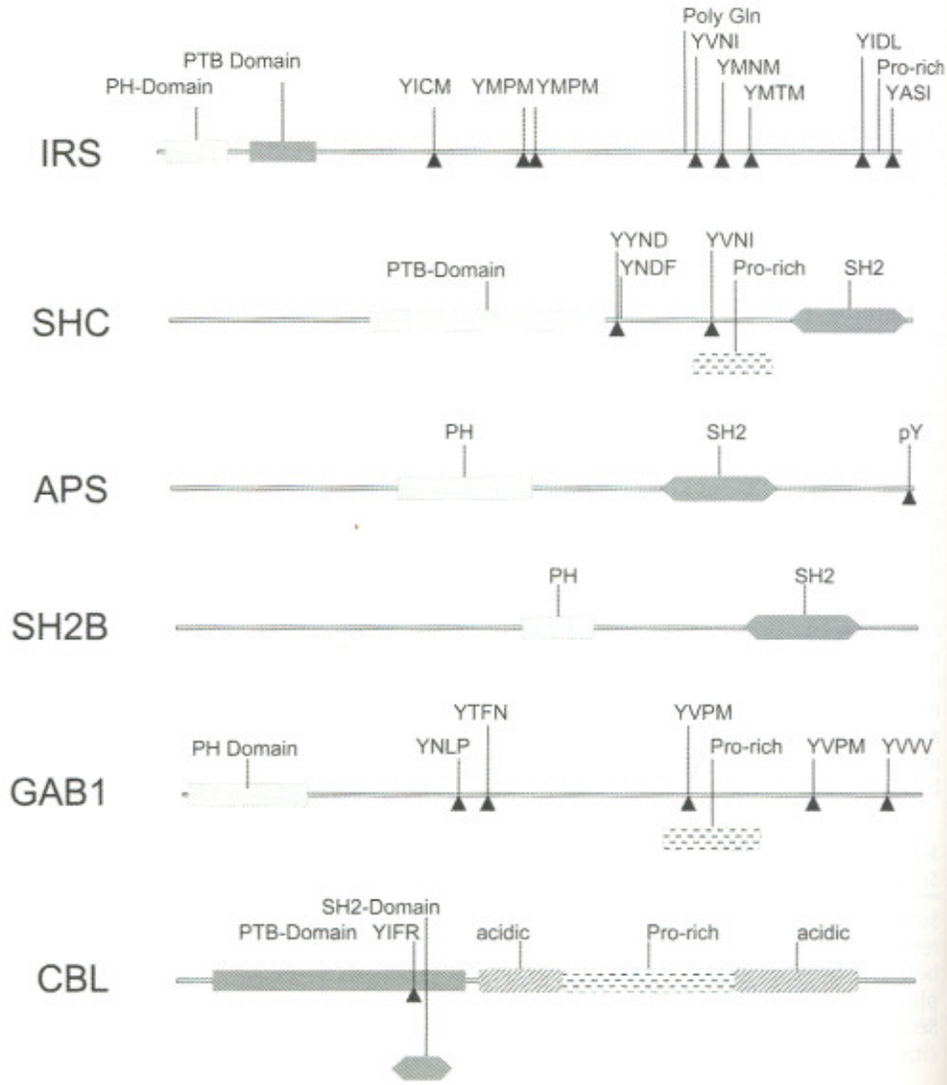

Figure 50-4 Diagrams (not drawn to scale) of various scaffold proteins that are reported to interact with the insulin receptor. The relative locations of protein interaction domains are shown: pleckstrin homology (PH), phosphotyrosine binding (PTB), src homology (SH2). Various amino acid sequence motifs are also shown: tyrosine phosphorylation sites, proline rich motifs (pro-rich), and acidic motifs.

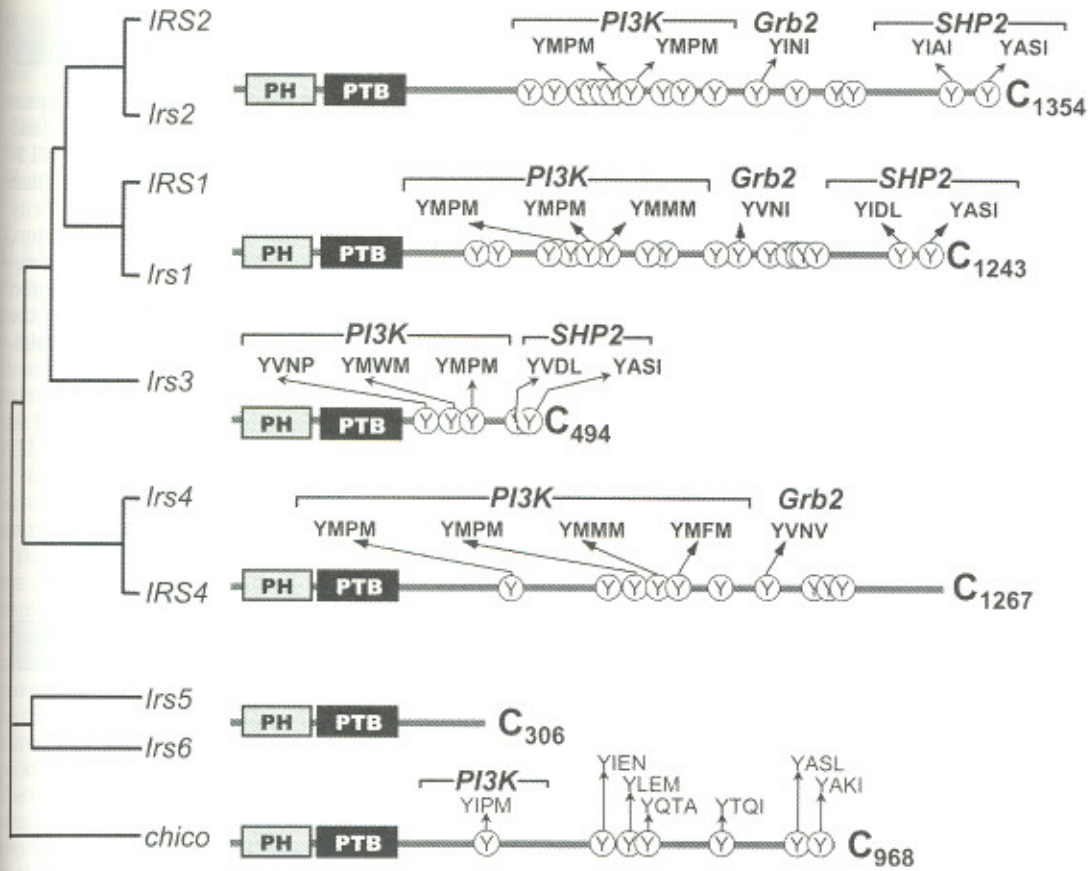

Figure 50-5 IRS-protein structures. A comparison (drawn to scale) of important sequence features of human IRS1, IRS2, IRS4, IRS5, and IRS6, mouse IRS3, and drosophila Chico. The relative position of the pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains are indicated. The relative positions of potential tyrosine phosphorylation sites are indicated.

small effects, mice with a combined deficiency of IRS1 and IRS3 developed severe early-onset lipoatrophy associated with marked hyperglycemia, hyperinsulinemia, and insulin resistance.[115] Since humans do not express a functional IRS3 gene, humans may be at increased risk for metabolic disorders.[119]

The IRS-protein isoforms display several important similarities (see Fig. 50-5). Mammalian IRS1, -2, -3 and -4, and the drosophila ortholog Chico contain an NH_2-terminal pleckstrin homology (PH) domain adjacent to a PTB domain (see Fig. 50-5). The structures of these domains are remarkably similar[120]; both appear to facilitate recruitment of IRS-proteins to the activated insulin and IGF-1 receptors.[121] Each IRS-protein recruits and activates PI 3-kinase during insulin stimulation, whereas the ability to activate Erk1 and -2 is variable.[122,123] IRS5/DOK4 and IRS6/DOK5 were recognized in the human genome owing to their NH_2-terminal tandem PH-PTB domains.[124]

Deletion of the PH and PTB domains in IRS1 or IRS2 almost completely prevents insulin-stimulated tyrosine phosphorylation of the tail, even when insulin receptors are expressed at high levels.[125] The PTB domain binds to a phosphorylated NPEY-motif in the β subunit of the activated insulin or IGF-1 receptor[121,125,126]; a similar motif is also phosphorylated in the IL-4 receptor, explaining its strong recruitment of IRS1 or IRS2.[127] At ordinary expression levels, deletion of the PTB domain reduces the ability of insulin to promote tyrosine phosphorylation of IRS1 or IRS2; however, overexpression of the insulin receptor restores phosphorylation and signaling, suggesting that the PH domain is sufficient.[125]

The mechanism of coupling employed by the PH domain is not understood; however, it promotes interaction between IRS-proteins and insulin receptors at physiologic levels. The PH domains in PKB and PDK1 bind membrane phospholipids with high affinity to provide unambiguous membrane targeting.[128,129] However the PH domain in IRS-proteins, like PH domains in most proteins, binds phospholipids poorly. PH domains can be exchanged among IRS-proteins without noticeable loss of bioactivity[130]; however, heterologous PH domains inhibit IRS1 function when substituted for the normal PH domain.[130] Since IRS-protein PH domains do not bind to the insulin receptor or to phospholipids, other targets might be involved. Yeast two-hybrid screens reveal a few potential binding partners, including nucleolin.[130] However, the interaction with nucleolin might mediate translocation of IRS1 into the nucleus rather than coupling to the insulin receptor.[131–133] The PH domain also binds to PHIP, an uncharacterized conserved protein that contains a WD40 repeat and BROMO domains.[24,134]

The tyrosine phosphorylation sites in the COOH-terminal end of each IRS-protein recruit and regulate various downstream signaling proteins (see Fig. 50-5). IRS1 and IRS2 have the longest tails, containing 20 potential tyrosine phosphorylation sites each; however, only a few sites that bind p85, Grb2, or SHP3 have been formally identified.[104] Many of the tyrosine residues cluster into common motifs that recruit or activate enzymes (PI 3-kinase, SHP2, fyn) or adapter molecules (Grb2, nck, crk, SH2B) (see Fig. 50-5). Grb2 and possibly SHP2 couple Grb2/SOS to IRS-proteins, which promotes the ras → raf cascade.[135] All IRS-proteins contain multiple p85-binding motifs that recruit the PI 3-kinase, which is the best studied insulin signaling pathway.

In addition to the tyrosine phosphorylation sites, sequence alignment of IRS-proteins reveals several conserved motifs that might be binding sites for other cellular proteins. IRS1, IRS2, and Chico contains a binding site for the c-Jun N-terminal kinase (JNK) that resembles the sites in the JNK-interacting proteins (JIP1 and JIP2).[136–139] During stimulation by proinflammatory cytokines or by insulin, activated JNK binds to IRS1 or IRS2 and promotes serine phosphorylation, which inhibits insulin-stimulated tyrosine phosphorylation.[138–141]

There are many unique amino acid sequence motifs between IRS1 and IRS2 that might create unique interaction sites for other partner proteins that fine-tune the biologic signals. IRS2 contains a unique region of undefined structure

that binds to the phosphorylated regulatory loop of the insulin receptor kinase called the kinase regulatory loop binding domain.[142] The discovery of this interaction was unexpected, as it maps to the portion of the COOH-terminal region between amino acid residues 591 and 786 that contains tyrosine phosphorylation sites. Two tyrosine residues in the kinase regulatory loop binding domain at positions 628 and 632 are crucial for this interaction. Phosphorylation of tyrosine residues in the kinase regulatory-loop binding domain by the insulin receptor inhibits the binding to the receptor, revealing a novel mechanism to regulate the interaction of the insulin receptor and IRS2 that might distinguish the signal of IRS2 from IRS1.[142]

THE PI 3-KINASE CASCADE

The PI 3-kinase is ubiquitous and used by nearly all receptor signaling systems to promote cell division, survival, and growth (Fig. 50-6). During insulin and IGF signaling, the PI 3-kinase cascade is accessed through tyrosine phosphorylation of the IRS-proteins. IRS-proteins introduce unique specificity and regulation upon the system for insulin and IGF action. Specificity is accomplished by dissociating IRS PI 3-kinase signaling complex from the intracellular itinerary of the insulin receptor. Differential regulation of the IRS-proteins at the level of gene expression and protein stability add an addi-

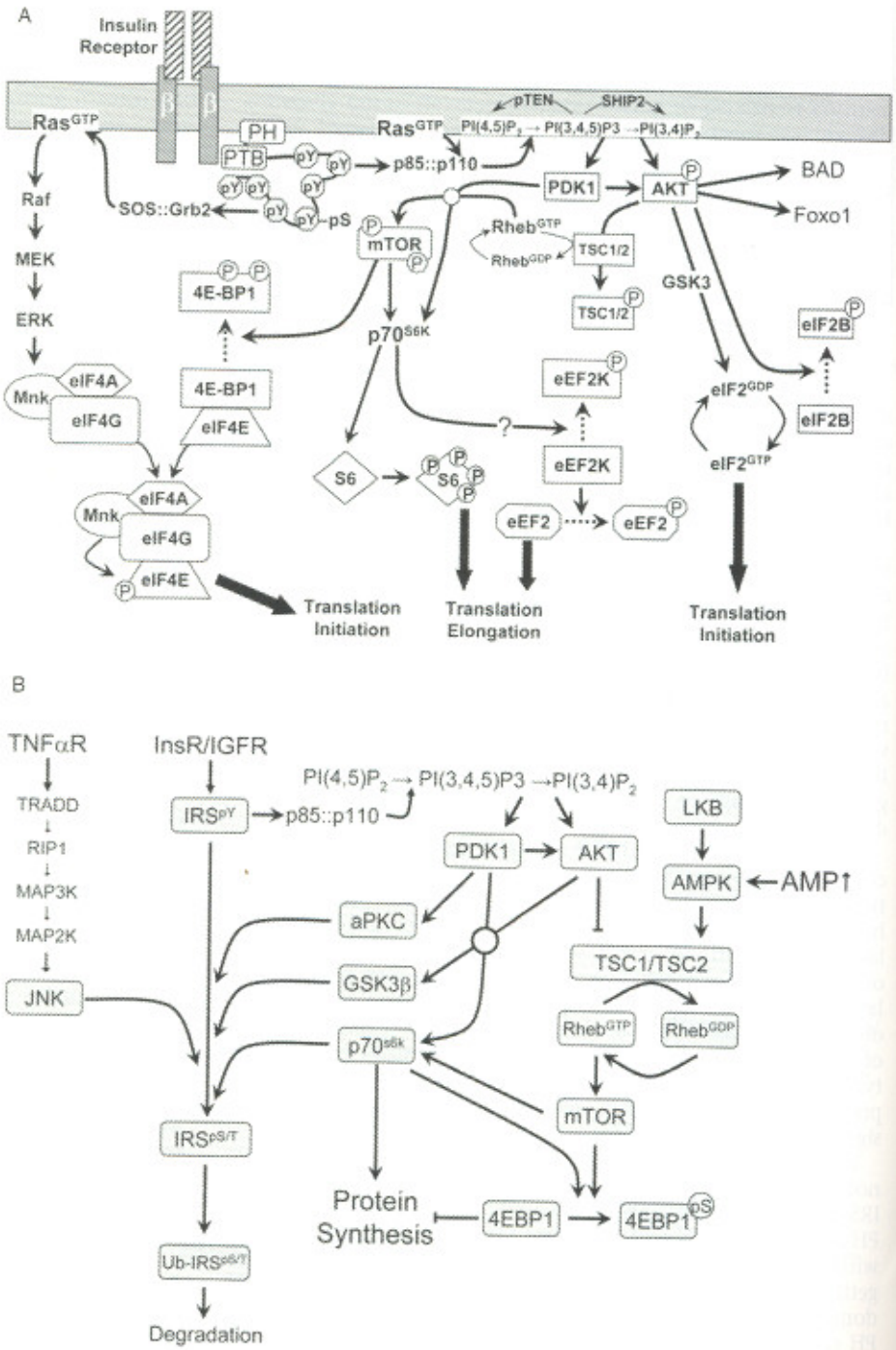

Figure 50-6 Activation of intracellular signaling pathways by insulin. **A,** There are two main limbs that propagate the signal generated through the IRS-proteins: the PI 3-kinase and the Grb2/Sos→ras cascade. Activation of the receptors for insulin and IGF-1 results in tyrosine phosphorylation of the IRS-proteins, which bind PI 3-kinase and Grb2/SOS. The GRB2/SOS complex promotes GDP/GTP exchange on p21^{ras}, which activates the ras raf MEK ERK1/2 cascade. The activated ERK stimulates transcriptional activity by direct phosphorylation of elk1 and by phosphorylation of fos through p90^{rsk}. The activation of PI 3-kinase by IRS-protein recruitment produces PI3,4P$_2$ and PI3,4,5P$_3$ (antagonized by the action of PTEN or SHIP2), which recruit PDK1 and PKB to the plasma membrane, where PKB is activated by PDK-mediated phosphorylation. The mTOR kinase is phosphorylated by Rheb^{GTP}, which accumulates upon inhibition of the GAP activity of the TSC1::TSC2 complex by PKB-mediated phosphorylation. The p70^{s6k} is primed through mTOR-mediated phosphorylation for activation by PDK1. PKB inactivates GSK3 by phosphorylation, which leads to the activation of glycogen synthesis and protein translation. PKB-mediated BAD phosphorylation inhibits apoptosis, and phosphorylation of the forkhead proteins results in their sequestration in the cytoplasm, in effect inhibiting their transcriptional activity. Insulin stimulates protein synthesis by altering the intrinsic activity or binding properties of key translation initiation and elongation factors (eIFs and eEFs, respectively) as well as critical ribosomal proteins. This occurs via phosphorylation and/or sequestration of repressive factors into inactive complexes. Components of the translational machinery that are targets of insulin regulation include eIF2B, eIF4E, eEF1, eEF2, and the S6 ribosomal protein.[215] **B,** Inhibition of IRS-protein signaling through nutrient sensing. High ATP levels, reflecting amino acid and glucose excess, inhibit the AMP kinase reducing its ability to activate the GAP activity of TSC1::TSC2, which mediates mTOR activity. Insulin activation of PDK1 together with mTOR leads to the activation of various kinases that phosphorylate IRS-protein, which targets them for poly ubiquitinylation and degradation. AKT, product of the akt proto-oncogene; GAP, guanosine triphosphatase associated protein; GLUT4, glucose transporter 4; GRB-2, factor receptor binding protein 2; GSK3, glycogen synthase kinase 3; IRS1, insulin receptor substrate 1; MAPKK, MAPK kinase; PDK, PI-dependent protein kinase; PH, pleckstrin homology domain; PKC, protein kinase C; PTB, phosphotyrosine binding domain; PTEN and SHIP2, phospholipid phosphatases; SOS, son-of-sevenless; TSC, tuberous sclerosis complex. See the text for details of the signaling.

tional level of control. One of the best examples of signaling specificity emerges from our work in pancreatic β cells. IRS2, but not IRS1, is strongly induced by cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) signaling in β cells, which regulates the PI 3-kinase Pkb/Akt cascade needed to promote growth, survival, and function of these insulin-producing cells.[1]

PI 3-KINASE

Phospholipids create platforms for cell signaling, and the products of the PI 3-kinase, PI(3,4) P_2 and PI(3,4,5) P_3, play a special role. These phospholipids recruit various serine kinases to the inner face of the plasma membrane, where signaling complexes interact and generate downstream signals (see Fig. 50-6). Various phospholipid phosphatases, including PTEN and SHIP2, control the steady-state phosphorylation of these membrane lipids, which modulates the capacity to recruit PDK1 and PKB/AKT and the strength of the downstream signals (see Fig. 50-6).

