Insulin Action: Molecular Basis of Diabetes

Secondary article

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Unbalanced regulation of the signalling cascades that mediate insulin action in the cell leads to diabetes mellitus. Thus, identification of the basic mechanism of insulin action promotes our understanding of the molecular basis of diabetes and the development of better therapeutic interventions.

Article Contents

Introduction

Insulin is secreted from pancreatic β cells upon absorption of digested food products to exert multiple immediate and long-term effects on the cell and organism. Insulin decreases the hepatic production of glucose, and induces fuel storage as glycogen in liver and muscle, and as triglycerides in adipose tissues. Together with the growth-promoting effects of insulin, the metabolic functions are initially mediated by insulin binding to its receptor at the cell surface membrane of insulin target tissues: liver, muscle and adipocytes. Insulin binding activates the tyrosine kinase of its receptor to phosphorylate the receptor and other endogenous substrates. Phosphorylation cascades constitute the basic mechanism of insulin action in the cell. Insulin signalling is terminated by many mechanisms that include receptor-mediated insulin endocytosis followed by degradation, activation of tyrosine phosphatases or serine/threonine kinases, sequestration of receptors from the plasma membrane and reducing the number of receptors in response to high insulin concentration (receptor down-regulation).

Abnormal processing of one or many of these steps leads to a deranged metabolic state commonly known as diabetes mellitus. This article reviews how these chains of molecular and cellular events are regulated, and how unbalanced regulation leads to diabetes.

Insulin: Structure and Synthesis

Insulin is a polypeptide hormone of about 5808 Da in humans. Gauged by circulating glucose levels, a combination of regulatory and basal elements works in concert to regulate tissue-specific expression of insulin in pancreatic β cells (Habener and Stoffers, 1998) (**Figure 1**). The most studied transcriptional factors that bind to deoxyribonucleic acid (DNA) sequences in the insulin promoter include hepatic nuclear factor (HNF) 1α (which is upregulated by HNF4α) and the islet duodenum homeobox 1 (IDX-1) (which is upregulated by HNF3β). In addition to its regulatory effect on insulin gene transcription, IDX-1, a homeodimer protein also known as insulin promoter factor 1 (IPF-1), functions as a chief regulator of the development of the endocrine pancreas. The DNA-binding sites in the insulin promoter and the basal elements include Sp1, a negative regulatory element between nucleotide -279 and -258 in the human insulin gene, cyclic adenosine monophosphate (cAMP) regulatory element (CRE) and others such as the E box, A, C1 and G elements.

Insulin is initially synthesized as preproinsulin in pancreatic β cells. About 5–10 min after its assembly in the endoplasmic reticulum, preproinsulin is processed into proinsulin before its transport to the trans-Golgi network (TGN) where immature granules are formed. Transport to the TGN may take about 30 min. Proinsulin, comprising an A and a B chain linked together by disulfide bonds and by a C-peptide bridge, undergoes maturation into active insulin through the action of endopeptidases. Endopeptidases cleave off C peptide from insulin by breaking the bonds between lysine 64 and arginine 65, and between arginine 31 and 32 (**Figure 2**). Mature insulin is packaged inside mature granules waiting for metabolic signals (such as leucine, arginine, glucose and mannose) and vagal nerve stimulation to be exocytosed from the cell into the circulation. Several signalling mechanisms regulate insulin secretion from the pancreatic β cell. The main mechanism involves phosphorylation of glucose by glucokinase upon its transport into β cells via glucose transporter 2 (Glut 2). Glucose 6-phosphate, the product of this rate-limiting step, undergoes stepwise metabolism in the cytosol followed by oxidative phosphorylation in the mitochondria to yield adenosine triphosphate (ATP). The raised intracellular ATP concentration inhibits the output of K^+ and consequently leads to β-cell depolarization. This causes the opening of voltage-sensitive Ca^{2+} channels and the subsequent inward movement of Ca^{2+} inside the cell. Increased intracellular levels of Ca^{2+} in turn activate kinases that upregulate insulin exocytosis and secretion into the circulation.

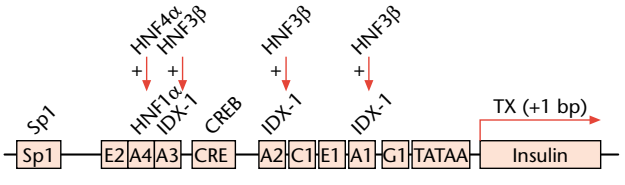

Figure 1 The insulin promoter. The genomic sequence elements are shown in boxes. Hepatic nuclear factors (HNF) regulate the expression of binding proteins (shown at an angle) that regulate the transcription of insulin in the β cell of the pancreas. TX denotes the transcription initiation site, and HNF the hepatic nuclear factors that regulate the expression of the binding proteins in a tissue-specific manner. CREB, cAMP regulatory element (binding protein); IDX, islet duodenum homeobox.