There are three types of PI 3-kinases in higher eukaryotes, but the class IA enzymes are activated by growth factors and insulin.[143] Class IA PI 3-kinase is a heterodimer composed of a regulatory subunit and catalytic subunit. The different isoforms of the catalytic subunit, p110α, β, and δ are encoded by separate genes; and there are five regulatory subunits isoforms encoded by three distinct genes.[144] The Pik3r1 gene encodes p85α, and through the use of alternative start sites encodes p55α and p50α[145]; Pik3r2 encodes p85β, which is similar in size to p85α. The third gene, Pik3r3, encodes p55γ (originally called p55 PIK), which is homologous to p55α.[146] p85α and p85β contain two SH2 domains, an inter-SH2 domain that is tightly bound to the p110 catalytic subunit, two proline-rich regions, a bcr-homology domain, and an SH3 domain; p55α and p50α lack the SH3 domain and Bcr-homology domain, which is replaced by a unique 34- or 6-amino acid peptide at the NH_2-terminus, respectively. The p55γ also contains a unique N-terminal terminus of 34 amino acids.[146] Each isoform associates noncovalently with a p110 catalytic subunits, which protects the p110 from proteolysis and inhibits its intrinsic catalytic activity[147,148]; Disruption of p85α, p55α, or p50α decreases the p110 protein levels in mice leading to diminished PI 3-kinase activity.[149]

The IRS1 and IRS2 are ideal regulators of PI 3-kinase because they contain multiple YXXM motifs that are phosphorylated by the activated insulin receptor and bind strongly to the regulatory subunits SH2 domains.[98] Occupancy of both SH2 domains by phosphorylated YXXM motifs in IRS-proteins activates the PI 3-kinase catalytic subunits.[150] Interestingly, phosphorylation of p85α at Tyr688 by Src tyrosine kinase also relieves the inhibition of the p110 catalytic subunit imposed by the p85 regulatory subunit, but this is not involved in insulin signaling.[151] Moreover, GTP-bound Ras binds directly to the p110 catalytic subunit to enhance PI 3-kinase activity.[152–155]

All five regulatory subunit isoforms (p85α, p55α, p50α, p85β, and p55γ) bind to phosphorylated IRS-proteins and mediate the activation of PI 3-kinase; however, it is very difficult to dissect unique roles for each isoform. Deletion of p85α reduces PI 3-kinase activity by 50% to 60% in skeletal muscle and adipocytes. Unexpectedly, insulin sensitivity increases in these tissues and the mutant mice develop hypoglycemia with hyperinsulinemia.[156] Part of the dysregulation might arise from upregulation of p50α, which has higher affinity for the p110.[156,157] Moreover, disruption of all three α isoforms (p85α, p55α, and p50α) causes hypoglycemia with hypoinsulinemia, even though PI 3-kinase activity is reduced 80% to 90% in the liver and muscles.[149] Importantly, the activation of Akt is nomal in the mutant mice, indicating that Akt activation does not always follow the apparent PI 3-kinase activity.[149]

It is not clear why disruption of all the p85α improves insulin sensitivity. At least one third of the p85 regulatory subunits exist as monomers in normal cells because they are more abundant than either IRS1 or p110.[157] Excessive p85 might block formation of active catalytic complexes by the IRS-protein scaffolds. The p85β accounts for about 20% and 30% of total p85 proteins in liver and muscle, respectively, but deletion of p85β increases insulin sensitivity in mice.[158,159] The disruption of p55γ has not yet been reported, but it is abundant in brain where it might play a special role in neuronal function or plasticity. Clearly, different isoforms share redundancy in regulating PI 3-kinase, while providing unique signaling features; however, cell-based experiments with embryonic stem cells reveal the sensitive balance achieved by the expression of p85 isoforms.[160]

It is difficult to establish unique roles for individual isoforms of the p110 catalytic subunits in vivo. Disruption of the p110α is lethal to embryo development, preventing the analysis of its role in insulin action.[161] Interestingly, the p110β is upregulated during adipose differentiation of 3T3-L1 cells, and appears to be more sensitive to insulin than p110α.[162] Microinjection of anti-p110β, but not p110α, blocks insulin-stimulated GLUT4 translocation.[162] PPAR-γ agonist troglitazone increases the expression of p110β, which correlates with the enhancement of the activation of PI 3-kinase and Akt and an improvement of insulin sensitivity in humans.[163] Understanding how the catalytic and regulatory subunits modulate the insulin signal needs to be resolved.

THE PDK1/AKT SIGNALING CASCADE

The AGC (cAMP-dependent protein kinase [PKA]/protein kinase G/protein kinase C) kinase family mediates many physiologic responses triggered by growth factors or insulin.[164] Members of the family that play a role during insulin action include PDK1,[165] AKT,[166–168] p70 ribosomal S6 kinase ($p70^{S6K}$),[169,170] p90 ribosomal S6 kinase ($p90^{RSK}$),[171,172] and the serum- and glucocorticoid-induced protein kinase (SGK).[173] PDK1 phosphorylates the A loop in these kinases, which is essential for their activation.[164,168,174] Since PDK1 is constitutively activated, regulation is achieved by controlling the interaction of the substrates with PDK1.[175]

AKT is one of the best-characterized insulin-stimulated enzymes as it is broadly implicated in growth and metabolism.[168,175] Three distinct AKT genes exists in people and rodents: AKT1 (PKBα), AKT2 (PKBβ), and AKT3 (PKBγ)[168,175–177] Each isoform shares a similar structure, including an NH_2 terminal PH domain and a COOH terminal catalytic domain. PI(3,4)P_2 and PI(3,4,5)P_3, produced in the plasma membrane by the activated PI 3-kinase, binds to the PH domain at the NH_2-terminus of AKT and PDK1, which recruits both enzymes to the plasma membrane.[168,175] At the plasma membrane, the A loop in PKB becomes accessible to PDK1, resulting in the phosphorylation of Thr308 and activation of AKT (see Fig. 50-6).

Many studies suggest that AKT is required for insulin-regulated glucose homeostasis. Overexpression of membrane targeted, constitutively active mutants of AKT promote GLUT4 translocation to the surface of the plasma membrane to stimulate glucose uptake in muscle and adipose cells.[178,179] Conversely, inhibition of endogenous AKT1 and AKT2 by microinjection of an AKT substrate peptide (KRPRAATF) or antibodies against AKT inhibits GLUT4 translocation in response to insulin in 3T3-L1 adipocytes.[180] A dominant-negative AKT mutant prevents phosphorylation of glycogen synthase kinase-3 (GSK3), which inhibits insulin stimulation of glycogen synthesis.[181] Similarly, insulin-stimulated GLUT4 translocation is blocked by expression of a dominant-negative AKT in GLUT4 vesicles, but not by the same mutant expressed in the cytosol, suggesting that AKT may directly target components in GLUT4 vesicles.[182] In the liver, insulin inhibits glucose production by suppressing the expression of gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase and glucose-6-phosphatase.[183]

Akt isoforms display distinct cellular roles in response to insulin or IGF-1. One of the best examples is the effect of AKT knockout upon growth and metabolism. Overexpression

of constitutively active AKT1 increases heart size, whereas overexpression of dominant-negative AKT1 inhibits cardiac myocyte growth induced by constitutively active PI 3-kinase in transgenic mice.[184] Consistent with its role in mediating growth and survival signals, deletion of AKT1 causes growth retardation in mice; $AKT1^{-/-}$ cells are more susceptible to apoptosis.[185] These results are similar to the effects of Irs1 knockout,[117] and suggest functional importance of an IRS1→Akt1 cascade with respect to growth control during IGF stimulation.

Mice lacking Akt2 develop hyperglycemia, hyperinsulinemia, and glucose intolerance, resembling the phenotype of type 2 diabetes in humans.[186] $Akt1^{-/-}$ mice maintain normal blood glucose and insulin sensitivity, indicating that Akt1 is not required for metabolic effect of insulin and IGF-1.[186a,b] Recently, a mutation in the gene encoding human Akt2 explains the autosomal-dominant inheritance of severe insulin resistance and diabetes mellitus, validating the central importance of Akt signaling to insulin sensitivity in humans.[187]

SIGNALING BY AGC KINASES

PDK1 is a master regulator of many AGC kinases activated by insulin.[164] AGC kinases do not generally contain a PH domain for recruitment to the plasma membrane, so a different mechanism is needed to regulate their interaction with PDK1. A docking site, called the PIF pocket, is located on the small lobe of the PDK1 kinase domain, which interacts with a hydrophobic region in the COOH terminus of $p70^{S6K}$, SGK, and $p90^{RSK}$.[188] Ser/Thr phosphorylation of the hydrophobic motif (Phe-Xaa-Xaa-Phe/Tyr-*Ser/Thr*-Phe/Tyr) in these kinases promotes binding to the PIF pocket of PDK1 and phosphorylation of the A loop. The mammalian target of rapamycin (mTOR) can phosphorylate the hydrophobic motif of p70 S6K, promoting its interaction with PDK1[188]; phosphorylation of RSK isoforms by the Erk1/Erk2 mitogen-activated protein kinases (MAPKs) activates its second catalytic domain that phosphorylates the hydrophobic motif to promote its interaction with PDK1.[189,190] SGK is also activated by PDK1-mediated phosphorylation; however, the kinase that phosphorylates the hydrophobic motif in SGK is unknown.[188]

AKT SUBSTRATES MEDIATED DIVERSE BIOLOGIC PROCESSES

AKT phosphorylates many substrates that regulate various aspects of cellular activity, including CREB and FOXO proteins (gene expression); GSK3, RAF, eNOS, and IKK (cell function and growth); p27 kip1 (cell division); RHEB-GAP (mTOR activity); and BAD and caspase-9 (cell survival).[166,190-198] In many cases, Akt phosphorylation inhibits the target protein function: Akt phosphorylation inactivates the proapoptotic protein BAD, releasing BCL2 to inhibit apoptosis[199,200]; phosphorylation of caspase-9 by AKT inhibits its protease activity, which promotes cell survival[201]; AKT phosphorylates p27 KIP1 at Thr157, which retains p27KIP1 in cytoplasm to prevent the inhibition of cell-cycle progression.[202] AKT phosphorylates and inhibits serine/threonine kinases RAF and GSK3.[196,197] Since GSK3 phosphorylates and inhibits glycogen synthase, AKT promotes glycogen synthase activity by inhibiting GSK3 (see Fig. 50-6).

FORKHEAD TRANSCRIPTION FACTORS LINK PI 3-KINASE PKB/Akt TO THE NUCLEUS

Among the major nuclear regulators of metabolic gene expression, the so-called forkhead transcription factors, FOXO1, FOXO3a, FOXO4 (previously termed FKHR, FKHRL1, and AFX1) and FOXA1, FOXA2, and FOXA3 (previously termed HNF-3α, HNF-3β, and HNF-3γ), play major regulatory roles in many tissues, including pancreatic β cells, adipose tissue, and liver.[203] FOXO1, FOXO3a, and FOXO4 are phosphorylated by AKT and SGK during insulin stimulation. AKT phosphorylates FOXO1 at Ser253, which facilitates the subsequent phosphorylation of Thr24 and Ser316, resulting in the cytosolic accumulation that blocks transcriptional activity.[194] FOXA2, but not FOXA1 and FOXA3, contains a single threonine site that is phosphorylated by Akt, which also localizes it to the cytosol.[203]

FOXO1 is abundantly expressed in the liver and binds to a CAAAA(C/T)AA motif present in hepatic enzymes required for gluconeogenesis.[204] Phosphoenolpyruvate carboxykinase and glucose-6-phosphatase are dramatically reduced in $Foxo1^{+/-}$ mice owing to insufficient nuclear Foxo1. Conversely, overexpression of Foxo1 in liver increases the levels of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, and induces insulin resistance and diabetes in the transgenic mice.[205] FOXO1 interacts with the peroxisome proliferator-activated receptor-γ coactivator (PGC)1α, a transcriptional coactivator of CREB, HNF-4α, and the glucocorticoid receptor, to fully upregulate phosphoenolpyruvate carboxykinase.[206] Association of Foxo1 with PGC1α is prevented by Akt-mediated phosphorylation, which inhibits the effect of PGC1α on gene expression.[206]

FOXO1 also plays an important role in coupling insulin signaling to adipocyte differentiation. FOXO1 expression is upregulated in preadipocytes coincidently with growth arrest and peaks at the onset of terminal differentiation. However, constitutively active FOXO1 blocks adipocyte differentiation, suggesting that inactivation of FOXO1 by AKT-mediated phosphorylation is probably essential once preadipocytes are in growth arrest. The inability to properly phosphorylate Foxo1 in mice lacking Irs1 and Irs2 can explain the lipodystrophic in $Irs1^{-/-}$::$Irs3^{-/-}$ mice.[115] Disruption of the insulin receptor also reduces adipocytes mass in mice, suggesting that it is an essential upstream kinase in this regulatory mechanism.[44]

Nuclear FOXO1 also inhibits β-cell function. Expression of a constitutively active FOX1 protein causes β-cell failure that dysregulates glucose homeostasis in mice.[10] By contrast, heterozygous disruption of Foxo1 promotes β-cell function and survival, and β-cell function in $Foxo1^{+/-}$::$Irs2^{-/-}$ mice is restored to normal, preventing the progression to diabetes.[10] Thus, in β cells, the IRS-2/PI 3-kinase branch of the insulin/IGF signaling cascade is an important pathway FOXO1 phosphorylation by AKT.

THE REGULATION OF PROTEIN SYNTHESIS

THE mTOR CASCADE

The mTOR cascade integrates nutrient availability with insulin signaling to control protein synthesis and cell growth. mTOR was originally isolated as the target of the immunosuppressive agent rapamycin, which binds to FKBP12 to form a complex that inhibits mTOR activity.[185] Components of the mTOR cascade are highly conserved from yeast to mammals, providing a common mechanism that is sensitive to the nutrients such as amino acids and glucose.[185,207]

Downstream effectors of mTOR—$p70^{S6K}$ and 4E-BP1—control protein synthesis and cell growth. The $p70^{S6K}$ occurs as two isoforms called $p70^{S6K1}$ and $p70^{S6K2}$. Disruption of S6K1 gene in mice causes glucose intolerance due to reduced size of pancreatic islet β cells; however, peripheral insulin action is enhanced suggesting that $p70^{S6K1}$ contributes to feedback inhibition of insulin signaling.[208] $p70^{S6K1}$ is activated by multisite phosphorylation events from various kinase activities in response to insulin and other mitogens when amino acids are available.[209] Nutrient sensitivity arises because mTOR mediates the phosphorylation of the hydrophobic motif needed for interaction of $p70^{S6K1}$ with PDK1 (see Fig. 50-6).

The regulation of the mTOR cascade is complex, but recent discoveries reveal how AKT acts to regulate mTOR function

during insulin stimulation, and how energy depletion inhibits mTOR through the adenosine monophosphate kinase (AMPK). The ubiquitously expressed small G protein, Rheb (RAS homologue expressed in brain) is an mTOR activator.[185,210] During GTP binding, Rheb promotes mTOR activity, whereas GTP hydrolysis inactivates Rheb-stimulated mTOR activity (see Fig. 50-6*B*). GTP hydrolysis is catalyzed by TSC2, a GTPase-activating protein for RHEB.[210] TSC2 complexes with TSC1 to form a heterodimer that is essential to prevent constitutive activation of mTOR (see Fig. 50-6*B*). Mutations in TSC1 or TSC2 that dysregulate GTP hydrolysis cause nonmalignant tumors in various tissues called hamartomas.[211]

TSC1::TSC2 → Rheb cascade is required for mTOR to sense energy depletion (see Fig. 50-6*B*). Cellular AMP levels rise during an energy deficit, which activates AMPK.[212,213] During AMP binding, the A loop of the AMPK, is accessible to the constitutively active LKB1. Recent results suggest that Rheb-GTPase-activating protein activity of the TSC1::TSC2 complex is increased by AMPK phosphorylation.[214] This regulatory model is consistent with the effects of inactivating LKB1 mutations that lead to hamartomas with similar characteristics to those observed in tuberous sclerosis patients.[212,213]

REGULATION OF PROTEIN SYNTHESIS BY INSULIN

Insulin stimulates protein synthesis by altering the intrinsic activity or binding properties of key translation initiation and elongation factors (eIFs and eEFs, respectively), as well as critical ribosomal proteins. This occurs via phosphorylation and/or sequestration of repressive factors into inactive complexes. Components of the translational machinery that are targets of insulin regulation include eIF2B, eIF4E, eEF1, eEF2, and the S6 ribosomal protein.[215] The eIF2B multi-subunit guanine nucleotide exchange factor for eIF2 is kept inactive via phosphorylation of the eIF2Bε subunit at Ser535 by GSK3.[216] Inhibition of GSK3 during insulin-stimulated Akt phosphorylation reduces eIF2B phosphorylation promoting the formation of eIF2 GTP that recruits the initiator methionyl-tRNA to the ribosome.[216–218] The insulin-stimulated activation of eIF2B leads to an overall increase in translation initiation.[219] Diabetic rats have significantly lower eIF2B activity in muscle.[220]

The eIF4F complex, including eIF4A, 4G, 4E and other proteins, is required for cap-dependent translation initiation. The mRNA cap-binding protein, eIF4E, is inactive during association with 4E-BP1 (see Fig. 50-6). Insulin activates eIF4E by stimulating mTOR-mediated phosphorylation of 4E-BP1. Phosphoyrlated 4E-BP1 dissociates to facilitate the interaction between eIF4E and eIF4G, the scaffold protein for the eIF4F complex.[221] MNK, an insulin-stimulated kinase activated through the RAS/ERK cascade, also resides in the eIF4F complex where it phosphorylates eIF4E at Ser209 (see Fig. 50-6).[222,223] Phosphorylation of eIF4E increases the binding affinity for mRNA caps, enhancing translation initiation. The phosphorylation of 4E-BP1 has an important systemic role in insulin action, as deletion of its gene increases insulin sensitivity and dramatically decreases white adipose tissue depots.[224]

Insulin also stimulates translation elongation by phosphorylation of eEF1 by an as yet undetermined mechanism. Insulin-stimulated phosphorylation of the ribosomal S6 protein by $p70^{S6K}$ may promote elongation of specific mRNAs corresponding to components of the translational machinery.[225] An elongation factor critical for ribosomal translocation along the mRNA, eEF2, is inactive when phosphorylated at Thr56 by the eEF2 kinase (eEF2K).[226,227] Insulin stimulates the dephosphorylation of eEF2 via a rapamycin-sensitive route potentially involving the phosphorylation and inactivation of eEF2K by $p70^{S6K}$.[226] In vitro, $p70^{S6K}$ phosphorylates eEF2K at Ser366, a modification that greatly reduces the activity of the kinase.[226] Activation of PKCζ is also required for insulin-stimulated protein synthesis although its downstream effectors remain undetermined.[228] Convergence between nutritional and insulin signals occurs at the level of mTOR activity regulation (see Fig. 50-6*B*).[225,229,230]

THE REGULATION OF GLUCOSE TRANSPORT AND METABOLISM

GLUCOSE TRANSPORTERS

Glucose transport is the prototype insulin response. The molecular mechanisms linking insulin signals to increased glucose influx into adipose and muscle has been difficult to resolve. All cells express one or more members of the glucose transporter family, including 12 isoforms organized into 3 classes: class I includes the well-characterized GLUT1, GLUT2, GLUT3, and GLUT4 transporters; class II includes GLUT5, GLUT7, GLUT9, and GLUT11; and class III includes GLUT6, GLUT8, GLUT10, and GLUT12.

GLUT4 is an insulin-responsive glucose transporter found in skeletal muscle, adipose cells, and heart. GLUT4 is glycosylated in the first exofacial loop, and contains a phosphorylation site (Ser488) and a di-leucine motif in its COOH terminus that plays a role in endocytosis.[231] Before insulin stimulation, GLUT4 resides in intracellular vesicles where it is unavailable to transport glucose across the plasma membrane. However, insulin stimulation promotes translocation of GLUT4-containing vesicles to the plasma membrane to increase the rate of glucose influx (Fig. 50-7).

Insulin does not regulate the translocation of other class 1 glucose transporters. GLUT1 is expressed in most cells and tissues and resides permanently on the plasma membrane where it constitutively transports glucose from the extracellular space into the cell. Although insulin does not stimulate translocation of GLUT1, chronic insulin treatment increases the levels of cellular GLUT1 via the $p21^{RAS}$ → ERK kinase pathway.[232,233] GLUT2 is mainly expressed in liver and pancreatic β cells, where its relatively low affinity but high transport capacity provides a constant flux of glucose into these organs at physiologic plasma glucose concentrations (5 mM). In the β cell, the uptake of glucose through GLUT2 is the first step in the detection of circulating glucose levels needed to stimulate insulin secretion. GLUT3 has a relatively high affinity for glucose and is most abundant in the central nervous system where glucose concentrations are lower than in the bloodstream. GLUT8 might be insulin sensitive as it contains GLUT4-like intracellular targeting motifs and is widely expressed in many insulin-responsive (as well as insulin-independent) tissues[234,235]; GLUT12 is expressed in prostate, small intestine, placenta, skeletal muscle, and adipocytes and might also be insulin responsive.[236,237]

THE REGULATION OF GLUT4 BY INSULIN

During insulin stimulation, GLUT4-containing vesicles move from their intracellular storage compartment and reach the cell surface through targeted exocytosis (see Fig. 50-7). Simultaneously, GLUT4 endocytosis is repressed, leading to an overall increase in glucose uptake.[238] It is not clear whether the effect of insulin to stimulate glucose uptake in muscle and fat can be accounted for in its entirety by translocation, as other factors that increase transporter activity could be involved. Substantial evidence exists to suggest that at least two distinct pathways mediate the effect of insulin on glucose transport. One pathway relies on PI 3-kinase activation of downstream effectors, including PDK1, AKT, and atypical protein kinase C (PKC) isoforms PKCζ and PKCλ.[239–241] The PI 3-kinase-independent pathway includes the activation of TC10, a Rho family G protein localized to lipid rafts in the plasma membrane.[242]

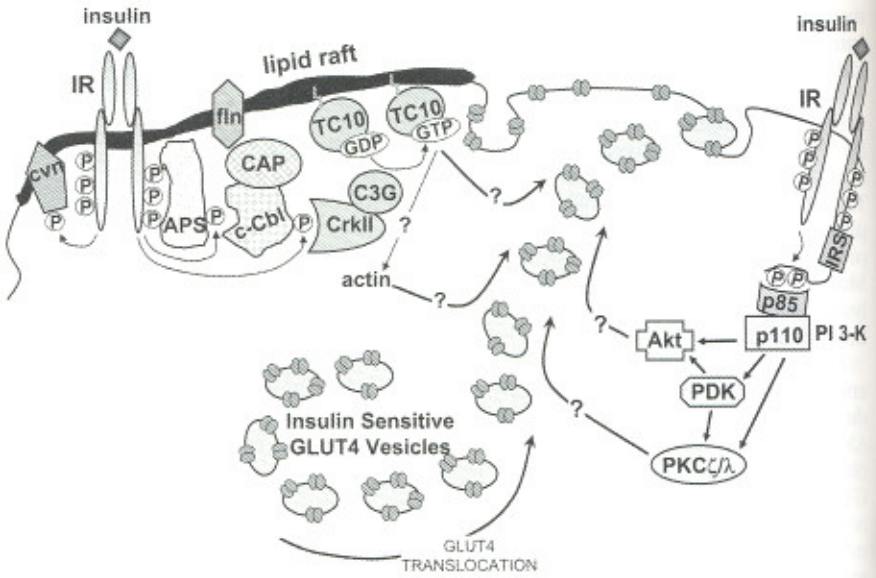

Figure 50-7 A diagram of components that mediate insulin-stimulated glucose transport. The PI 3-kinase sensitive branch of the pathway is shown on the right, including IRS-proteins, AKT and the atypical protein kinase C isoforms, and PKCζ/λ and PKCτ. The PI 3-kinase independent branch of the pathway is shown on the left and includes the APSCbl cascade. Activation of these pathways by insulin promotes the accumulation of GLUT4-containing vesicle in the plasma membrane. See the text for a detailed description of these pathways.

ATYPICAL PROTEIN KINASE C REGULATES GLUT4 TRANSLOCATION

PKCζ and PKCλ are implicated in the regulation of GLUT4 translocation in adipose tissue and muscle.[180,243,244] Constitutively active PKCλ or PKCζ promote GLUT4 translocation and glucose uptake in adipocytes and muscles in the absence of insulin.[244–247] Overexpression of inactive PKCλ inhibits insulin-stimulated activation of endogenous PKCλ, which inhibits GLUT4 translocation and glucose.[246] Microinjection of PKCλ antibodies inhibits insulin-induced GLUT4 translocation.[248] PKCζ also promotes insulin-stimulated glucose uptake. Insulin-promoted activation of PKCζ is reduced in obese patients with insulin resistance[249]; impaired PKCζ activity is associated with obesity-induced insulin resistance in monkeys.[245,250] It will be important to understand how the atypical PKC isoforms, PKB and other related AGC kinases, interact with cellular components to regulate GLUT4 translocation (see Fig. 50-7).

PI 3-KINASE-INDEPENDENT REGULATION OF GLUT4 TRANSLOCATION

The activation of TC10, a small G protein of the RHO family localized to lipid rafts in the plasma membrane might also regulate GLUT4 translocation.[242] Lipid rafts are microdomains on the cell surface containing specific glycolipids, sphingolipids, proteins, and cholesterol that do not mix with other lipids within the plasma membrane. The insulin receptor is localized to caveolae, a specific lipid raft subset, where it associates with caveolin (cvn) and phosphorylates it on tyrosine residues.[242] The localization of the insulin receptor to caveolae might be critical for the activation of the PI 3-kinase-independent signals leading to GLUT4 translocation in adipocytes.[251] During insulin stimulation, APS binds to the A loop of the activated insulin receptor. Upon tyrosyl phosphorylation, APS recruits c-Cbl for tyrosine phosphorylation by the insulin receptor.[239,252] In insulin-responsive cells, c-Cbl is constitutively associated with the CAP::flotilin complex, which stabilizes the growing caveolae-localized insulin receptor complex.[239] The tyrosine-phosphorylated c-Cbl also recruits the CRK II, which constitutively binds C3G, a guanine nucleotide-exchange factor that catalyzes the exchange of GTP for GDP in the small G protein TC10.[112] The GTP-bound and activated TC10 results from the localization of the CRK II-C3G complex to the lipid raft microdomain where the small G protein resides.[253] Proper localization to the lipid raft through specific posttranslational modifications of TC10 and its activation appears to promote insulin-stimulated PI 3-kinase-dependent GLUT4 translocation and glucose transport.[253] This mechanism requires careful validation, as recent evidence suggests that depletion of Cbl isoforms, CAP, or CRK II does not impair insulin-stimulated GLUT4 translocation.[254]

GENETIC, METABOLIC, AND REGULATORY ASPECTS OF INSULIN SIGNALING

Insulin resistance is a common occurrence among human populations, and its association with obesity, advancing age, and physical inactivity is especially common in industrialized nations.[255] Increased insulin secretion can compensate for insulin resistance, but type 2 diabetes occurs when sufficient insulin is no longer secreted quickly enough.[1] A predominant cause for the imbalance between insulin action and insulin secretion in type 2 diabetes is difficult to establish, suggesting that a combination of defects is involved. Moreover, environmental challenges owing to chronic inflammatory or metabolic stress contribute to insulin resistance.

Polymorphisms have been identified in human genes encoding proximal signaling components that might contribute to metabolic disease. Although insulin receptor polymorphisms provide important insight into receptor function, they fail to uncover a general cause of type 2 diabetes.[256] A few polymorphisms in the gene for IRS-1 have been found, some of which are more common in type 2 diabetic patients[257]; however, they do not reveal a simple genetic basis for insulin resistance.[258] Gly972Arg mutation moderately decreases insulin-stimulated PI 3-kinase activation in cultured cells,[259,260] and associates with peripheral insulin resistance and impaired insulin secretion in certain backgrounds.[261,262] Two polymorphisms in IRS2, including Gly1057Asp and Gly879Ser substitutions, associate in females with impaired glucose tolerance, polycystic ovarian syndrome, and obesity.[261,263,264] A common polymorphism in p85α associates with a moderately reduced insulin sensitivity during an intravenous glucose tolerance test.[265] Similarly, mutations in AKT2 validate the importance of this kinase for insulin signaling in humans, but they do not explain insulin resistance encountered frequently in people.[187] Thus, genetic defects in the insulin signaling system validate the importance of the insulin signaling cascade, but they fail to explain the common causes of type 2 diabetes.

LESSONS LEARNED FROM INSULIN RECEPTOR MUTATIONS

Humans with rare mutations in the insulin receptor gene confirm that the insulin receptor mediates critical growth and metabolic signals. The informative polymorphisms impair synthesis or translocation of the receptor to the plasma membrane surface, insulin binding, transmembrane signaling, or endocytosis.[258] Depending on the allele, homozygous or double heterozygous individuals develop severe syndromes of insulin resistance with altered growth, such as leprechaunism or Rabson-Mendenhall syndrome.[256] Most affected individuals are heterozygous for the defective allele, but display severe insulin resistance owing to the dominant-negative effects on the functional receptor in covalent dimmers.[258] Interestingly, males with severe insulin resistance owing to receptor defects are hyperinsulinemic and usually not diabetic, suggesting that compensatory β-cell function is not impaired by dysregulated insulin receptor function. Thus, insulin resistance alone is insufficient to cause diabetes while β-cells secrete sufficient insulin to compensate for the resistance, reinforcing the role of β-cell failure in common type 2 diabetes.

Genetically altered mice reinforce these notions, and provide insight into how and where defects in insulin sensitivity and secretion occur. Disruption of the insulin receptor gene in mice does not significantly alter development; however, $IR^{-/-}$ mice develop hyperinsulinemia, hyperglycemia, and ketoacidosis immediately after birth.[30] By contrast, mice lacking the insulin receptor in skeletal muscle almost completely eliminates insulin signaling and insulin-stimulated glucose transport in isolated muscle; however, the mice display nearly normal glucose homeostasis and never develop hyperinsulinemia or physiologic insulin resistance.[266] This result contrasts the usual view that muscle is responsible for the vast majority of insulin-stimulated glucose disposal in the body, and the failure of insulin signaling in muscle in a primary cause of type 2 diabetes in humans.[267,268] By contrast, genetically altered mice deficient in GLUT4 in skeletal muscle develop insulin resistance and glucose intolerance.[269,270] Increases in IGF-1 receptor function in skeletal muscle apparently compensate in the absence of the insulin receptor gene.[271]

The progression of glucose intolerance to diabetes occurs when the β cells fail to produce enough insulin to overcome systemic insulin resistance. Mice with β-cell-specific deletion of the insulin receptor display a loss of first-phase insulin secretion in response to glucose.[272] This resembles the defect in insulin secretion observed in humans with type 2 diabetes.[273] Both insulin and IGF-1 receptors appears to contribute to β-cell function, possibly through tyrosine phosphorylation of Irs2.

ADIPOKINES AND INSULIN SIGNALING

Communication between adipocytes, other peripheral tissues, and the central nervous system reveals new insight into the relation between obesity and insulin resistance.[273,274] Adipose tissue secretes various proteins and metabolites that inhibit insulin signaling such as free fatty acids (FFAs), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and resistin, or those that promote insulin signaling such as adipocyte complement-related protein of 30 kilodaltons (adiponectin) and leptin.[50,275–280]

Leptin correlates closely with fasting insulin concentrations and the percentage of body fat, revealing leptin levels as a marker of obesity and insulin resistance.[277] Leptin binding to the long form of the leptin receptor promotes tyrosine phosphorylation of the cytoplasmic domain and the associated Janus kinase.[281,282] One of the phosphorylation sites in the leptin receptor (Tyr1138) binds STAT3, which is required for nutrient homeostasis by promoting melanocortin signaling.[283] Leptin might influence β-cell physiology by regulating levels of triglycerides and/or free fatty acids in the β cell, and reduce insulin secretion.[284] Thus, leptin signaling frames a rational basis to link obesity to the disruption of β-cell function at the molecular level.

TNF-α is an endogenous cytokine produced by macrophages and lymphocytes after inflammatory stimulation. Adipocytes of obese animals and humans overexpress TNF-α in positive correlation to body mass index and hyperinsulinemia; weight reduction decreases TNF-α expression.[285,286] TNF-α production in adipose tissue arises, at least in part, from the recruitment of macrophages.[287] TNF-α treatment increases serine phosphorylation of IRS-proteins, which inhibits insulin-stimulated tyrosine phosphorylation and impairs insulin signaling.[280,288,289] Disruption of both TNF-α receptor isoforms improves insulin sensitivity.[290] Troglitazone reduces the ability of TNF-α to cause insulin resistance, providing a rational mechanism by which thiazolidinediones might enhance insulin action.[291] Thus, localized production of TNF-α might link obesity to insulin resistance.

Adipose tissue macrophages increase in obesity and are responsible for significant amounts of IL-6 expression.[287] IL-6 derived from omental fat depots drains directly into the portal venous system, which can regulate hepatic triglyceride production, as well as insulin-stimulated glycogenesis.[292–294] Moreover, IL-6 treatment of primary hepatocytes and 3T3-L1 adipocytes impairs insulin signaling by reducing IRS-1 tyrosine phosphorylation and PI 3-kinase activity.[293–295] IL-6 administration into rodents and humans induces hepatic gluconeogenesis leading to hyperglycemia.[296] IL-6 inhibits lipoprotein lipase activity, which increases lipolysis and lipodystrophy.[297–299] Thus, locally produced IL-6 could regulate adipocyte function through an autocrine/paracrine mechanism. Since IL-6 is opposing the action of insulin under these conditions, it might induce lipolysis, at least in part, by inhibiting insulin action. IL-6 might cause insulin resistance by exerting inhibitory effects on IRS-1, glucose transporter GLUT4, and proxisome proliferation-activated receptor (PPAR-γ) gene transcription in 3T3-L1 adipocytes.[295]

Adiponectin is expressed exclusively by differentiated adipocytes.[300] Adiponectin normally circulates at a high concentration (1.9–17.0 μg/mL), which promotes insulin sensitivity. Adiponectin levels fall during obesity, but can be normalized upon weight loss, caloric restriction, or thiazolidinedione treatment that also increases insulin sensitivity. In general, adiponectin influences whole-body metabolism by enhancing insulin sensitivity in muscle and liver, and by increasing fatty acid oxidation in muscle.[301,302] Long-term administration of adiponectin to mice fed a high-fat diet causes profound weight loss by enhancing free fatty acid oxidation in muscles without affecting food intake. Some of these effects might be linked to the activation of AMP kinase, the phosphorylation of acetyl coenzyme A carboxylase, increased glucose uptake and fatty-acid oxidation, and reduced hepatic gluconeogenesis.[301] Adiponectin mediates these effects during association with membrane receptor, adipo1 or adipo2.[303] The mechanism that couples these receptors to AMP kinase and other signals is important to understand.