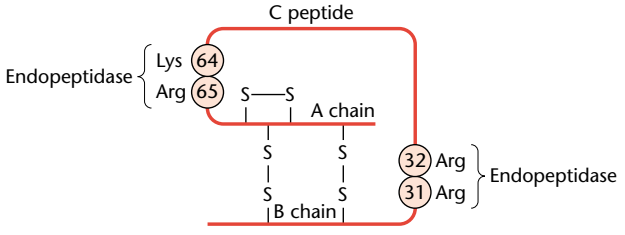

Figure 2 The proinsulin molecule, which consists of a C-peptide bridge linking the A and B chains. Cleavage of the C-peptide bridge by endopeptidases produces mature and active insulin. S–S denotes the disulfide bonds.

Insulin Action: Basic Mechanism

Insulin action in peripheral tissues

Insulin stimulates glucose turnover by stimulating its transport across the plasma membrane, followed by either oxidative or nonoxidative disposal, the latter being associated with glycogen synthesis. The effect of insulin on glucose transport is observed only in skeletal muscle, adipose tissues and heart. Insulin generally promotes protein synthesis in virtually all tissues, by virtue of a combined effect on gene transcription, messenger ribonucleic acid (mRNA) translation and amino acid uptake. Insulin also exerts mitogenic effects, mediated through increased DNA synthesis and prevention of programmed cell death (apoptosis). In addition, insulin stimulates ion transport across the plasma membrane of multiple tissues. Finally, insulin stimulates lipid synthesis in fat cells, skeletal muscle and liver, and prevents lipolysis by inhibiting hormone-sensitive lipase. Increasingly, there is evidence for a direct role of insulin, acting through the insulin receptor, to regulate insulin release from the pancreatic β cell (**Figure 3**).

The effects of insulin are tissue specific

Different tissues respond differently to insulin. Although tissue sensitivity to insulin correlates with the levels of insulin receptors expressed on the plasma membrane, it has

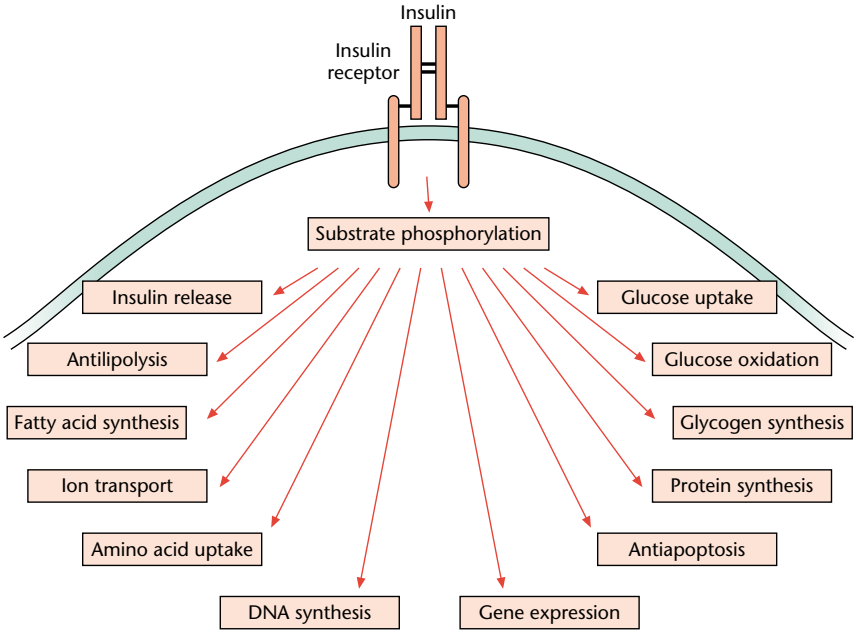

Figure 3 Insulin action in the cell. Insulin exerts multiple effects in the cell. Insulin action is mediated by the binding of insulin to its receptor, and the subsequent phosphorylation of the receptor and other substrates by the receptor tyrosine kinase.

become clear that the assembly of different components of the insulin signalling pathway is also responsible for bestowing specificity of insulin signalling upon target cells. Thus, insulin-dependent glucose transport is observed only in skeletal muscle and adipose cells, because these cells possess the insulin-dependent Glut4 glucose transporters. Likewise, inhibition of gluconeogenesis by insulin is specific to liver and kidney. On the other hand, the effects on ion transport, DNA synthesis and protein synthesis appear to be ubiquitous.