MULTISITE Ser/Thr PHOSPHORYLATION OF IRS-PROTEINS

Considerable data suggests that various pathologic conditions associated with insulin resistance promote serine/tyrosine phosphorylation of IRS-proteins. Stress-induced cytokines like TNF-α cause insulin resistance, at least in part, by serine phosphorylation of IRS-1 and IRS-2.[286,304] Disruption of the TNF-α receptor improves insulin sensitivity and glucose tolerance in obese mice.[290,305] Thus, the inhibitory effect of TNF-α appears to function through serine phosphorylation of the IRS-proteins.[53]

IRS-1 and IRS-2 each contain more than 100 potential serine/threonine phosphorylation sites; therefore, mapping

the physiologically relevant ones is difficult. Many Ser/Thr-kinases phosphorylate IRS-proteins, including Raf, MEK, MAPK, p90^{rsk}, Rho kinase (ROK-α), JNK, and PKC isoforms, and kinases downstream of the PI 3-kinase cascade: PDK1, AKT, mTOR, p70^{S6K}, GKK3β.[225,306–314] The inhibitory role of the PI 3-kinase/AKT cascade might explain why partial inhibition of PI 3-kinase activity by disruption of various regulatory subunits increases insulin sensitivity.[157–159,315]

JNK binds directly to IRS1 and phosphorylates Ser307, which reveals a direct mechanism for inhibition of insulin signaling.[139] Ser307 phosphorylation inhibits tyrosine phosphorylation of IRS1 and the ability of IRS1 to activate the PI 3-kinase/AKT pathway in response to insulin.[140,306] Free fatty acids, which contribute to insulin resistance in obesity, also promote Ser307 phosphorylation through the activation of PKC.[316] The role of Ser307 phosphorylation has become a target of intense investigation by various groups.[141,316–320] Ser307 is phosphorylated in response to insulin or TNF-α in cultured adipocytes and muscles from mouse, rat, and human.[140] It is poorly phosphorylated in JNK$^{-/-}$ mice, suggesting that JNK-mediated phosphorylation of IRS1 is physiologically important (Fig. 50-8).[321]

IκB kinase β (IKKβ) cascade, which is implicated in inflammation-related insulin resistance, also mediates Ser307 phosphorylation.[322,323] IKKβ promotes TNF-α signaling during chronic obesity and trauma; heterozygous disruption of IKKβ protects against the development of insulin resistance during high-fat feeding and in obese leptin-deficient (ob/ob) mice.[318] IKKβ inhibitors (aspirin and salicylates) block TNF-α-induced Ser307 phosphorylation,[141] improving insulin sensitivity in obese rodents and in type 2 diabetes patients.[322,324,325]

The phosphorylation of other residues in IRS1 also contribute to inhibition of insulin signaling: Ser612, Ser632, Ser636, Ser662, Ser731, and Ser789.[326] Recently, PKCδ was shown to phosphorylate several serine residues that inhibit IRS1 tyrosine phosphorylation- Ser307, Ser323, and Ser574.[327] AMP kinase, which is activated by increased AMP levels during energy depletion, associates with IRS1 and phosphorylates S789, which promotes insulin-stimulated tyrosine phosphorylation. The phosphorylation of Ser302 might prime IRS1 for tyrosine phosphorylation, revealing a positive role for serine phosphorylation.[328]

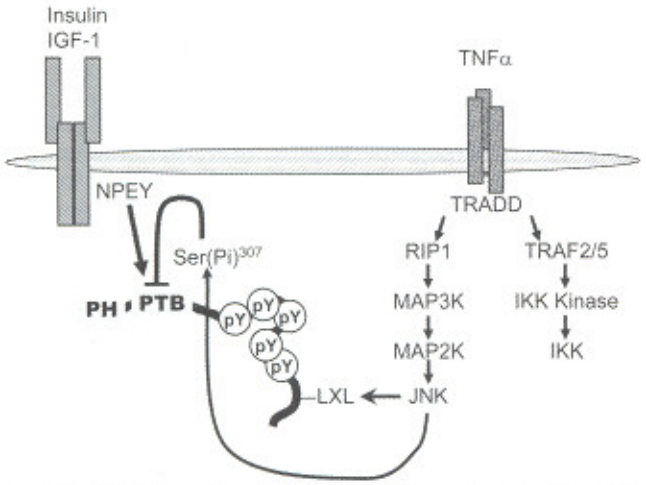

Figure 50-8 TNF-α-induced inhibition of IRS-protein signaling. TNF-α binding to TNFR1 results in recruitment of TRAF2/5, RIP1, and FADD through the adaptor protein TRADD. TRAF2/5 and RIP1 appear to lead to activation of the protein kinases JNK and IKK. Activated JNK associates with IRS1 and the JNK-binding LXL motif and promotes phosphorylation of Ser307. Phosphorylation of Ser307 inhibits PTB domain function and inhibits insulin/IGF stimulated tyrosine phosphorylation and signal transduction. FADD, FAS-associated death domain protein; IKK, IκB kinase; JNK, c-Jun N-terminal kinase; RIP1, receptor-interacting protein 1; TNF-α , tumor necrosis factor-alpha; TNFR1, TNF receptor type 1; TRAF2, TNF-receptor-associated factor 2.

Several mechanisms have been proposed to explain how serine phosphorylation can regulate IRS1 signaling. Ser307 phosphorylation inhibits PTB domain function, which uncouples IRS1 from the insulin receptor.[138,329,330] Other sites might electrostatically block access to nearby tyrosine phosphorylation sites. Some phosphoserine residues in IRS1 bind 14-3-3 isoforms, which can target IRS1 to subcellular compartments.[331] Under conditions that activate the mTOR cascade, including prolonged insulin-stimulation but also other mTOR agonists, IRS-proteins are strongly degraded by the 26S proteasome.[332] IRS1 and IRS2 degradation is inhibited in various cell backgrounds by rapamycin, suggesting that the mTOR cascade plays a central role in this process.[332–334] Consistent with this model, constitutive activation of the mTOR cascade by mutations in TSC1 or TSC2 promote hyperphosphorylation of IRS1 and IRS2, leading to their degradation and insulin resistance (see Fig. 50-6*B*).[335] Degradation of IRS-proteins through the constitutively activated mTOR cascade reveals a potential explanation for the inhibitory effect of excess nutrients upon insulin signaling, and the positive effects of adiponectin upon insulin action (see Fig 50-6*B*).

SOCS1- AND SOCS3-MEDIATED IRS-PROTEIN DEGRADATION

Whereas Ser/Thr phosphorylation is a reversible processes that might be ideal for short-term negative regulation of insulin action, proteolysis of IRS1 and IRS2 causes long-term inhibition. In cultured cells, degradation of IRS1 and IRS2 is stimulated by TNF-α, interferon-γ, insulin, IGF-1, osmotic shock, platelet-derived growth factor, endothelin-1, free fatty acids, PMA, and inhibitors of serine/threonine phosphatase; and the degradation is blocked by 26S proteasome inhibitors.[336] In addition, ubiquitinylation of IRS-proteins is increased in response to chronic insulin and IGF-1, indicating that the ubiquitin/proteasome system is involved.[337,338]

One way that IRS-proteins are recruited to the elongin B/C-based ubiquitin-ligase is through SOCS1 or SOCS3 (Fig. 50-9).[336] These SOCS isoforms are cytokine-inducible gene products that suppress activity of various cytokine receptors by binding via their SH2 domain to the associated Janus kinase tyrosine kinases.[339] Overexpression of SOCS1 or SOCS3 also promotes ubiquitinylation and degradation of IRS1 and IRS2.[336] These SOCS isoforms are composed of an SH2 domain that binds to IRS1 or IRS2, and a BC box within the canonical SOCS box that binds to elongin C. Elongin C is a component of an E3-ubiquitin ligase that includes elongin B, a cullin family member, and Rbx-1.[340] This complex also associates with an ubiquitin-conjugating enzyme (E2) that catalyzes the transfer of ubiquitin from the ATP-dependent ubiquitin-activating enzyme (E1) to the SOCS-targeted protein.[339] Ubiquitin, a 76-amino acid peptide can also be ubiquitinylated on Lys48, forming polyubiquitin chains that target IRS-proteins for proteasome degradation. SOCS1 or SOCS3 mutants that fail to bind elongin BC-based ubiquitin ligase prevent ubiquitinylation and degradation of IRS1 and IRS2. The expression of recombinant SOCS1 in mouse liver by adenovirus-mediated gene transfer dramatically reduces hepatic IRS1 and IRS2, causing insulin resistance, whereas dysfunctional SOCS1 mutants have no effect.[336] Thus, one mechanism of IRS-protein degradation is through SOCS1- or SOCS3-mediated recruitment of a bc-based ubiquitin ligase (see Fig. 50-9).

PHOSPHOTYROSINE PHOSPHATASE 1B

Phosphotyrosine phosphatase 1B (PTP1B) directly dephosphorylates the insulin receptor, so it has a major effect on strength and duration of the insulin response.[341,342] PTP1B might also dephosphorylate IRS-proteins during their interaction with

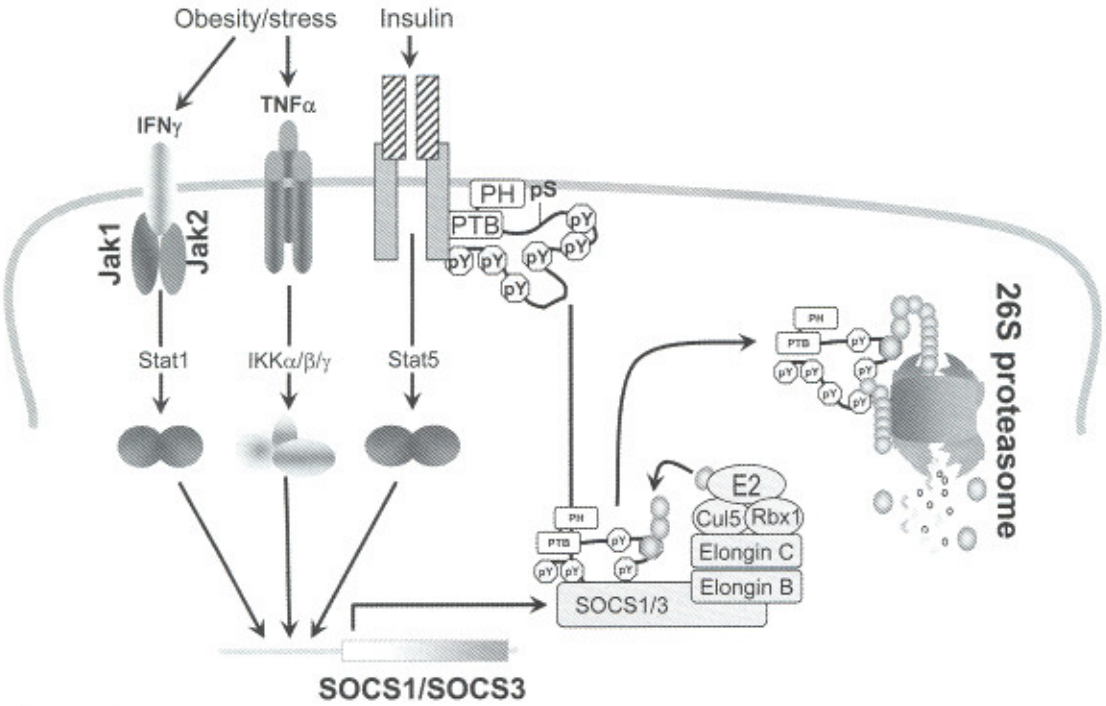

Figure 50-9 A potential mechanism of cytokine-induced insulin resistance based on the induced expression of SOCS1/3. Most proinflammatory cytokines that cause insulin resistance also induce the expression of SOCS family members.[384,385] SOCS family members contain an NH_2-terminal SH2 domain and a COOH-terminal SOCS box.[384,385] SOCS proteins might target proteins for ubiquitinylation and degradation, because the conserved SOCS box associates with elongin BC-containing ubiquitin ligase E3.[386–388] Ubiquitinylation is expected to promote degradation of IRS-protein through the 26S proteasome.

the insulin receptor, as PTP1B exhibits the highest activity toward IRS-1 among four other candidate protein-tyrosine phosphatases (PTP1B, SHP2, LAR, and LRP).[343] Overexpression of PTP1B inhibits insulin-stimulated phosphorylation of the insulin receptor and IRS-1, whereas neutralization of PTP1B by antibodies enhances insulin signaling.[344,345] $PTP1B^{-/-}$ mice display increased insulin sensitivity as revealed by enhanced phosphorylation of the insulin receptor and IRS-1 in muscle and liver.[52] PTP1B also plays a role in hypothalamic sensing of nutrient homeostasis, as $PTP1B^{-/-}$ mice are resistant to diet-induced obesity and insulin resistance; and energy dissipation in the $PTP1B^{-/-}$ mice is increased.[52,346] In ob/ob or db/db genetic background, reduction of PTP1B using antisense oligonucleotides improves insulin sensitivity in liver and fat, normalizing hyperglycemia.[347]

PTP1B has broad substrate specificity, but disruption of the PTP1B gene has its greatest effect on the insulin receptor. The functional specificity toward the insulin receptor appears to be determined by a combination of PTP1B targeting and the itinerary of the activated insulin receptor. Although PTP1B dephosphorylates epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors, they are largely degraded on internalization so dephosphorylation might not be important physiologically. By contrast, the insulin receptors are recycled to the plasma membrane so inactivation might be more dependent on dephosphorylation.[348] Consistent with this hypothesis, PTP1B associates rapidly with activated insulin receptor, as revealed by bioluminescence resonance energy transfer.[349] This approach to measure protein-protein interactions is very sensitive and reveals an almost immediate association between the activated insulin receptor and PTP1B. Transient interactions also occur before insulin stimulation suggesting that PTP1B might be important for maintaining the insulin receptor in an unphosphorylated inactive state. The insulin receptor associates most strongly with endoplasmic reticulum associated PTP1B, suggesting that internalization of the insulin receptor is an important step in its inactivation by the constitutively active PTP1B.[348,349]

PTEN

PTEN was identified as a tumor suppressor located at 10q23.[350] PTEN contains a phosphatase domain at its N terminus and C2 domain and a PDZ-binding motif at the C terminus. The C2 domain might mediate its interaction with membrane lipids, whereas the PDZ-binding motif may bind to PDZ domain-containing proteins that associated with actin cytoskeleton to localize PTEN at plasma membrane subdomains. PTEN inhibits accumulation of both $PI(3,4)P_2$ and $PI(3,4,5)P_3$ induced by both constitutively active p110 and insulin stimulation.[351] Since PTEN efficiently dephosphorylates phosphoinositide at the 3 position both in vitro and in vivo, it is a negative regulator of PDK1, AKT, and their downstream targets (see Fig. 50-6).[314,352,353] Consistent with this conclusion, AKT activity is elevated in PTEN hypomorphs in accordance with the elevated $PI(3,4,5)P_3$. $PTEN^{+/-}$ mice die before birth, and $PTEN^{+/-}$ mice display increased tumor incidence.[354]

The expression of PTEN in 3T3L1 adipocytes inhibits insulin-stimulated phosphorylation and activation of Akt and p70 S6K; and it inhibits GLUT4 translocation and glucose uptake in response to insulin. As expected, PTEN does not inhibit tyrosine phosphorylation of IRS1 or its associated PI 3-kinase.[355] By contrast, inactive PTEN mutants enhance insulin-stimulated accumulation of $PI(3,4)P_2$ and $PI(3,4,5)P_3$, activation of Akt and glucose uptake in 3T3-L1 adipocytes.[351] In addition, specific inhibition of PTEN expression by antisense oligonucleotides normalized blood glucose concentrations in db/db and ob/ob mice, dramatically reduced insulin concentrations in ob/ob mice, and improved glucose tolerance of db/db mice.[356] Thus, PTEN activity downregulates insulin pathways and may contribute to insulin resistance and β-cell failure in diabetes.[7]

IRS-2 SIGNALING AND THE COMMON PATH TO DIABETES

Mice lacking the gene for $IRS1^{-/-}$ or $IRS2^{-/-}$ show marked effects of insulin resistance, including impaired peripheral glucose utilization.[116,357,358] However, metabolic dysregulation is more severe in $IRS2^{-/-}$ mice owing to excessive gluconeogenesis, decreased hepatic glycogen synthesis, and unsuppressed plasma free fatty acid/glycerol levels during the hyperinsulinemic-euglycemic clamp.[358] Progression to diabetes also depends on failure of pancreatic β cells to secrete sufficient insulin quickly enough to maintain normal serum glucose levels relative to the prevailing insulin sensitivity.

The insulin/IGF-1/IRS2 pathway has a major role in β-cell development and survival, especially during compensation for peripheral insulin resistance.[117] The progeny of intercrossed mice heterozygous for null alleles of IGF-1R and IRS2 reveal that IGF-1 receptors promote β-cell development and survival through the IRS2 signaling pathway.[117] Targeted deletion of the IGF-1R in β-cells promotes age-dependent glucose intolerance owing to decreased glucose- and arginine-stimulated insulin release; however, there is no effect on β-cell growth.[359,360] Thus, the effect of IGF-1 on IRS2-medited β-cell growth and survival might not be β-cell autonomous, but might be more closely related to the growth and differentiation of precursors.

Many factors are required for proper β-cell function, including the homeodomain transcription factor PDX1. PDX1 mutations cause autosomal forms of MODY, because PDX1 regulates downstream genes needed for β-cell growth and function (see Fig. 50-1).[361,362] PDX1 is reduced in IRS2$^{-/-}$ islets, and PDX-1 haploinsufficiency further diminishes the function of β cells lacking IRS2.[10,363] Unexpectedly, transgenic PDX1 expressed in IRS2$^{-/-}$ mice restores β-cell function and normalizes glucose tolerance for at least 20 months.[363] Moreover, transgenic upregulation of IRS2 in wild-type or IRS2$^{-/-}$ islets increases PDX1 levels, supporting the hypothesis that PDX1 is regulated by IRS2 signaling cascades in β cells (Fig. 50-10).[8]

Some strains of C57Bl/6 IRS2$^{-/-}$ mice express sufficient PDX1 to prevent diabetes without additional genetic manipulations.[364] This apparent rescue might arise from natural variations in β-cell gene expression that increases the apparent activity of signals lying downstream of IRS2. For example, Foxo1 haploinsufficiency upregulates PDX1 in IRS2 $^{-/-}$ islets/β-cells and prevents diabetes in the IRS2$^{-/-}$ mice (see Fig. 50-10).[10] Similarly, haploinsufficiency for PTEN also prevents diabetes in IRS2$^{-/-}$ mice, owing to the simulation of the PI 3-kinase PKB/Akt cascade that upregulates PDX1 (see Fig. 50-10). Thus, variations of downstream IRS2-mediated signals, through natural genetic drift or directed genetic manipulations, strongly compensate for IRS2 deletion and restore glucose tolerance.