The insulin receptor

Roth and colleagues first identified the insulin receptor in 1971. Insulin receptors are expressed in virtually all cells, ranging in number from a few hundred in red blood cells to several hundred thousand in liver and adipose cells. In 1985, Ullrich and Ebina cloned the complementary DNA (cDNA) and the gene encoding the receptor. This has led to an intense area of research, investigating the basic mechanism of insulin action and the identification of intracellular mediators of insulin action.

The insulin receptor is initially synthesized as a single-chain polypeptide precursor which undergoes posttranslational cleavage into separate α and β subunits, followed by dimerization and export to the plasma membrane. The mature receptor is a homodimer composed of two α and β subunits (α_2 and β_2) (**Figure 4**). The α subunit is extracellular and contains the ligand-binding domain. The β subunit spans the plasma membrane, and is linked to the α subunit via disulfide bridges as well as noncovalent interactions. The intracellular part of the β subunit contains the tyrosine kinase domain. Insulin binding to the α subunit results in a conformational modification in the intracellular domain, such that the tyrosine kinase is activated and the receptor binds ATP and undergoes autophosphorylation. Several tyrosine residues in the β subunit are phosphorylated. These include tyrosine 972 in the juxtamembrane domain, which plays a key role in the binding and phosphorylation of most substrates; tyrosines 1158, 1162 and 1163 in the catalytic domain, which are essential to promote the kinase activity of the receptor towards other protein substrates; and tyrosines 1328 and 1334 in the C-terminal domain. The role of the latter phosphorylation sites is more controversial, with some investigations suggesting that they play a role in mediating the growth-promoting activity of insulin.

The genomic organization of the insulin receptor is similar in mice and humans. The insulin receptor gene is housekeeping, containing no TATA or CAAT boxes and several Sp1-binding sites in addition to two novel nuclear factors. The gene spans more than 150 kilobases, and is composed of 22 exons, the 11th of which is alternatively spliced to form two isoforms that either contain (isoform B) or lack (isoform A) the 12-amino-acid peptide encoded

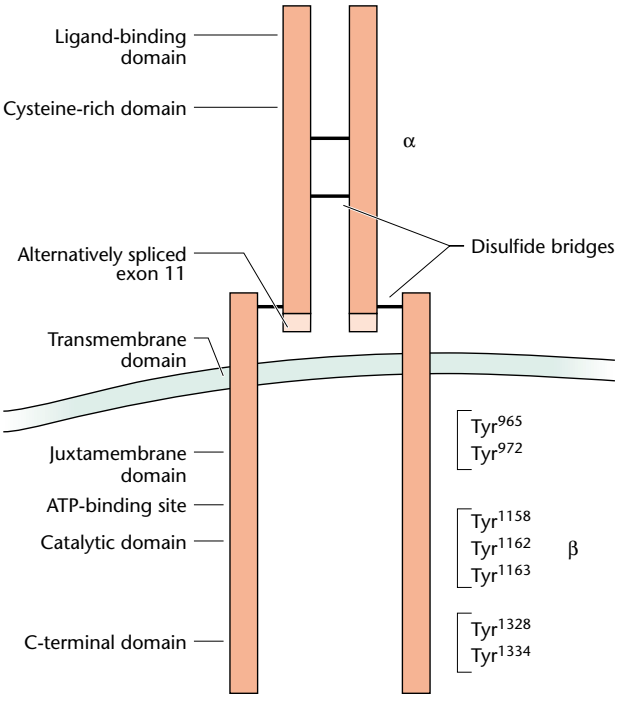

Figure 4 The insulin receptor, a heterotetrameric structure containing two α and two β subunits. The α subunit contains the insulin-binding domain and the β subunit contains the catalytic tyrosine kinase domain. Binding of insulin (ligand) to its receptor activates the kinase to phosphorylate the receptor on tyrosine (Tyr) residues in the intracellular domain of the receptor. ATP, adenosine triphosphate.

by exon 11 at the C-terminus of the α subunit. The two isoforms are expressed at a different relative ratio in a tissue-specific manner, with isoform A predominating in haematopoietic cells and isoform B predominating in hepatocytes. The two isoforms are comparably distributed in muscle and adipocytes. The role of alternative splicing in receptor function is not clear, but isoform B has been shown to bind insulin with lower affinity than isoform A.