Activation of signals upstream of IRS2 only temporarily restores the function of β cells lacking IRS2. As outlined previously, inhibition of PTP1B improves systemic insulin sensitivity and reduced systemic adiposity.[347] Moreover, disruption of the PTP1B gene temporarily restores β-cell function in IRS2$^{-/-}$ mice; however, compound IRS2$^{-/-}$::PTP1B$^{-/-}$ mice eventually develop glucose intolerance and die when the β cells fail to compensate for age-related increases of insulin resistance.[7] These results suggest that IRS2, rather than the upstream receptor kinases, could be the "gatekeeper" for β-cell plasticity and function.

The discovery that cAMP agonists upregulate IRS2 reveals an unexpected mechanism to promote the growth function and survival of a variety of tissues. GLP1 secreted from the intestinal L cells during meals promotes β-cell function and peripheral insulin sensitivity in diabetic patients.[365] GLP1 promotes cAMP production and upregulates IRS2 in islets, hepatocytes, brain, and probably other tissues. Thus, inhibition of cAMP-regulated gene expression in β cells with a transgenic ACREB, a dominant-negative form of the cAMP response element binding protein (CREB), strongly suppresses IRS2 expression and causes β-cell apoptosis and glucose intolerance by 15 weeks of age.[366] We hypothesize that IRS2 is upregulated in β-cells and other tissues during the counterregulatory phase, which ensures β-cell function and peripheral insulin sensitivity during the next meal (see Fig. 50-10). Similar mechanism might occur in the brain and liver.

Finally, IRS2 signaling reveals a molecular link between obesity and peripheral insulin resistance. Dysregulated signaling, rather than antidotal consumption of high-calorie diets, might contribute to the early development of obesity that progresses to diabetes.[3,367,368] Insulin, leptin, and adiponectin are important peripheral signals that inform the brain of short- and long-term nutrient availability.[3,369,370] Pharmacologic inhibition of insulin signaling in the hypothalamus increases food intake, and conditional knockout of the insulin receptor in the brain causes obesity in mice on high-fat diets.[371–374] Leptin secreted from adipocytes promotes satiety and energy utilization, at least in part, by promoting alpha melanocyte-stimulating hormone (α-MSH) production in the hypothalamus.[367] Mutations that disrupt neuronal leptin or melanocortin signaling increase food intake, body weight, and peripheral insulin resistance in mice and people that progresses to diabetes if β-cell function also deteriorates.[375–377] Adiponectin, another adipocyte-derived hormone, enhances hepatic and muscle insulin action and promotes energy expenditure through signaling in the hypothalamus[303,369]; however, adiponectin is reduced in obese people and rodents which might promote disease progression.[378,379]

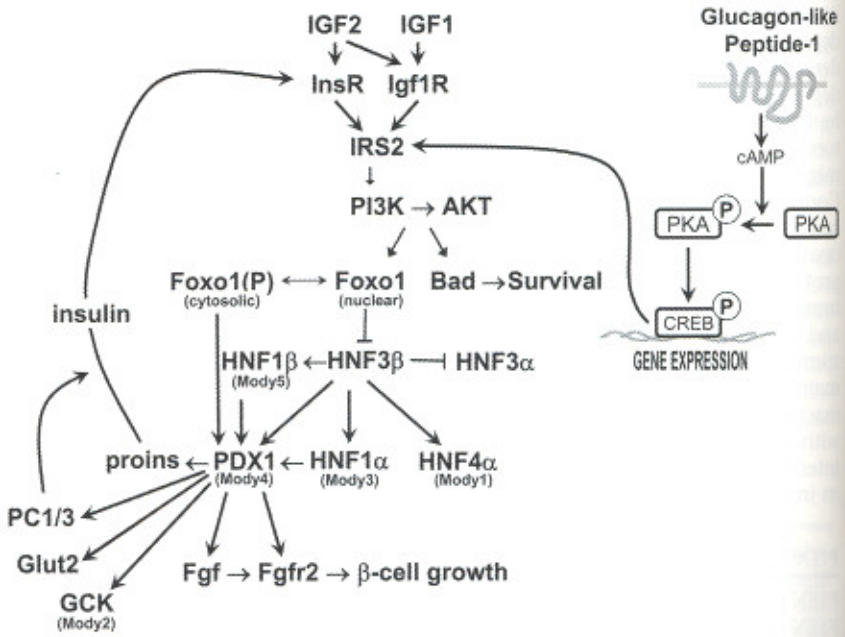

Figure 50-10 A potential pathway linking IRS2 signaling to the expression and function of the homeodomain transcription factor PDX1. The diagram shows the relation between the MODY genes, especially PDX1, and the IRS2 branch of the insulin signaling pathway.[363] Drugs that promote IRS2 signaling are expected to promote PDK1 function in β cells, including the phosphorylation of BAD and FOXO1, which will promote β-cell growth, function, and survival. Induction of PDX1 promotes the expression of genes products that enhance glucose sensing and insulin secretion. Activation of the cAMP CREB cascade induces IRS2 expression in β-cells, revealing a mechanism that promotes β-cell growth, function, and survival.

Previous work suggests that IRS2 signaling plays an important role in the central nervous system for brain growth, female fertility, and nutrient homeostasis.[380] Since IRS2 is highly expressed in the hypothalamus, its signaling cascade could integrate central control of nutrient homeostasis and appetite regulation with peripheral insulin action and β-cell function.[8] Female IRS2$^{-/-}$ mice, which develop diabetes more slowly that male mice, are hyperphagic and obese until severe diabetes causes weight loss.[380] To test the role of selective IRS2 dysregulation in obesity and diabetes, we flanked the IRS2 gene with loxP recombination sites (fIrs2) and crossed these mice with transgenic mice (TgN[Ins2Cre]25Mgn) expressing Cre recombinase under control of the rat insulin-2 promoter. Cre recombinase is expressed strongly in β cells and weakly in certain brain regions of these transgenic mice (cr^2 mice) including the hypothalamus.[381,382] Thus, our strategy strongly deletes fIrs2 alleles from β cells, and weakly deletes them from brain and certain neurons of the hypothalamus.

Our results suggest that partial dysregulation of IRS2 signaling in β cells and brain (hypothalamus) can explain the close association between obesity, peripheral insulin resistance, and β-cell failure that characterizes type 2 diabetes.[383] Whether dysregulation of IRS2 signaling contributes to type 2 diabetes and obesity in humans is unknown. However, many mechanisms are described that dysregulate IRS2 signaling in various tissues. Strategies to promote IRS2 expression in β cells and hypothalamus, or alleviate inhibition of IRS2 signaling in these tissues could be a rational approach to prevent or cure type 2 diabetes.

REFERENCES

1. White MF: Insulin signaling in health and disease. Science 302:1710–1711, 2003.
2. DeFronzo RA, Ferrannini E: Regulation of intermediary metabolism during fasting and feeding. In DeGroot LJ, Jameson JL (eds): Endocrinology, 4th ed. Philadelphia, WB Saunders, 2001, pp 737–755.
3. Schwartz MW, Woods SC, Porte D Jr, et al: Central nervous system control of food intake. Nature 404:661–671, 2000.
4. Fajans SS, Bell GI, Polonsky KS: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med 345:971–980, 2001.
5. Frayling TM, Evans JC, Bulman MP, et al: Beta-cell genes and diabetes: Molecular and clinical characterization of mutations in transcription factors. Diabetes 50 (Suppl 1):S94–S100, 2001.
6. Froguel P, Velho G: Molecular genetics of maturity-onset diabetes of the young. Trends Endocrinol Metab 10:142–146, 1999.
7. Kushner JA, Haj FG, Klaman LD, et al: Islet-sparing effects of protein tyrosine phosphatase-1b deficiency delays onset of diabetes in IRS2 knockout mice. Diabetes 53:61–66, 2004.
8. Hennige AM, Burks DJ, Ozcan U, et al: Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. J Clin Invest 112:1521–1532, 2003.
9. Otani K, Kulkarni RN, Baldwin AC, et al: Reduced beta-cell mass and altered glucose sensing impair insulin-secretory function in betaIRKO mice. Am J Physiol Endocrinol Metab 286:E41–E49, 2004.
10. Kitamura T, Nakae J, Kitamura Y, et al: The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. J Clin Invest 110:1839–1847, 2002.
11. Roth J, Kahn CR, De Meyts P, et al: Receptors for insulin and other peptide hormones in disease states. In Bajaj JS (ed): Insulin and Metabolism. Amsterdam, Excerpta Medica, 1977, pp 73–80.
12. Brogiolo W, Stocker H, Ikeya, T et al: An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Curr Biol 11:213–221, 2001.
13. Frasca F, Pandini G, Scalia P, et al: 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.
14. Sciacca L, Prisco M, Wu A, et al: Signaling differences from the A and B isoforms of the insulin receptor (IR) in 32D cells in the presence or absence of IR substrate-1. Endocrinology 144:2650–2658, 2003.
15. Mosthaf L, Grako K, Dull TJ, et al: Functionally distinct insulin receptors generated by tissue-specific alternative splicing. EMBO J 9:2409–2413, 1990.
16. Moller DE, Yokota A, Caro JF, Flier JS: Tissue -specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol 3:1263–1269, 1989.
17. Goldstein BJ, Kahn CR: Analysis of mRNA heterogeneity by ribonuclease H mapping: Application to the insulin receptor. Biochem Biophys Res Commun 159:664–669, 1989.
18. Seino S, Bell GI: Alternative splicing of human insulin receptor messenger RNA. Biochem Biophys Res Commun 159:312–316, 1989.
19. Savkur RS, Philips AV, Cooper TA: Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. Nat Genet 29:40–47, 2001.
20. Louvi A, Accili D, Efstratiadis A: Growth-promoting interaction of IGF-II with the insulin receptor during mouse embryonic development. Dev Biol 189:33–48, 1997.
21. Pandini G, Frasca F, Mineo R, et al: Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem 277:39684–39695, 2002.
22. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G: daf -2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans [see comments]. Science 277:942–946, 1997.
23. Chen C, Jack J, Garofalo RS: The Drosophila insulin receptor is required for normal growth. Endocrinology 137:846–856, 1996.
24. Schubert M, Brazil DP, Burks DJ, et al: Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. J Neurosci 23:7084–7092, 2003.
25. Werner H, Beitner-Johnson D, Roberts CT, LeRoith D: Molecular comparisions of the insulin and IGF-1 receptors. In Draznin B, LeRoith D (eds): Molecular Biology of Diabetes II. Insulin Action, Effects on Gene Expression and Regulation, and Glucose Transport. Totowa, Humana, 1994, pp 377–392.
26. Beguinot F, Kahn CR, Moses AC, Smith RJ: Distinct biologically active receptors for insulin, insulin-like growth factor I, and insulin-like growth factor II in cultured skeletal muscle cells. J Biol Chem 260:15892–15898, 1985.
27. Kitamura T, Kahn CR, Accili D: Insulin receptor knockout mice. Annu Rev Physiol 65:313–332, 2003.
28. Dunger DB, Ong KK, Sandhu MS: Serum insulin-like growth factor-I levels and potential risk of type 2 diabetes. Horm Res 60(Suppl 3):131–135, 2003.
29. Okamoto H, Nakae J, Kitamura T, et al: Transgenic rescue of insulin receptor-deficient mice. J Clin Invest 114:214–223, 2004.
30. Accili D, Drago J, Lee EJ, et al: Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 12:106–109, 1996.
31. Joshi RL, Lamothe B, Cordonnier N, et al: Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality. EMBO J 15:1542–1547, 1996.
32. Baker J, Liu JP, Robertson EJ, Efstratiadis A: Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75:73–82, 1993.
33. DeChiara TM, Efstratiadis A, Robertson EJ: A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 345:78–80, 1990.
34. Morrione A, Valentinis B, Xu SQ, et al: Insulin-like growth factor II stimulates cell proliferation through the insulin receptor. Proc Natl Acad Sci U S A 94:3777–3782, 1997.

35. Ozaki K: Insulin receptor-related receptor in rat islets of Langerhans. Eur J Endocrinol 139:244–247, 1998.
36. Nef S, Verma-Kurvari S, Merenmies J, et al: Testis determination requires insulin receptor family function in mice. Nature 426:291–295, 2003.
37. Hone J, Accili D, Psiachou H, et al: Homozygosity for a null allele of the insulin receptor gene in a patient with leprechaunism. Hum Mutat 6:17–22, 1995.
38. Kitamura T, Kitamura Y, Nakae J, et al: Mosaic analysis of insulin receptor function. J Clin Invest 113:209–219, 2004.
39. Clancy DJ, Gems D, Harshman LG, et al: Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. Science 292:104–106, 2001.
40. Arantes-Oliveira N, Berman JR, Kenyon C: Healthy animals with extreme longevity. Science 302:611, 2003.
41. Finch CE, Ruvkun G: The genetics of aging. Annu Rev Genomics Hum Genet 2:435–462, 2001.
42. Libina N, Berman JR, Kenyon C: Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. Cell 115:489–502, 2003.
43. Holzenberger M, Dupont J, Ducos B, et al: IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421:182–187, 2003.
44. Bluher M, Kahn BB, Kahn CR: Extended longevity in mice lacking the insulin receptor in adipose tissue. Science 299:572–574, 2003.
45. Ullrich A, Bell JR, Chen EY, et al: Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313:756–761, 1985.
46. Ebina Y, Ellis L, Jarnagin K, et al: The human insulin receptor cDNA: The structural basis for hormone activated transmembrane signalling. Cell 40:747–758, 1985.
47. Schlessinger J: Cell signaling by receptor tyrosine kinases. Cell 103:211–225, 2000.
48. Myers MG Jr, White MF: The new elements in insulin signaling. Insulin receptor substrate-1 and proteins with SH2 domains. Diabetes 42:643–650, 1993.
49. White MF, Kahn CR: The insulin signaling system. J Biol Chem 269:1–4, 1994.
50. Rosen ED, Spiegelman BM: Tumor necrosis factor-alpha as a mediator of the insulin resistance of obesity. Curr Opin Endocrinol Diab 6:170–176, 1999.
51. Chen H, Wertheimer SJ, Lin CH, et al: Protein-tyrosine phosphatases PTP1B and syp are modulators of insulin-stimulated translocation of GLUT4 in transfected rat adipose cells. J Biol Chem 272:8026–8031, 1997.
52. Elchebly M, Payette P, Michaliszyn E, et al: Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene [see comments]. Science 283:1544–1548, 1999.
53. Zick Y: Role of Ser/Thr kinases in the uncoupling of insulin signaling. Int J Obes Relat Metab Disord 27 (Suppl 3):S56–S60, 2003.
54. Yang Feng TL, Francke U, Ullrich A: Gene for human insulin receptor: Localization to site on chromosome 19 involved in pre-B-cell leukemia. Science 228:728–731, 1985.
55. Seino S, Seino M, Nishi S, Bell GI: Structure of the human insulin receptor gene and characterization of its promoter. Proc Natl Acad Sci U S A 86:114–118, 1989.
56. Kasuga M, Hedo JA, Yamada KM, Kahn CR: The structure of the insulin receptor and its subunits: Evidence for multiple non-reduced forms and a 210K possible proreceptor. J Biol Chem 257:10392–10399, 1982.
57. Hedo JA, Kahn CR, Hayoshi M, et al: Biosynthesis and glycosylation of the insulin receptor. Evidence for a single polypeptide precursor of the two major subunits. J Biol Chem 258:10020–10026, 1983.
58. De Meyts P, Whittaker J: Structural biology of insulin and IGF1 receptors: Implications for drug design. Nat Rev Drug Discov 1:769–783, 2002.
59. Schumacher R, Soos MA, Schlessinger J, et al: A. Signaling-competent receptor chimeras allow mapping of major insulin receptor binding domain determinants. J Biol Chem 268:1087–1094, 1993.
60. Mynarcik DC, Yu GQ, Whittaker J: Alanine-scanning mutagenesis of a C-terminal ligand binding domain of the insulin receptor alpha subunit. J Biol Chem 271:2439–2442, 1996.
61. Yip CC, Ottensmeyer P: Three-dimensional structural interactions of insulin and its receptor. J Biol Chem 278:27329–27332, 2003.
62. Luo RZ, Beniac DR, Fernandes A, et al: Quaternary structure of the insulin-insulin receptor complex. Science 285:1077–1080, 1999.
63. De Meyts P: Insulin and insulin-like growth factors: The paradox of signaling specificity. Growth Horm IGF Res 12:81–83, 2002.
64. Kasuga M, Zick Y, Blithe DL, et al: Insulin stimulation of phosphorylation of the β-subunit of the insulin receptor: Formation of both phosphoserine and phosphotyrosine. J Biol Chem 257:9891–9894, 1982.
65. Rosen OM, Herrera R, Olowe Y, et al: Phosphorylation activates the insulin receptor tyrosine protein kinase. Proc Natl Acad Sci U S A 80:3237–3240, 1983.
66. White MF, Haring HU, Kasuga M, Kahn CR: Kinetic properties and sites of autophosphorylation of the partially purified insulin receptor from hepatoma cells. J Biol Chem 259:255–264, 1984.
67. Krook A, O'Rahilly S: Mutant insulin receptors in syndromes of insulin resistance. Baillieres Clin Endocrinol Metab 10:97–122, 1996.
68. Chou CK, Dull TJ, Russell DS, et al: Human insulin receptors mutated at the ATP-binding site lack protein tyrosine kinase activity and fail to mediate postreceptor effects of insulin. J Biol Chem 262:1842–1847, 1987.
69. Cama A, de la Luz Sierra M, Ottini L, et al: A mutation in the tyrosine kinase domain of the insulin receptor associated with insulin resistance in an obese woman. J Clin Endocrinol Metab 73:894–901, 1991.
70. White MF, Shoelson SE, Keutmann H, Kahn CR: A cascade of tyrosine autophosphorylation in the β-subunit activates the insulin receptor. J Biol Chem 263:2969–2980, 1988.
71. Wilden PA, Siddle K, Haring E, et al: The role of insulin receptor kinase domain autophosphorylation in receptor-mediated activities. J Biol Chem 267:13719–13727, 1992.
72. Cann AD, Kohanski RA: Cis-autophosphorylation of juxtamembrane tyrosines in the insulin receptor kinase domain. Biochemistry 36:7681–7689, 1997.
73. Myers MG Jr, Backer JM, Siddle K, White MF: The insulin receptor functions normally in Chinese hamster ovary cells after truncation of the C-terminus. J Biol Chem 266:10616–10623, 1991.
74. Maegawa H, McClain DA, Freidneberg G, et al: Properties of a human insulin receptor with a COOH-terminal trancation II. Truncated receptors have normal kinase activity but are defective in signaling metabolic effects. J Biol Chem 263:8912–8917, 1998.
75. McClain DA, Maegawa H, Levy J, et al: Properties of a human insulin receptor with a COOH-terminal truncation. I. Insulin binding, autophosphorylation and endocytosis. J Biol Chem 263:8904–8912, 1988.
76. Backer JM, Shoelson SE, Weiss MA, et al: The insulin receptor juxtamembrane region contains two independent tyrosine/beta-turn internalization signals. J Cell Biol 118:831–839, 1992.
77. Baron V, Gautier N, Kaliman P, et al: The carboxyl-terminal domain of the insulin receptor: Its potential role in growth-promoting effects. Biochemistry 30:9365–9370, 1991.
78. Levy-Toledano R, Blaettler DH, Larochelle WJ, Taylor SI: Insulin-induced activation of phosphatidylinositol (PI) 3-kinase. J Biol Chem 270:30018–30022, 1995.
79. Van Horn DJ, Myers MG Jr, Backer JM: Direct activation of the phosphatidylinositol 3′-kinase by the insulin receptor. J Biochem 269:29–32, 1994.
80. Yenush L, Fernandez R, Myers MG Jr, et al: The drosophila insulin receptor activates multiple signaling pathways but requires IRS-proteins for DNA synthesis. Mol Cell Biol 16:2509–2517, 1996.
81. Wilden PA, Backer JM, Kahn CR, et al: The insulin receptor with phenylalanine

replacing tyrosine-1146 provides evidence for separate signals regulating cellular metabolism and growth. Proc Natl Acad Sci U S A 87:3358–3362, 1990.
82. Wilden PA, Kahn CR, Siddle K, White MF: Insulin receptor kinase domain autophosphorylation regulates receptor enzymatic function. J Biol Chem 267:16660–16668, 1992.
83. Hubbard SR: Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. EMBO J 16:5572–5581, 1997.
84. Li S, Covino ND, Stein EG, et al: Structural and biochemical evidence for an autoinhibitory role for tyrosine 984 in the juxtamembrane region of the insulin receptor. J Biol Chem 278:26007–26014, 2003.
85. Till JH, Ablooglu AJ, Frankel M, et al: Crystallographic and solution studies of an activation loop mutant of the insulin receptor tyrosine kinase: Insights into kinase mechanism. J Biol Chem 276:10049–10055, 2001.
86. Hubbard SR, Wei L, Ellis L, Hendrickson WA: Crystal structure of the tyrosine kinase domain of the human insulin receptor. Nature 372:746–754, 1994.
87. Sawka-Verhelle D, Tartare-Deckert S, White MF, Van Obberghen E: IRS-2 binds to the insulin receptor through its PTB domain and through a newly identified domain comprising amino acids 591 to 786. J Biol Chem 271:5980–5983, 1996.
88. Sawka-Verhelle D, Baron V, Mothe I, et al: Tyr624 and Tyr628 in insulin receptor substrate-2 mediate its association with the insulin receptor. J Biol Chem 272:16414–16420, 1997.
89. Liu F, Roth RA: Grb-IR: A SH2 domain-containing protein that binds to the insulin receptor and inhibits its function. Proc Natl Acad Sci U S A 92:10287–10291, 1995.
90. Hu J, Liu J, Ghirlando R, et al: Structural basis for recruitment of the adaptor protein APS to the activated insulin receptor. Mol Cell 12:1379–1389, 2003.
91. Minami A, Iseki M, Kishi K, et al: Increased insulin sensitivity and hypoinsulinemia in APS knockout mice. Diabetes 52:2657–2665, 2003.
91a. Kotani K, Wilden P, Pillay TS: SH2-B alpha is an insulin-receptor adapter protein and substrate that interacts with the activation loop of the insulin-receptor kinase. Biochem J 335(Pt 1):103–109, 1998.
91b. Moodie SA, Ieman-Sposeto J, Gustafson TA: Identification of the APS protein as a novel insulin receptor substrate. J Biol Chem 274(16):11186–11193, 1999.
91c. Nelms K, O'Neill TJ, Li S, et al: Alternative splicing, gene localization, and binding of SH2-B to the insulin receptor kinase domain. Mamm Genome 10(12):1160–1167, 1999.
91d. Ahmed Z, Pillay TS: Adapter protein with a pleckstrin homology (PH) and an Src homology 2 (SH2) domain (APS) and SH2-B enhance insulin-receptor autophosphorylation, extracellular-signal-regulated kinase and phosphoinositide 3-kinase-dependent signalling. Biochem J 371(Pt 2):405–412, 2003.
92. Duan C, Yang H, White MF, Rui L: Disruption of SH2-B Causes Age-dependent Insulin Resistance and Glucose Intolerance. Mol Cell Biol 24(17):7435–7443, 2004.
93. White MF, Livingston JN, Backer JM, et al: Mutation of the insulin receptor at tyrosine 960 inhibits signal transmission but does not affect its tyrosine kinase activity. Cell 54:641–649, 1988.
94. Backer JM, Kahn CR, Cahill DA, et al: Receptor-mediated internalization of insulin requires a 12-amino acid sequence in the juxtamembrane region of the insulin receptor β-subunit. J Biol Chem 265:16450–16454, 1990.
95. Hubbard SR: Juxtamembrane autoinhibition in receptor tyrosine kinases. Nat Rev Mol Cell Biol 5:464–471, 2004.
96. Feener EP, Backer JM, King GL, et al: Insulin stimulates serine and tyrosine phosphorylation in the juxtamembrane region of the insulin receptor. J Biol Chem 268:11256–11264, 1993.
97. Songyang Z, Carraway KL III, Eck MJ, et al: Catalytic specificity of protein-tyrosine kinases is critical for selective signalling. Nature 373:536–539, 1995.
98. Songyang Z, Cantley LC: Recognition and specificity in protein tyrosine kinase-mediated signaling. TIBS 20:470–475, 1995.
99. Shoelson SE, Chatterjee S, Chaudhuri M, White MF: YMXM motifs of IRS-1 define the substrate specificity of the insulin receptor kinase. Proc Natl Acad Sci U S A 89:2027–2031, 1992.
100. Kasuga M, Karlsson FA, Kahn CR: Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. Science 215:185–187, 1982.
101. Kasuga M, Zick Y, Blithe DL, et al: Insulin stimulates tyrosine phosphorylation of the insulin receptor in a cell-free system. Nature 298:667–669, 1982.
102. White MF, Maron R, Kahn CR: Insulin rapidly stimulates tyrosine phosphorylation of a Mr 185,000 protein in intact cells. Nature 318:183–186, 1985.
103. Sun XJ, Rothenberg PL, Kahn CR, et al: The structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352:73–77, 1991.
104. Sun XJ, Crimmins DL, Myers MG Jr, et al: Pleiotropic insulin signals are engaged by multisite phosphorylation of IRS-1. Mol Cell Biol 13:7418–7428, 1993.
105. Backer JM, Myers MG Jr, Shoelson SE, et al: Phosphatidylinositol 3'-kinase is activated by association with IRS-1 during insulin stimulation. EMBO J 11:3469–3479, 1992.
106. Nelms K, O'Neill TJ, Li S, et al: Alternative splicing, gene localization, and binding of SH2-B to the insulin receptor kinase domain. Mamm Genome 10:1160–1167, 1999.
107. Yenush L, White MF: The IRS-signaling system during insulin and cytokine action. Bio Essays 19:491–500, 1997.
108. Pawson T, Scott JD: Signaling through scaffold, anchoring, and adaptor proteins. Science 278:2075–2080, 1997.
109. Kotani K, Wilden P, 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(Pt 1):103–109, 1998.
110. Lock P, Casagranda F, 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.
111. Noguchi T, Matozaki T, Inagaki K, et al: Tyrosine phosphorylation of p62(Dok) induced by cell adhesion and insulin: Possible role in cell migration. EMBO J 18:1748–1760, 1999.
112. Chiang SH, Baumann CA, Kanzaki M, et al: Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. Nature 410:944–948, 2001.
113. Baumann CA, Ribon V, Kanzaki M, et al: CAP defines a second signalling pathway required for insulin-stimulated glucose transport [see comments]. Nature 407:202–207, 2000.
114. Uchida T, Myers MG Jr, White MF: IRS-4 mediates activation of PKB/Akt during insulin stimulation without inhibition of apoptosis. Mol Cell Biol 20:126–138, 2000.
115. Laustsen PG, Michael MD, Crute BE, et al: Lipoatrophic diabetes in Irs1(−/−)/Irs3(−/−) double knockout mice. Genes Dev 16:3213–3222, 2002.
116. Withers DJ, Gutierrez JS, Towery H, et al: Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900–904, 1998.
117. Withers DJ, Burks DJ, Towery HH, et al: Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat Genet 23:32–40, 1999.
118. Kido Y, Burks DJ, Withers DJ, et al: Tissue-specific insulin resistance in mice with mutations of the insulin receptor, IRS-1 and IRS-2. J Clin Invest 105:199–205, 2000.
119. Bjornholm M, He AR, Attersand A, et al: Absence of functional insulin receptor substrate-3 (IRS-3) gene in humans. Diabetologia 45:1697–1702, 2002.
120. Dhe-Paganon S, Ottinger EA, Nolte RT, et al: Crystal structure of the pleckstrin homology-phosphotyrosine binding (PH-PTB) targeting region of insulin

receptor substrate 1. Proc Natl Acad Sci U S A 96:8378–8383, 1999.
121. Yenush L, Zanella C, Uchida T, et al: 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.
122. Tsuruzoe K, Emkey R, Kriauciunas KM, et al: Insulin receptor substrate 3 (IRS-3) and IRS-4 impair IRS-1- and IRS-2-mediated signaling. Mol Cell Biol 21:26–38, 2000.
123. Uchida T, Myers MG Jr, White MF: IRS-4 mediates protein kinase B signaling during insulin stimulation without promoting antiapoptosis. Mol Cell Biol 20:126–138, 2000.
124. Cai D, Dhe-Paganon S, Melendez PA, et al: Two new substrates in insulin signaling, IRS5/DOK4 and IRS6/DOK5. J Biol Chem 278:25323–25330, 2003.
125. Yenush L, Makati KJ, Smith-Hall J, et al: The pleckstrin homology domain is the principle link between the insulin receptor and IRS-1. J Biol Chem 271:24300–24306, 1996.
126. Burks DJ, Pons S, Towery H, et al: Heterologous PH domains do not mediate coupling of IRS-1 to the insulin receptor. J Biol Chem 272:27716–27721, 1997.
127. Keegan AD, Nelms K, White MF, et al: An IL-4 receptor region containing an insulin receptor motif is important for IL-4-mediated IRS-1 phosphorylation and cell growth. Cell 76:811–820, 1994.
128. Lemmon MA, Ferguson KM, Schlessinger J: PH domains: Diverse sequences with a common fold recruit signaling molecules to the cell surface. Cell 85:621–624, 1996.
129. Lemmon MA, Ferguson KM, Abrams CS: Pleckstrin homology domains and the cytoskeleton. Growth Regul 513:71–76, 2002.
130. Burks DJ, Wang J, Towery H, et al: IRS pleckstrin homology domains bind to acidic motifs in proteins. J Biol Chem 273:31061–31067, 1998.
131. Tu X, Baffa R, Luke S, et al: Intracellular redistribution of nuclear and nucleolar proteins during differentiation of 32D murine hemopoietic cells. Exp Cell Res 288:119–130, 2003.
132. Tu X, Wu A, Maiorana A, et al: Subcellular localization of IRS-1 in cell proliferation and differentiation. Horm Metab Res 35:734–739, 2003.
133. Wu A, Sciacca L, Baserga R: Nuclear translocation of insulin receptor substrate-1 by the insulin receptor in mouse embryo fibroblasts. J Cell Physiol 195:453–460, 2003.
134. Farhang-Fallah J, Yin X, Trentin G, et al: Cloning and characterization of PHIP, a novel insulin receptor substrate-1 pleckstrin homology domain interacting protein. J Biol Chem 275:40492–40497, 2000.
135. Skolnik EY, Lee CH, Batzer AG, et al: The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine-phosphorylated IRS-1 and Shc: Implications for insulin control of ras signalling. EMBO J 12:1929–1936, 1993.
136. Weston CR, Lambright DG, Davis RJ: Signal transduction. MAP kinase signaling specificity. Science 296:2345–2347, 2002.
137. Ip YT, Davis RJ: Signal transduction by the c-Jun N-terminal kinase (JNK)—from inflammation to development. Curr Opin Cell Biol 10:205–219, 1998.
138. Aguirre V, Werner ED, Giraud J, et al: Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem 277:1531–1537, 2002.
139. Lee YH, Giraud J, Davis RJ, White MF: c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem 278:2896–2902, 2003.
140. Rui L, Aguirre V, Kim JK, et al: Insulin/IGF-1 and TNF-alpha stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. J Clin Invest 107:181–189, 2001.
141. Jiang G, Dallas-Yang Q, Liu F, et al: Salicylic acid reverses phorbol 12-myristate-13-acetate (PMA) and tumor necrosis factor alpha (TNFalpha)-induced insulin receptor substrate 1 (IRS1) serine 307 phosphorylation and insulin resistance in human embryonic kidney 293 (HEK293) cells. J Biol Chem 278:180–186, 2003.
142. Sawka-Verhelle D, Baron V, Mothe I, et al: Tyr624 and Tyr628 in insulin receptor substrate-2 mediate its association with the insulin receptor. Am Soc Biochem Mol Biol 272:16414-16420,1997.
143. Fruman DA, Meyers RE, Cantley LC: Phosphoinositide kinases. Annu Rev Biochem 67:481–507, 1998.
144. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD: Phosphoinositide 3-kinases: A conserved family of signal transducers. Trends Biochem Sci 22:267–272, 1997.
145. Fruman DA, Cantley LC, Carpenter CL: Structural organization and alternative splicing of the murine phosphoinositide 3-kinase p85α gene. Genomics 37:113–121, 1996.
146. Pons S, Asano T, Glasheen EM, et al: The structure and function of $p55^{PIK}$ reveals a new regulatory subunit for the phosphatidylinositol-3 kinase. Mol Cell Biol 15:4453–4465, 1995.
147. Woscholski R, Kodaki T, McKinnon M, et al: A comparison of demethoxyviridin and wortmannin as inhibitors of phosphatidylinositol 3-kinase. FEBS Lett 342:109–114, 1994.
148. Yu J, Wjasow C, Backer JM: Regulation of the p85/p110alpha phosphatidylinositol 3′-kinase. Distinct roles for the n-terminal and c-terminal SH2 domains. J Biol Chem 273:30199–30203, 1998.
149. Fruman DA, Mauvais-Jarvis F, Pollard DA, et al: Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85 alpha. Nat Genet 26:379–382, 2000.
150. Rordorf-Nikolic T, Van Horn DJ, Chen D, et al: Regulation of phosphatidylinositol 3-kinase by tyrosyl phosphoproteins. Full activation requires occupancy of both SH2 domains in the 85 kDa regulatory subunit. J Biol Chem 270:3662–3666, 1995.
151. Cuevas BD, Lu Y, Mao M, et al: Tyrosine phosphorylation of p85 relieves its inhibitory activity on phosphatidylinositol 3-kinase. J Biol Chem 276:27455–27461, 2001.
152. Rodriguez-Viciana P, Warne PH, Dhand R, et al: Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature 370:527–532, 1994.
153. Rodriguez-Viciana P, Warne PH, Vanhaesebroeck B, et al: Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation. EMBO J 15:2442–2451, 1996.
154. Marte BM, Rodriguez-Viciana P, Wennstrom S, et al: R-Ras can activate the phosphoinositide 3-kinase but not the MAP kinase arm of the Ras effector pathways [published erratum appears in Curr Biol 7:197, 1997]. Curr Biol 7:63–70, 1997.
155. Khwaja A, Rodriguez-Viciana P, Wennstrom S, et al: Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. EMBO J 16:2783–2793, 1997.
156. Terauchi Y, Tsuji T, Satoh S, et al: Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. Nat Genet 21:230–235, 1999.
157. Ueki K, Fruman DA, Brachmann SM, et al: Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. Mol Cell Biol 22:965–977, 2002.
158. Ueki K, Yballe CM, Brachmann SM, et al: Increased insulin sensitivity in mice lacking p85beta subunit of phosphoinositide 3-kinase. Proc Natl Acad Sci U S A 99:419–424, 2002.
159. Ueki K, Fruman DA, Yballe CM, et al: Positive and negative roles of p85 alpha and p85 beta regulatory subunits of phosphoinositide 3-kinase in insulin signaling. J Biol Chem 278:48453–48466, 2003.
160. Hallmann D, Trumper K, Trusheim H, et al: Altered signaling and cell cycle regulation in embryonal stem cells with a disruption of the gene for phosphoinositide 3-kinase regulatory subunit p85alpha. J Biol Chem 278:5099–5108, 2003.
161. Bi L, Okabe I, Bernard DJ, et al: Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of