Intracellular signalling pathways

The different signalling pathways activated by the hormone in part explain the diversity of insulin action in different tissues. There are two main limbs that propagate the signal generated through the insulin receptor: the insulin receptor substrates/phosphatidylinositol 3-kinase pathway (IRS/PI3-K) and the Ras/mitogen-activated protein kinase (MAPK) pathway (reviewed in Di Guglielmo *et al.*, 1998; Ogawa *et al.*, 1998 and White, 1998) (**Figure 5**). The IRS/PI3-K pathway leads to the generation of phosphatidylinositol 3-phosphate, and the consequent activation of PI-dependent kinases, such as the PI-

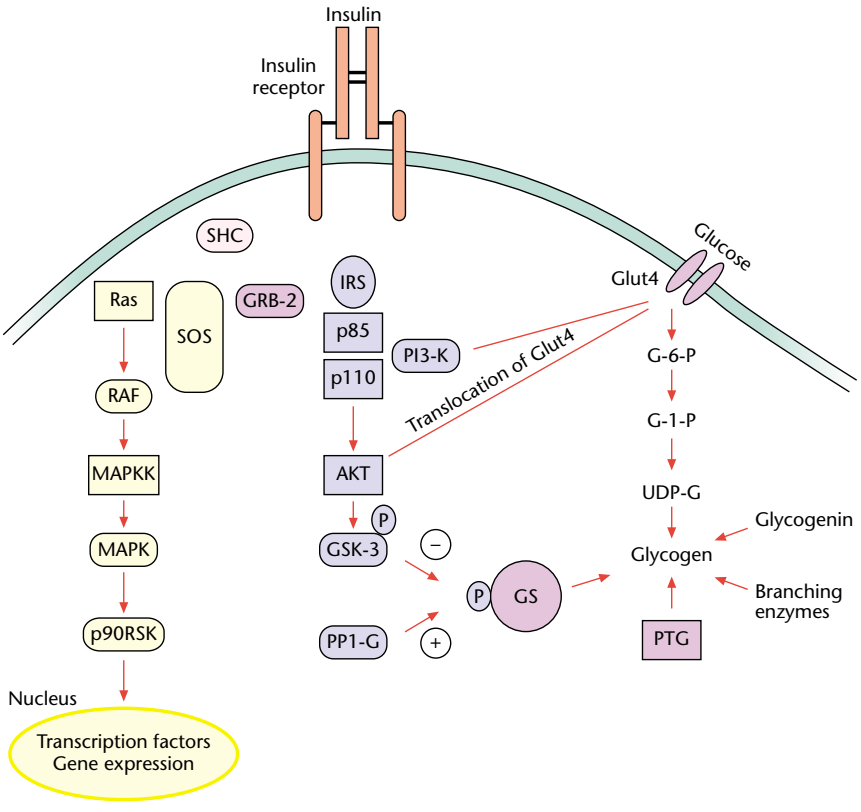

Figure 5 Intracellular insulin signalling pathways. Insulin binding to its receptor activates different signalling pathways. Two main limbs propagate insulin signals: the insulin receptor substrate (IRS)/phosphatidylinositol 3-kinase (PI3-K) pathway, and the Ras/mitogen-activated protein kinase (MAPK) pathway. The IRS/PI3-K pathway mediates glycogen synthesis and the translocation of glucose transporters (Glut) to the cell surface. The Ras/MAPK pathway, which is activated by coupling of growth factor receptor-binding protein 2 (GRB-2) to the receptor either by IRS proteins or by SHC, mediates the effects of insulin on cell growth and proliferation. GS(K), glycogen synthase (kinase); G-6-P, glucose-6-phosphate; MAPKK, MAPK kinase; Ras, a GTPase protooncogene product; Raf, a serine/threonine kinase protoconcogene product; RSK, ribosomal S6 kinase; PP1-G, glycogen-associated protein phosphatase-1; UDP-G, uridine diphosphate-glucose.

dependent kinases 1 and 2 (PDK-1, PDK-2) and protein kinase C (PKC). Some of these kinases may be required to activate downstream kinases, such as the serine/threonine kinase AKT (the product of the *AKT* proto-oncogene). It is thought that AKT may directly phosphorylate and inactivate glycogen synthase kinase 3 (GSK-3), thus leading to dephosphorylation and activation of glycogen synthase and increased glycogen synthesis. There is also evidence linking AKT to translocation of glucose transporters. Insulin, through the formation of complexes between the exchange factor SOS (son-of-sevenless) and growth factor receptor-binding protein 2 (GRB-2), can activate the Ras/MAPK pathway. GRB-2 can be activated by IRS or SHC, two direct substrates of the insulin receptor kinase. It appears that the acute metabolic effects of insulin require activation of the IRS/PI3-K pathway, whereas the Ras/MAPK pathway may play a role in certain tissues to stimulate the long-term effects of insulin on growth and proliferation.

Insulin receptor substrate proteins act as docking molecules to mediate insulin signalling

The IRSs were initially identified as tyrosine-phosphorylated proteins in insulin-treated cells (White, 1998). In addition to insulin, other agents (e.g. insulin-like growth factors (IGFs) and interleukins) can stimulate IRS phosphorylation. Tyrosine phosphorylation occurs at signature Y-x-x-M motifs (where x is any amino acid, and M is methionine) (**Figure 6**). The phosphorylated tyrosines become binding sites for src-homology 2 (SH2) motifs in downstream signalling molecules. SH2 motifs are 50–100 amino acids long; they act as high-affinity phosphotyrosine-binding sites and are found in many intracellular signalling molecules. Binding of phosphotyrosine to SH2 domains leads to the formation of signalling complexes. In this manner, the IRS proteins function as docking molecules to mediate the indirect interaction between the insulin receptor and downstream signalling

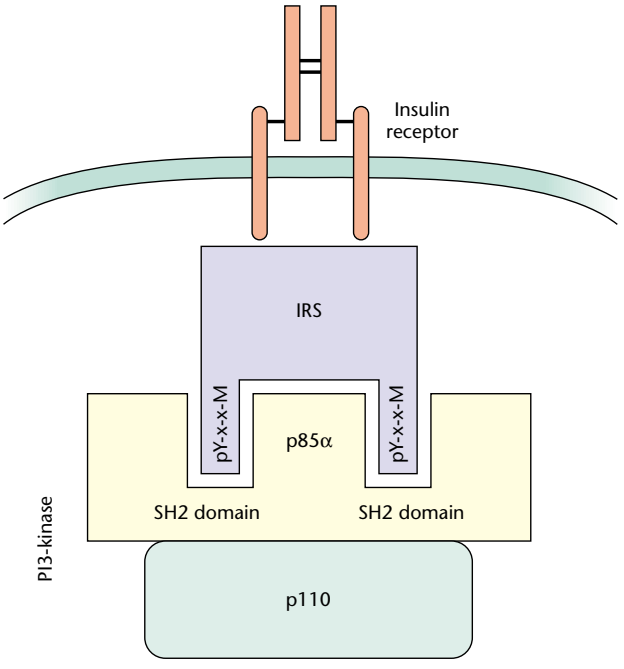

Figure 6 The docking function of proteins of the insulin receptor substrate family. Phosphorylated tyrosine residues of insulin receptor substrate (IRS) proteins at signature Y-x-x-M motifs (where Y is tyrosine, x is any amino acid, and M is methionine) become binding sites for src homology 2 (SH2) domains of the p85α subunit of phosphatidylinositol 3-kinase (PI3-kinase). This leads to the activation of the p110 catalytic subunit of PI3-kinase and the propagation of insulin signal through the IRS/PI3-kinase pathway.

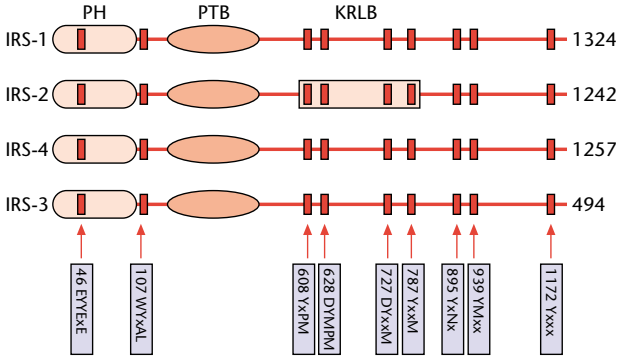

Figure 7 Alignment of the members of the insulin receptor substrate (IRS) family of proteins, showing the four clones of IRS proteins. The length of the polypeptide chain is shown to the right of each bar. Conserved phosphorylation sites are indicated by small red boxes. The figure also includes the pleckstrin homology (PH) domain, the phosphotyrosine-binding (PTB) domain and the kinase regulatory loop-binding (KRLB) domain, which is found only in IRS-2.

molecules. A key signalling complex in insulin action is formed between IRSs and the regulatory subunit (p85) of the enzyme PI3-K (**Figure 6**). There are two SH2 domains in the structure of p85. Binding of IRSs to the two SH2 domains of p85 leads to activation of the p110 (catalytic) subunit of PI3-K. The catalytic activity of p110 stimulates phosphorylation of phosphatidylinositol on the D3 position of the inositol ring, leading to the generation of PI 3-phosphate. There is increasing evidence that 3-phosphorylated inositides act as intracellular messengers, leading to activation of PDKs, changes in intracellular trafficking, and growth stimulation.