phosphoinositide 3-kinase. J Biol Chem 274:10963–10968, 1999.
162. Asano T, Kanda A, Katagiri H, et al: p110beta is up-regulated during differentiation of 3T3-L1 cells and contributes to the highly insulin-responsive glucose transport activity. J Biol Chem 275:17671–17676, 2000.
163. Kim YB, Ciaraldi TP, Kong A, et al: Troglitazone but not metformin restores insulin-stimulated phosphoinositide 3-kinase activity and increases p110beta protein levels in skeletal muscle of type 2 diabetic subjects. Diabetes 51:443–448, 2002.
164. Collins BJ, Deak M, Arthur JS, et al: In vivo role of the PIF-binding docking site of PDK1 defined by knock-in mutation. EMBO J 22:4202–4211, 2003.
165. Mora A, Komander D, van Aalten DM, Alessi DR: PDK1, the master regulator of AGC kinase signal transduction. Semin Cell Dev Biol 15:161–170, 2004.
166. Brazil DP, Park J, Hemmings BA: PKB binding proteins. Getting in on the Akt. Cell 111:293–303, 2002.
167. Scheid MP, Woodgett JR: PKB/AKT: Functional insights from genetic models. Nat Rev Mol Cell Biol 2:760–768, 2001.
168. Lawlor MA, Alessi DR: PKB/Akt: A key mediator of cell proliferation, survival and insulin responses? J Cell Sci 114(Pt 16):2903–2910, 2001.
169. Avruch J, Belham C, Weng Q, et al: The p70 S6 kinase integrates nutrient and growth signals to control translational capacity. Prog Mol Subcell Biol 26:115–154, 2001.
170. Thomas G: The S6 kinase signaling pathway in the control of development and growth. Biol Res 35:305–313, 2002.
171. Frodin M, Gammeltoft S: Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol Cell Endocrinol 151:65–77, 1999.
172. Frodin M, Jensen CJ, Merienne K, Gammeltoft S: A phosphoserine-regulated docking site in the protein kinase RSK2 that recruits and activates PDK1. EMBO J 19:2924–2934, 2000.
173. Lang F, Cohen P: Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms. Sci STKE RE17, 2001.
174. Storz P, Toker A: 3′-phosphoinositide-dependent kinase-1 (pdk-1) in pi 3-kinase signaling. Front Biosci 7:D886–D902, 2002.
175. Alessi DR: Discovery of PDK1, one of the missing links in insulin signal transduction. Colworth Medal Lecture. Biochem Soc Trans 29(Pt 2):1–14, 2001.
176. Coffer PJ, Jin J, Woodgett JR: Protein kinase B (c-Akt): A multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J 335(Pt 1):1–13, 1998.
177. Murthy SS, Tosolini A, Taguchi T, Testa JR: Mapping of AKT3, encoding a member of the Akt/protein kinase B family, to human and rodent chromosomes by fluorescence in situ hybridization. Cytogenet Cell Genet 88:38–40, 2000.
178. Kohn AD, Summers SA, Birnbaum MJ, Roth RA: Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 271:31372–31378, 1996.
179. Hajduch E, Alessi DR, Hemmings BA, Hundal HS: Constitutive activation of protein kinase B alpha by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase 3 in L6 muscle cells. Diabetes 47:1006–1013, 1998.
180. Hill MM, Clark SF, Tucker DF, et al: A role for protein kinase Bbeta/Akt2 in insulin-stimulated GLUT4 translocation in adipocytes. Mol Cell Biol 19:7771–7781, 1999.
181. Takata M, Ogawa W, Kitamura T, et al: Requirement for Akt (protein kinase B) in insulin-induced activation of glycogen synthase and phosphorylation of 4E-BP1 (PHAS-1). J Biol Chem 274:20611–20618, 1999.
182. Ducluzeau PH, Fletcher LM, Welsh GI, Tavare JM: Functional consequence of targeting protein kinase B/Akt to GLUT4 vesicles. J Cell Sci 115(Pt 14):2857–2866, 2002.
183. Cichy SB, Uddin S, Danilkovich A, et al: Protein kinase B/Akt mediates effects of insulin on hepatic insulin-like growth factor-binding protein-1 gene expression through a conserved insulin response sequence. J Biol Chem 273:6482–6487, 1998.
184. Shioi T, McMullen JR, Kang PM, et al: Akt/protein kinase B promotes organ growth in transgenic mice. Mol Cell Biol 22:2799–2809, 2002.
185. Cho H, Thorvaldsen JL, Chu Q, et al: Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276:38349–38352, 2001.
186. Cho H, Mu J, Kim JK, et al: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292:1728–1731, 2001.
186a. Cho H, Mu J, Kim JK, et al: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292(5522):1728–1731, 2001.
186b. Cho H, Thorvaldsen JL, Chu Q, et al: Akt1/PKB alpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276(42):38349-38352, 2001.
187. George S, Rochford JJ, Wolfrum C, et al: A family with severe insulin resistance and diabetes due to a mutation in AKT2. Science 304:1325–1328, 2004.
188. Biondi RM, Kieloch A, Currie RA, et al: The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB. EMBO J 20:4380–4390, 2001.
189. Frodin M, Antal TL, Dummler BA, et al: A phosphoserine/threonine-binding pocket in AGC kinases and PDK1 mediates activation by hydrophobic motif phosphorylation. EMBO J 21:5396–5407, 2002.
190. Jensen CJ, Buch MB, Krag TO, et al: 90-kDa ribosomal S6 kinase is phosphorylated and activated by 3-phosphoinositide-dependent protein kinase-1. J Biol Chem 274:27168–27176, 1999.
191. Du K, Montminy M: CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem 273:32377–32379, 1998.
192. Anderson KE, Coadwell J, Stephens LR, Hawkins PT: Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. Curr Biol 8:684–691, 1998.
193. Anderson MJ, Viars CS, Czekay S, et al: Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. Genomics 47:187–199, 1998.
194. Brunet A, Bonni A, Zigmond MJ, et al: Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96:857–868, 1999.
195. Cross DA, Alessi DR, Cohen P, et al: Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378:785–789, 1995.
196. Guan KL, Figueroa C, Brtva TR, et al: Negative regulation of the serine/threonine kinase B-Raf by Akt. J Biol Chem 275:27354–27359, 2000.
197. Zimmermann S, Moelling K: Phosphorylation and regulation of Raf by Akt (protein kinase B). Science 286:1741–1744, 1999.
198. Brazil DP, Hemmings BA: Ten years of protein kinase B signalling: A hard Akt to follow. Trends Biochem Sci 26:657–664, 2001.
199. Datta SR, Dudek H, Tao X, et al: Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231–241, 1997.
200. Datta SR, Brunet A, Greenberg ME: Cellular survival: A play in three Akts. Genes Dev 13:2905–2927, 1999.
201. Cardone MH, Roy N, Stennicke HR, et al: Regulation of cell death protease caspase-9 by phosphorylation. Science 282:1318–1321, 1998.
202. Liang J, Zubovitz J, Petrocelli T, et al: PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. Nat Med 8:1153–1160, 2002.
203. Czech MP: Insulin's expanding control of forkheads. Proc Natl Acad Sci U S A 100:11198–11200, 2003.
204. Nakae J, Kitamura T, Silver DL, Accili D: The forkhead transcription

factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. J Clin Invest 108:1359–1367, 2001.
205. Nakae J, Biggs WH III, Kitamura T, et al: Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. Nat Genet 32:245–253, 2002.
206. Puigserver P, Rhee J, Donovan J, et al: Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. Nature 423:550–555, 2003.
207. Long X, Muller F, Avruch J: TOR action in mammalian cells and in Caenorhabditis elegans. Curr Top Microbiol Immunol 279:115–138, 2004.
208. Pende M, Kozma SC, Jaquet M, et al: Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. Nature 408:994–997, 2000.
209. Isotani S, Hara K, Tokunaga C, et al: Immunopurified mammalian target of rapamycin phosphorylates and activates p70 S6 kinase alpha in vitro. J Biol Chem 274:34493–34498, 1999.
210. Garami A, Zwartkruis FJ, Nobukuni T, et al: Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. Mol Cell 11:1457–1466, 2003.
211. Young J, Povey S: The genetic basis of tuberous sclerosis. Mol Med Today 4:313–319, 1998.
212. Woods A, Vertommen D, Neumann D, et al: Identification of phosphorylation sites in AMP-activated protein kinase (AMPK) for upstream AMPK kinases and study of their roles by site-directed mutagenesis. J Biol Chem 278:28434–28442, 2003.
213. Woods A, Johnstone SR, Dickerson K, et al: LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Curr Biol 13:2004–2008, 2003.
214. Corradetti MN, Inoki K, Bardeesy N, et al: Regulation of the TSC pathway by LKB1: Evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. Genes Dev 18:1533–1538, 2004.
215. Rhoads RE: Signal transduction pathways that regulate eukaryotic protein synthesis. J Biol Chem 274:30337–30340, 1999.
216. Hartley D, Cooper GM: Role of mTOR in the degradation of IRS-1: Regulation of PP2A activity. J Cell Biochem 85:304–314, 2002.
217. Plas DR, Thompson CB: Akt activation promotes degradation of tuberin and FOXO3a via the proteasome. J Biol Chem 278:12361–12366, 2003.
218. Kim JK, Fillmore JJ, Chen Y, et al: Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A 98:7522–7527, 2001.
219. Proud CG, Denton RM: Molecular mechanisms for the control of translation by insulin. Biochem J 328(Pt 2):329–341, 1997.
220. Jaeschke A, Hartkamp J, Saitoh M, et al: Tuberous sclerosis complex tumor suppressor-mediated S6 kinase inhibition by phosphatidylinositide-3-OH kinase is mTOR independent. J Cell Biol 159:217–224, 2002.
221. Brunn GJ, Hudson CC, Sekulic A, et al: Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. Science 277:99–101, 1997.
222. Waskiewicz AJ, Johnson JC, Penn B, et al: Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. Mol Cell Biol 19:1871–1880, 1999.
223. Minich WB, Balasta ML, Goss DJ, Rhoads RE: Chromatographic resoluation of in vivo phosphorylated and nonphorphorylated eukaryotic translation initiation factor eIF-4E: Increased cap affinity of the phosphorylated form. Proc Natl Acad Sci U S A 91:7668–7672, 1996.
224. Tsukiyama-Kohara K, Poulin F, Kohara M, et al: Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. Nat Med 7:1128–1132, 2001.
225. Begum N, Sandu OA, Ito M, et al: Active Rho kinase (ROK-alpha) associates with insulin receptor substrate-1 and inhibits insulin signaling in vascular smooth muscle cells. J Biol Chem 277:6214–6222, 2002.
226. Proud CG, Wang X, Patel JV, et al: Interplay between insulin and nutrients in the regulation of translation factors. Biochem Soc Trans 29(Pt 4):541–547, 2001.
227. Redpath NT, Price NT, Severinov KV, Proud CG: Regulation of elongation factor-2 by multisite phosphorylation. Eur J Biochem 213:689–699, 1993.
228. Mendez R, Kollmorgen G, White MF, Rhoads RE: Requirement of protein kinase C zeta for stimulation of protein synthesis by insulin. Mol Cell Biol 17:5184–5192, 1997.
229. Lu Z, Hu X, Li Y, et al: Human papillomavirus 16 E6 oncoprotein interferences with insulin signaling pathway by binding to tuberin. J Biol Chem 279(34):35664–35670, 2004.
230. Stefan N, Fritsche A, Machicao F, et al: The Gly1057Asp polymorphism in IRS-2 interacts with obesity to affect beta cell function. Diabetologia 47(4):759–761, 2004.
231. Czech MP: Molecular actions of insulin on glucose transport. Annu Rev Nutr 15:441–471, 1995.
232. Fingar DC, Birnbaum MJ: A role for raf-1 in the divergent signaling pathways mediating insulin-stimulated glucose transport. J Biol Chem 269:10127–10132, 1994.
233. Haney PM, Levy MA, Strube MS, Mueckler M: Insulin-sensitive targeting of the GLUT4 glucose transporter in L6 myoblasts is conferred by its COOH-terminal cytoplasmic tail. J Cell Biol 129:641–658, 1995.
234. Carayannopoulos MO, Chi MM, Cui Y, et al: GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. Proc Natl Acad Sci U S A 97:7313–7318, 2000.
235. Wood IS, Trayhurn P: Glucose transporters (GLUT and SGLT): Expanded families of sugar transport proteins. Br J Nutr 89:3–9, 2003.
236. Rogers S, Macheda ML, Docherty SE, et al: Identification of a novel glucose transporter-like protein-GLUT-12. Am J Physiol Endocrinol Metab 282:E733–E738, 2002.
237. Gude NM, Stevenson JL, Rogers S, et al: GLUT12 expression in human placenta in first trimester and term. Placenta 24:566–570, 2003.
238. Pessin JE, Thurmond DC, Elmendorf JS, et al: Molecular basis of insulin-stimulated GLUT4 vesicle trafficking. Location! Location! Location! J Biol Chem 274:2593–2596, 1999.
239. Khan AH, Pessin JE: Insulin regulation of glucose uptake: A complex interplay of intracellular signalling pathways. Diabetologia 45:1475–1483, 2002.
240. Farese RV: Function and dysfunction of aPKC isoforms for glucose transport in insulin-sensitive and insulin-resistant states. Am J Physiol Endocrinol Metab 283:E1–E11, 2002.
241. Bandyopadhyay G, Standaert ML, Sajan MP, et al: Protein kinase C-lambda knockout in embryonic stem cells and adipocytes impairs insulin-stimulated glucose transport. Mol Endocrinol 18:373–383, 2004.
242. Saltiel AR, Pessin JE: Insulin signaling in microdomains of the plasma membrane. Traffic 4:711–716, 2003.
243. Kotani K, Ogawa W, Matsumoto M, et al: Requirement of atypical protein kinase clambda for insulin stimulation of glucose uptake but not for akt activation in 3T3-L1 adipocytes. Mol Cell Biol 18:6971–6982, 1998.
244. Bandyopadhyay G, Kanoh Y, Sajan MP, et al: Effects of adenoviral gene transfer of wild-type, constitutively active, and kinase-defective protein kinase C-lambda on insulin-stimulated glucose transport in L6 myotubes. Endocrinology 141:4120–4127, 2000.
245. Bandyopadhyay G, Sajan MP, Kanoh Y, et al: PKC-zeta mediates insulin effects on glucose transport in cultured preadipocyte-derived human adipocytes. J Clin Endocrinol Metab 87:716–723, 2002.
246. Kotani K, Ogawa W, Matsumoto M, et al: Requirement of atypical protein kinase Cκ for insulin stimulation of glucose uptake but not for akt activation in 3t3-l1 adipocytes. Mol Cell Biol 18(12):6971–6982, 1998.
247. Etgen GJ, Valasek KM, Broderick CL, Miller AR: In vivo adenoviral delivery of recombinant human protein kinase C-zeta stimulates glucose transport activity in rat skeletal muscle. J Biol Chem 274:22139–22142, 1999.

248. Imamura T, Vollenweider P, Egawa K, et al: G alpha-q/11 protein plays a key role in insulin-induced glucose transport in 3T3-L1 adipocytes. Mol Cell Biol 19:6765–6774, 1999.
249. Vollenweider P, Menard B, Nicod P: Insulin resistance, defective insulin receptor substrate 2-associated phosphatidylinositol-3′ kinase activation, and impaired atypical protein kinase C (zeta/lambda) activation in myotubes from obese patients with impaired glucose tolerance. Diabetes 51:1052–1059, 2002.
250. Standaert ML, Ortmeyer HK, Sajan MP, et al: Skeletal muscle insulin resistance in obesity-associated type 2 diabetes in monkeys is linked to a defect in insulin activation of protein kinase C-zeta/lambda/iota. Diabetes 51:2936–2943, 2002.
251. Cohen AW, Razani B, Wang XB, et al: Caveolin-1-deficient mice show insulin resistance and defective insulin receptor protein expression in adipose tissue. Am J Physiol Cell Physiol 285:C222–C235, 2003.
252. Liu J, Kimura A, Baumann CA, Saltiel AR: APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. Mol Cell Biol 22:3599–3609, 2002.
253. Watson RT, Shigematsu S, Chiang SH, et al: Lipid raft microdomain compartmentalization of TC10 is required for insulin signaling and GLUT4 translocation. J Cell Biol 154:829–840, 2001.
254. Mitra P, Zheng X, Czech MP: RNAi-based analysis of CAP, Cbl and CrkII function in the regulation of GLUT4 by insulin. J Biol Chem 279(36):37431–37435, 2004.
255. Roth J: Diabetes and obesity. Diab Met Rev 13:1–2, 1998.
256. Taylor SI: Lilly lecture: Molecular mechanisms of insulin resistance—Lessons from patients with mutations in the insulin receptor gene. Diabetes 41:1473–1490, 1992.
257. Virkamaki A, Ueki K, Kahn CR: Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest 103:931–943, 1999.
258. Taylor SI, Accili D: Mutations in the genes encoding the insulin receptor and insulin receptor substrate-1. In LeRoith D, Taylor SI, Olefsky JM (eds): Diabetes Mellitus: A Fundamental and Clinical Text. Philadelphia, Lippincott-Raven, 1996, pp 575–583.
259. Almind K, Bjorbaek C, Vestergaard H, et al: Amino acid polymorphisms of insulin receptor substrate-1 in non-insulin-dependent diabetes mellitus. Lancet 342:828–832, 1993.
260. Almind K, Inoue G, Pedersen O, Kahn CR: A common amino acid polymorphism in insulin receptor substrate-1 causes impaired insulin signaling. Evidence from transfection studies. J Clin Invest 97:2569–2575, 1996.
261. El Mkadem SA, Lautier C, Macari F, et al: Role of allelic variants Gly972Arg of IRS-1 and Gly1057Asp of IRS-2 in moderate-to-severe insulin resistance of women with polycystic ovary syndrome. Diabetes 50:2164–2168, 2001.
262. Marchetti P, Lupi R, Federici M, et al: Insulin secretory function is impaired in isolated human islets carrying the Gly(972)→Arg IRS-1 polymorphism. Diabetes 51:1419–1424, 2002.
263. Lautier C, El Mkadem SA, Renard E, et al: Complex haplotypes of IRS2 gene are associated with severe obesity and reveal heterogeneity in the effect of Gly1057Asp mutation. Hum Genet 113:34–43, 2003.
264. Ehrmann DA, Tang X, Yoshiuchi I, et al: Relationship of insulin receptor substrate-1 and -2 genotypes to phenotypic features of polycystic ovary syndrome. J Clin Endocrinol Metab 87:4297–4300, 2002.
265. Hansen T, Andersen CB, Echwald SM, et al: Identification of a common amino acid polymorphism in the p-85alpha regulatory subunit of phosphatidylinositol 3-kinase: Effects on gluccose disappearance constant, glucose effectiveness and the insulin sensitivity index. Diabetes 46:494–501, 1997.
266. Bruning JC, Michael MD, Winnay JN, et al: A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell 2:559–569, 1998.
267. Shulman GI: Cellular mechanisms of insulin resistance. J Clin Invest 106:171–176, 2000.
268. Petersen KF, Shulman GI: Cellular mechanism of insulin resistance in skeletal muscle. J R Soc Med 95 (Suppl 42):8–13, 2002.
269. Galuska D, Ryder J, Kawano Y, et al: Insulin signaling and glucose transport in insulin resistant skeletal muscle. Special reference to GLUT4 transgenic and GLUT4 knockout mice. Adv Exp Med Biol 441:73–85, 1998.
270. Charron MJ, Katz EB: Metabolic and therapeutic lessons from genetic manipulation of GLUT4 [In Process Citation]. Mol Cell Biochem 182:143–152, 1998.
271. Shefi-Friedman L, Wertheimer E, Shen S, et al: Increased IGFR activity and glucose transport in cultured skeletal muscle from insulin receptor null mice. Am J Physiol Endocrinol Metab 281:E16–E24, 2001.
272. Kulkarni RN, Bruning JC, Winnay JN, et al: 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.
273. DeFronzo RA: Pathogenesis of type 2 diabetes: Metabolic and molecular implications for identifying diabetes genes. Diabetes Rev 5:177–269, 1997.
274. Hansen PA, Han DH, Marshall BA, et al: A high fat diet impairs stimulation of glucose transport in muscle. Functional evaluation of potential mechanisms. J Biol Chem 273:26157–26163, 1998.
275. Nawrocki AR, Scherer PE: The delicate balance between fat and muscle: Adipokines in metabolic disease and musculoskeletal inflammation. Curr Opin Pharmacol 4:281–289, 2004.
276. Flier JS: Leptin expression and action: New experimental paradigms. Proc Natl Acad Sci U S A 94:4242–4245, 1997.
277. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432, 1994.
278. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46:3–10, 1997.
279. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. Science 274:1185–1188, 1996.
280. Hotamisligil GS, Peraldi P, Budvari A, et al: IRS-1 mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. Science 271:665–668, 1996.
281. Banks AS, Davis SM, Bates SH, Myers MG Jr: Activation of downstream signals by the long form of the leptin receptor. Control of ERK, c-fos, and SOCS3 by LRb. J Biol Chem 275(19):14563–14572, 2000.
282. Feener EP, Rosario F, Dunn SL, et al: Tyrosine phosphorylation of Jak2 in the JH2 domain inhibits cytokine signaling. Mol Cell Biol 24:4968–4978, 2004.
283. Bates SH, Stearns WH, Dundon TA, et al: STAT3 signalling is required for leptin regulation of energy balance but not reproduction. Nature 421:856–859, 2003.
284. Wang MY, Koyama K, Shimabukuro M, et al: OB-Rb gene transfer to leptin-resistant islets reverses diabetogenic phenotype. Proc Natl Acad Sci U S A 95:714–718, 1998.
285. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: A key component of the obesity-diabetes link. Diabetes 43:1271–1278, 1994.
286. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. Science 259:87–91, 1993.
287. Weisberg SP, McCann D, Desai M, et al: Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808, 2303.
288. Peraldi P, Hotamisligil GS, Buurman WA, et al: 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.
289. Kanety H, Feinstein R, Papa MZ, et al: Tumor necrosis factor α-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism

for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. J Biol Chem 270:23780–23784, 1995.
290. Uysal KT, Wiesbrock SM, Hotamisligil GS: Functional analysis of tumor necrosis factor (TNF) receptors in TNF-alpha-mediated insulin resistane in genetic obesity. Endocrinology 139:4832–4838, 1998.
291. Miles PD, Romeo OM, Higo K, et al: TNF-alpha-induced insulin resistance in vivo and its prevention by troglitazone. Diabetes 46:1678–1683, 1997.
292. Nonogaki K, Fuller GM, Fuentes NL, et al: Interleukin-6 stimulates hepatic triglyceride secretion in rats. Endocrinology 136:2143–2149, 1995.
293. Senn JJ, Klover PJ, Nowak IA, Mooney RA: Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 51:3391–3399, 2002.
294. Senn JJ, Klover PJ, Nowak IA, et al: Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. J Biol Chem 278:13740–13746, 2003.
295. Rotter V, Nagaev I, Smith U: Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 278:45777–45784, 2003.
296. Tsigos C, Papanicolaou DA, Kyrou I, et al: Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. J Clin Endocrinol Metab 82:4167–4170, 1997.
297. Kern PA, Ranganathan S, Li C, et al: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 280:E745–E751, 2001.
298. Lyngso D, Simonsen L, Bulow J: Metabolic effects of interleukin-6 in human splanchnic and adipose tissue. J Physiol 543(Pt 1):379–386, 2002.
299. van Hall G, Steensberg A, Sacchetti M, et al: Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab 88:3005–3010, 2003.
300. Scherer PE, Williams S, Fogliano M, et al: Serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270:26746–26749, 1995.
301. Wong GW, Wang J, Hug C, et al: A family of Acrp30/adiponectin structural and functional paralogs. Proc Natl Acad Sci U S A 101:10302–10307, 2004.
302. Rajala MW, Scherer PE: Minireview: The adipocyte—at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144:3765–3773, 2003.
303. Yamauchi T, Kamon J, Ito Y, et al: Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769, 2003.
304. Hotamisligil GS: Mechanisms of NF-alpha-induced insulin resistance. Exp Clin Endocrinol Diabetes 107:119–125, 1999.
305. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS: Protection from obesity-induced insulin resistance in mice lacking TNF-α function. Nature 389:610–614, 1997.
306. Aguirre V, Uchida T, Yenush L, et al: The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J Biol Chem 275:9047–9054, 2000.
307. De Fea K, Roth RA: Protein kinase C modulation of insulin receptor substrate-1 tyrosine phosphorylation requires serine 612. Biochemistry 36:12939–12947, 1997.
308. Eldar-Finkelman H, Krebs EG: Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. Proc Natl Acad Sci U S A 94:9660–9664, 1997.
309. Staubs PA, Nelson JG, Reichart DR, Olefsky JM: Platelet-derived growth factor inhibits insulin stimulation of insulin receptor substrate-1 associated phosphatidylinositol 3-kinase in 3T3-L1 adipocytes without affecting glucose transport. J Biol Chem 273:25139–25147, 1998.
310. Hemi R, Paz K, Wertheim N, et al: Transactivation of ErbB2 and ErbB3 by tumor necrosis factor-alpha and anisomycin leads to impaired insulin signaling through serine/threonine phosphorylation of IRS proteins. J Biol Chem 277:8961–8969, 2002.
311. Egawa K, Nakashima N, Sharma PM, et al: Persistent activation of phosphatidylinositol 3-kinase causes insulin resistance due to accelerated insulin-induced insulin receptor substrate-1 degradation in 3T3-L1 adipocytes. Endocrinology 141:1930–1935, 2000.
312. Ravichandran LV, Chen H, Li Y, Quon MJ: Phosphorylation of PTP1B at Ser(50) by Akt impairs its ability to dephosphorylate the insulin receptor. Mol Endocrinol 15:1768–1780, 2001.
313. Liu YF, Paz K, Herschkovitz A, et al: Insulin stimulates PKCzeta-mediated phosphorylation of insulin receptor substrate-1 (IRS-1). A self-attenuated mechanism to negatively regulate the function of IRS proteins. J Biol Chem 276:14459–14465, 2001.
314. Ozes ON, Akca H, Mayo LD, et al: A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. Proc Natl Acad Sci U S A 98:4640–4645, 2001.
315. Mauvais-Jarvis F, Ueki K, Fruman DA, et al: Reduced expression of the murine p85alpha subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. J Clin Invest 109:141–149, 2002.
316. Yu C, Chen Y, Cline GW, et al: Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem 277:50230–50236, 2002.
317. Greene MW, Garofalo RS: Positive and negative regulatory role of insulin receptor substrate 1 and 2 (IRS-1 and IRS-2) serine/threonine phosphorylation. Biochemistry 41:7082–7091, 2002.
318. Gao Z, Hwang D, Bataille F, et al: Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. J Biol Chem 277:48115–48121, 2002.
319. Gao Z, Zuberi A, Quon MJ, et al: Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. J Biol Chem 278:24944–24950, 2003.
320. Gual P, Gremeaux T, Gonzalez T, et al: MAP kinases and mTOR mediate insulin-induced phosphorylation of insulin receptor substrate-1 on serine residues 307, 612 and 632. Diabetologia 46:1532–1542, 2003.
321. Hirosumi J, Tuncman G, Chang L, et al: A central role for JNK in obesity and insulin resistance. Nature 420:333–336, 2002.
322. Yuan M, Konstantopoulos N, Lee J, et al: Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 293:1673–1677, 2001.
323. Konstantopoulos N, Lee J, Yuan M, et al: Inhibition of IKKb reverses "Insulin Resistance" in cultured cells. Diabetes 49:A14, 2000.
324. Kim JK, Kim YJ, Fillmore JJ, et al: Prevention of fat-induced insulin resistance by salicylate. J Clin Invest 108:437–446, 2001.
325. Hundal RS, Petersen KF, Mayerson AB, et al: Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 109:1321–1326, 2002.
326. Mothe I, Van Obberghen E: Phosphorylation of insulin receptor substrate-1 on multiple serine residues, 612, 632, 662, and 731, modulates insulin action. J Biol Chem 271:11222–11227, 1996.
327. Greene MW, Morrice N, Garofalo RS, Roth RA: Modulation of human insulin receptor substrate-1 tyrosine phosphorylation by protein kinase Cdelta. Biochem J 378(Pt 1):105–116, 2004.
328. Giraud J, Leshan R, Lee YH, White MF: Nutrient-dependent and insulin-stimulated phosphorylation of insulin receptor substrate-1 on serine 302 correlates with increased insulin signaling. J Biol Chem 279:3447–3454, 2004.

329. Paz K, Hemi R, LeRoith D, et al: A molecular basis for insulin resistance. Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. J Biol Chem 272:29911–29918, 1997.
330. Folli F, Kahn CR, Hansen H, et al: Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels: A potential role for serine phosphorylation in insulin/angiotensin II "crosstalk." J Clin Invest 100:2158–2169, 1997.
331. Xiang X, Yuan M, Song Y, et al: 14-3-3 facilitates insulin-stimulated intracellular trafficking of insulin receptor substrate 1. Mol Endocrinol 16:552–562, 2002.
332. Rui L, Fisher TL, Thomas J, White MF: Regulation of insulin/insulin-like growth factor-1 signaling by proteasome-mediated degradation of insulin receptor substrate-2. J Biol Chem 276:40362–40367, 2001.
333. Haruta T, Uno T, Kawahara J, et al: A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. Mol Endocrinol 14:783–794, 2000.
334. Takano A, Usui I, Haruta T, et al: Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. Mol Cell Biol 21:5050–5062, 2001.
335. Harrington LS, Findlay GM, Grgay A, et al: The TSC1-2 tumor supressor controls insulin-PI3K signaling via regulation of IRS proteins. J Cell Biol 166(2):213–223, 2004.
336. Rui L, Yuan M, Frantz D, et al: SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem 277:42394–42398, 2002.
337. Krebs DL, Uren RT, Metcalf D, et al: SOCS-6 binds to insulin receptor substrate 4, and mice lacking the SOCS-6 gene exhibit mild growth retardation. Mol Cell Biol 22:4567–4578, 2002.
338. Sadowski CL, Choi TS, Le MN, et al: Insulin induction of SOCS-2 and SOCS-3 mRNA expression in C2C12 skeletal muscle cells is mediated by Stat5. J Biol Chem 276(23): 20703–20710, 2001.
339. Krebs DL, Hilton DJ: A new role for SOCS in insulin action. Suppressor of cytokine signaling. Sci STKE E6, 2003.
340. Wojcik C: Inhibition of the proteasome as a therapeutic approach. Drug Discov Today 4:188–189, 1999.
341. Dadke S, Kusari A, Kusari J: Phosphorylation and activation of protein tyrosine phosphatase (PTP) 1B by insulin receptor. Mol Cell Biochem 221:147–154, 2001.
342. Dadke S, Kusari J, Chernoff J: Down-regulation of insulin signaling by protein-tyrosine phosphatase 1B is mediated by an N-terminal binding region. J Biol Chem 275:23642–23647, 2000.
343. Goldstein BJ, Bittner-Kowalcyk A, White MF, Harbeck M: Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein tyrosine phosphatase 1B: Possible facilitation by the formation of a ternary complex with the Grb-2 adaptor protein. J Biol Chem 275:4283–4289, 2000.
344. Bandyopadhyay D, Kusari A, Kenner KA, et al: Protein-tyrosine phosphatase 1B complexes with the insulin receptor in vivo and is tyrosine-phosphorylated in the presence of insulin. J Biol Chem 272:1639–1645, 1997.
345. Kenner KA, Anyanwu E, Olefsky JM, Kusari J: Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signaling. J Biol Chem 271:19810–19816, 1996.
346. Klaman LD, Boss O, Peroni OD, et al: Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 20:5479–5489, 2000.
347. Zinker BA, Rondinone CM, Trevillyan JM, et al: PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci U S A 99:11357–11362, 2002.
348. Haj FG, Markova B, Klaman LD, et al: Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatase-1B. J Biol Chem 278:739–744, 2003.
349. Boute N, Boubekeur S, Lacasa D, Issad T: Dynamics of the interaction between the insulin receptor and protein tyrosine-phosphatase 1B in living cells. EMBO Rep 4:313–319, 2003.
350. Li J, Yen C, Liaw D, et al: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943–1947, 1997.
351. Ono H, Katagiri H, Funaki M, et al: Regulation of phosphoinositide metabolism, Akt phosphorylation, and glucose transport by PTEN (phosphatase and tensin homolog deleted on chromosome 10) in 3T3-L1 adipocytes. Mol Endocrinol 15:1411–1422, 2001.
352. Maehama T, Dixon JE: The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 273:13375–13378, 1998.
353. Maehama T, Dixon JE: PTEN: A tumour suppressor that functions as a phospholipid phosphatase. Trends Cell Biol 9:125–128, 1999.
354. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP: Pten is essential for embryonic development and tumour suppression. Nat Genet 19:348–355, 1998.
355. Nakashima N, Sharma PM, Imamura T, et al: The tumor suppressor PTEN negatively regulates insulin signaling in 3T3-L1 adipocytes. J Biol Chem 275:12889–12895, 2000.
356. Butler M, McKay RA, Popoff IJ, et al: Specific inhibition of PTEN expression reverses hyperglycemia in diabetic mice. Diabetes 51:1028–1034, 2002.
357. Kubota N, Tobe K, Terauchi Y, et al: Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. Diabetes 49:1880–1889, 2000.
358. Previs SF, Withers DJ, Ren JM, et al: Contrasting effects of IRS-1 vs IRS-2 gene disruption on carbohydrate and lipid metabolism in vivo. J Biol Chem 275:38990–38994, 2000.
359. Xuan S, Kitamura T, Nakae J, et al: Defective insulin secretion in pancreatic beta cells lacking type 1 IGF receptor. J Clin Invest 110:1011–1019, 2002.
360. Kulkarni RN, Holzenberger M, Shih DQ, et al: Beta-cell-specific deletion of the Igf1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter beta-cell mass. Nat Genet 31:111–115, 2002.
361. Jonsson J, Carlsson L, Edlund T, Edlund H: Insulin-promoter-factor 1 is required for pancreas developement in mice. Nature 371:606–609, 1994.
362. Stoffers DA, Zinkin NT, Stanojevic V, et al: Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 15:106–110, 1997.
363. Kushner JA, Ye J, Schubert M, et al: Pdx1 restores beta cell function in Irs2 knockout mice. J Clin Invest 109:1193–1201, 2002.
364. Kubota T, Kubota N, Moroi M, et al: Lack of insulin receptor substrate-2 causes progressive neointima formation in response to vessel injury. Cirulation 107:3073–3080, 2003.
365. Perry T, Greig NH: The glucagon-like peptides: A double-edged therapeutic sword? Trends Pharmacol Sci 24:377–383, 2003.
366. Jhala US, Canettieri G, Screaton RA, et al: cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. Genes Dev 17:1575–1580, 2003.
367. Butler AA, Cone RD: Knockout studies defining different roles for melanocortin receptors in energy homeostasis. Ann N Y Acad Sci 994:240–245, 2003.

368. Farooqi IS, Keogh JM, Yeo GS, et al: Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med 348:1085–1095, 2003.
369. Qi Y, Takahashi N, Hileman SM, et al: Adiponectin acts in the brain to decrease body weight. Nat Med 10:524–529, 2004.
370. Myers MG Jr: Leptin receptor signaling and the regulation of mammalian physiology. Recent Prog Horm Res 59:287–304, 2004.
371. Woods SC, Lotter EC, McKay LD, Porte D Jr: Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 282:503–505, 1979.
372. Schwartz M, Figlewicz DP, Baskin DG, et al: Insulin in the brain: A hormonal regulator of energy balance. Endocr Rev 13:387–414, 1992.
373. Schwartz MW, Baskin DG, Kaiyala KJ, Woods SC: Model of the regulation of energy balance and adiposity by the central nervous system. Am J Clin Nutr 69:584–596, 1999.
374. Bruning JC, Gautam D, Burks DJ, et al: Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122–2125, 2000.
375. Huszar D, Lynch CA, Fairchild-Huntress V, et al: Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131–141, 1997.
376. Obici S, Feng Z, Tan J, et al: Central melanocortin receptors regulate insulin action. J Clin Invest 108:1079–1085, 2001.
377. Challis BG, Pritchard LE, Creemers JW, et al: A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. Hum Mol Genet 11:1997–2004, 2002.
378. Combs TP, Berg AH, Rajala MW, et al: Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. Diabetes 52:268–276, 2003.
379. Spranger J, Kroke A, Mohlig M, et al: Adiponectin and protection against type 2 diabetes mellitus. Lancet 361:226–228, 2003.
380. Burks DJ, de Mora JF, Schubert M, et al: IRS-2 pathways integrate female reproduction and energy homeostasis. Nature 407:377–382, 2000.
381. Gannon M, Shiota C, Postic C, et al: Analysis of the Cre-mediated recombination driven by rat insulin promoter in embryonic and adult mouse pancreas. Genesis 26:139–142, 2000.
382. Cui Y, Huang L, Elefteriou F, et al: Essential role of STAT3 in body weight and glucose homeostasis. Mol Cell Biol 24:258–269, 2004.
383. Lin X, Taguchi A, Park S, et al: Dysregulation of IRS2 in beta cells and brain causes obesity and diabetes. J Clin Invest 114(7):908–916, 2004.
384. Yasukawa H, Sasaki A, Yoshimura A: Negative regulation of cytokine signaling pathways. Annu Rev Immunol 18:143–164, 2000.
385. Krebs DL, Hilton DJ: SOCS: Physiological suppressors of cytokine signaling. J Cell Sci 113(Pt 16):2813–2819, 2000.
386. Kamura T, Sato S, Haque D, et al: The elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. Genes Dev 12:3872–3881, 1998.
387. Tyers M, Willems AR: One ring to rule a superfamily of E3 ubiquitin ligases [comment]. Science 284:601–604, 1999.
388. Zhang JG, Farley A, Nicholson SE, et al: The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. Proc Natl Acad Sci U S A 96:2071–2076, 1999.