Among the proteins that bind to IRSs, the p85 subunit of PI3-K is the most prominent. The regulatory subunit of PI3-K exists in several different isoforms (p85-a, -b, p55, p55PIK, p50). It is not clear whether different isoforms can mediate different effects of insulin. Another partner is GRB-2 (**Figure 5**), an adaptor molecule composed of one SH2 and two SH3 domains. GRB-2 links IRSs to the Ras signalling pathway. Several other proteins have been shown to bind IRSs, but their role in insulin action is unclear (White, 1998).

There are four cloned members of the IRS family of proteins, and a related protein called Gab-1 (Grb-associated binder-1). An alignment of the four cloned IRSs is shown (**Figure 7**). The length of the polypeptide chain is shown to the right of the figure. The main structural motifs of the IRS molecules are indicated by lightly shaded geometric structures: the pleckstrin homology (PH) domain, the phosphotyrosine-binding (PTB) domain and the kinase regulatory loop-binding domain (KRLB). Dark small boxes indicate the conserved phosphorylation sites. The PTB domain binds to tyrosine 972 in the juxtamembrane domain of the insulin receptor, and the other domains regulate this binding and association of IRSs to intracellular molecules (**Figure 8**). It is conceivable that the presence of a unique assortment of sites in each molecule would allow for the recruitment of different signalling molecules and lead to the formation of unique signalling complexes. In addition, there are distinctive patterns of tissue expression, such that each IRS protein may play a different role in different tissues (White, 1998).

Downstream effectors of insulin action: the AKT kinase

The rapid increase in intracellular concentrations of PI 3-phosphate following activation of the insulin receptor has led to the hypothesis that inositol 3-phosphate may act as an intracellular mediator of insulin action. There are several kinases that are activated by the rise in PI 3-phosphate. PDK-1 and PDK-2 are two members of this family that may be involved in mediating some of insulin action (**Figure 9**). PDK-1 and PDK-2 phosphorylate threonine 308 and serine 473 respectively, in the primary sequence of the AKT serine/threonine kinase. The AKT kinase has been linked to insulin stimulation of glycogen synthesis and glucose transport. The enzyme is composed of a PH domain and a kinase domain with the two PI-dependent phosphorylation sites. Maximal enzyme activ-

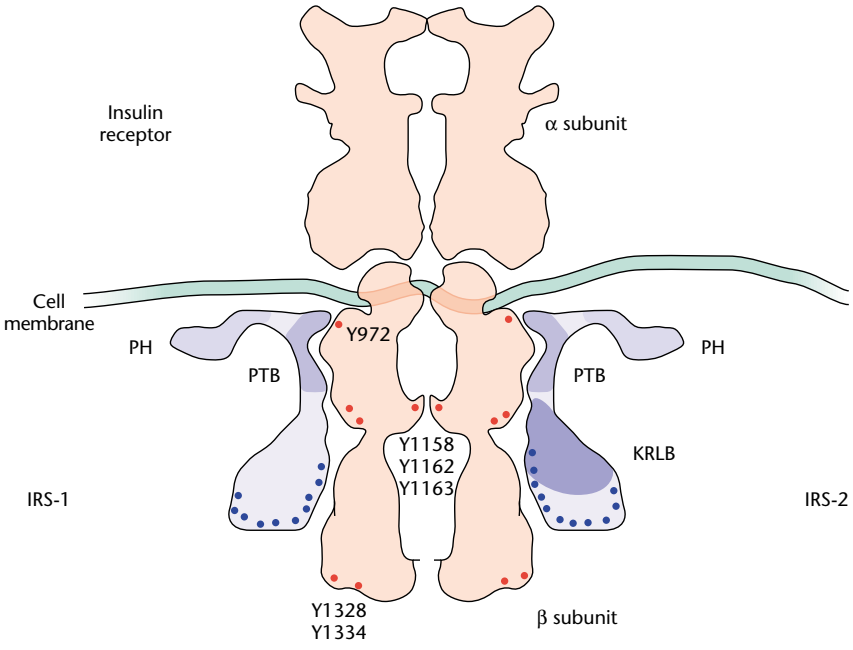

Figure 8 Interaction of insulin receptors and proteins from the insulin receptor substrates family. Association between the insulin receptor substrate (IRS) proteins and the insulin receptor occurs mainly between the phosphotyrosine-binding (PTB) domain of IRS proteins and the juxtamembrane region of the insulin receptor. It requires phosphorylation of tyrosine 972 in the juxtamembrane domain of the receptor. The pleckstrin homology (PH) domain appears to stabilize this conformation, perhaps by binding to the phospholipids of the surface membrane bilayer. Association of IRS-2 with the receptor also involves the binding of its kinase regulatory loop-binding (KRLB) domain to the receptor. This requires phosphorylation on tyrosines 1158, 1162 and 1163 in the catalytic domain of the receptor.

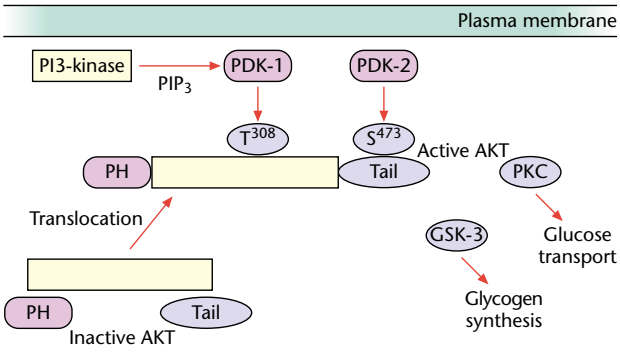

Figure 9 Substrates of the insulin receptor kinase. Various substrates undergo phosphorylation by the insulin receptor kinase. Whereas most substrates are cytoplasmic, pp120 is a plasma membrane protein that undergoes insulin-stimulated tryosine phosphorylation in its cytoplasmic tail. AKT, product of the *AKT* proto-oncogene; GSK, glycogen synthase kinase; PDK, phosphatidylinositol-dependent kinase; PH, pleckstrin homology domain; PI3-K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol triphosphate; PKC, protein kinase C.

ity requires that both sites be phosphorylated. The role of the PH domain is as yet unclear, but it may provide a targeting sequence for membrane translocation or for PI 3-phosphate binding. In addition to its role in glucose turnover, the serine/threonine kinase AKT is involved in the activation of p70 S6 kinase which, in turn, regulates protein synthesis and cellular growth and differentiation (**Figure 9**).

Downstream effectors of insulin action: protein kinase Cs

Members of the protein kinase C family of serine/threonine kinases have been implicated in several aspects of insulin action. There are four subgroups of PKCs: the 'classical' ones require calcium binding for activation, whereas the other three groups can be activated by diacylglycerol (DAG) binding, or by other phospholipids, such as PI 3,4,5-trisphosphate (PIP_3) (atypical PKCs). Insulin can activate different members of this kinase family through the formation of DAG and PIP_3. Insulin stimulates DAG formation through phosphatidylcholine hydrolysis into DAG and phosphatidic acid, or through the activity of a glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC), leading to the formation of DAG and inositolphosphoglycan (IPG).

Different isoforms of PKC have been shown to undergo translocation from the cytosol to the membrane in response to insulin stimulation in different tissues. There is evidence that this process may be important for the biological activity of PKCs. It is known that PKCs can

directly activate the MAPK pathway and the nuclear transcription factor NF-κB, leading to increased gene expression and protein synthesis. More recently, evidence has emerged that activation of atypical PKCs by PIP_3 may be important for the process of insulin-dependent glucose transport (**Figure 9**).

Other substrates of the insulin receptor kinase

In addition to the growing interest in IRS proteins as the main mediators of insulin action, there is substantial evidence that insulin receptors activate a whole host of cytosolic and membrane-bound proteins. Some of these proteins appear to be specific substrates of the insulin receptor, raising the possibility that they may be involved in mediating some actions of insulin. These include pp120, a plasma membrane glycoprotein in the liver (**Figure 10**) that is specifically phosphorylated by the insulin receptor, but not by its close relative, the IGF-1 receptor (Najjar *et al*., 1997). Likewise, GRB-IR, an adaptor protein that binds to the insulin receptor (IR) but not to the IGF-1 receptor, is weakly tyrosine-phosphorylated in response to insulin and has been shown to dampen insulin signalling. c-CBL is also a substrate of the insulin receptor in adipocytes, where it has been shown to direct insulin-dependent phosphorylation of caveolin.

Several substrates for phosphorylation by the insulin receptor in the 53–60-kDa range have also been cloned. Some of them are associated with molecules that play an important role in insulin action, such as PI3-K (p85), the tyrosine phosphatase SHP-2 and Ras-GAP (guanosine triphosphatase-associated protein). Thus, it is possible that phosphorylation of these substrates may affect insulin signalling in different tissues. Except for pp120, the phosphorylation of which requires the C-terminus of the β subunit, phosphorylation of other substrates requires an intact tyrosine 972 in the juxtamembrane domain of the insulin receptor.

Attenuation of insulin action

Termination of insulin signalling is largely mediated by rapid receptor endocytosis and degradation upon insulin binding to its receptor (Duckworth *et al*., 1998). To a significant extent, the action of insulin is also terminated by tyrosine phosphatases that dephosphorylate the tyrosine residues of the β subunit of the receptor, and by serine/threonine kinases that dampen its tyrosine kinase activity. Sequestration of insulin receptors and decrease in receptor number at the cell surface membrane are also associated with decreased insulin action, especially in obesity and hyperinsulinaemia.

Receptor internalization and turnover

Insulin binding to its receptor causes rapid endocytosis of insulin through its receptor. Receptor-mediated insulin endocytosis occurs via two distinct vesicular pathways: clathrin-coated pits and noncoated caveolae. When targeted for degradation in the liver, insulin is largely internalized through the clathrin-coated pathway of hepatocytes. Vesicular trafficking delivers the insulin–receptor complex to endosomes, where it undergoes dissociation in the acidic environment. Insulin is then targeted for degradation, whereas the receptor recycles back to the cell surface. This constitutes the basic mechanism of insulin clearance from the blood, which occurs mainly in the liver. Insulin-induced internalization

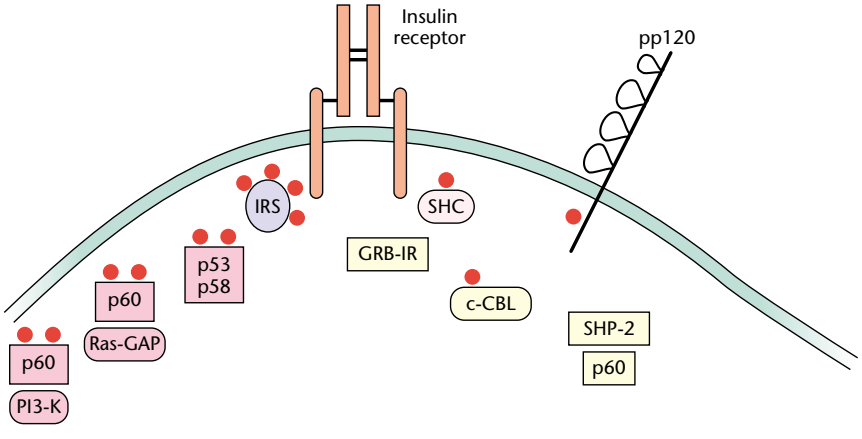

Figure 10 Downstream effectors of insulin signalling: the AKT serine/threonine kinase. In response to insulin, phosphatidylinositol 3-kinase (PI3-K) is activated, and subsequently the intracellular concentration of PI 3-phosphate is increased. This activates the PI-dependent kinases (PDK) 1 and 2 which, in turn, activate AKT kinase by phosphorylating its threonine 308 and serine 473 residues, respectively. Active AKT kinase mediates the effect of insulin on glycogen synthesis and glucose transport. GAP, GTPase-activator protein; GRB-IR, growth factor receptor-binding protein-insulin receptor; IRS, insulin receptor substrate; c-CBL, an adaptor protooncogene product; SHP-2, src homology domain containing phosphatase-2; Ras, a GTPase protooncogene product.

and recycling of the receptor is believed to regulate sensitivity of the cell to insulin. For instance, abnormally increased levels of insulin and more prolonged exposure of cells to insulin increase the rate of receptor degradation, providing a primary mechanism of insulin-induced receptor downregulation.

The molecular mechanism of insulin endocytosis is not well defined. Insulin-stimulated receptor autophosphorylation is involved in this internalization process. However, the requirement for substrates phosphorylation is disputed. Phosphorylation of IRS-1 and activation of PI3-K did not regulate insulin-receptor endocytosis in transfected cells. In contrast, phosphorylation of pp120 was associated with increased rate of receptor-mediated insulin endocytosis and degradation in transfected cells. Using transgenic mice, it has recently been shown in the author's laboratory that pp120 does function in insulin clearance from the circulation.

Tyrosine dephosphorylation

Phosphotyrosine phosphatases (PTPases) are known to dephosphorylate the insulin receptor to inactivate its kinase activity. The identity of the actual PTPases that mediate receptor inactivation is as yet unknown. Nonetheless, PTPases have been shown to dephosphorylate insulin receptor tyrosine-containing peptides. These enzymes are divided into two classes: (1) transmembrane PTPases, which are orphan receptors with the enzymatic activity in the intracellular domain, and (2) intracellular PTPases, some of which contain SH2 domains (Matozaki and Kasuga, 1996).

Serine/threonine phosphorylation

An important emerging area of investigation in insulin action is the role of serine/threonine phosphorylation in modulating the kinase activity of the insulin receptor and the subsequent biological response (Hotamisligil *et al.*, 1996). In the absence of insulin, the insulin receptor is phosphorylated on serine/threonine. In response to tumour neorosis factor α (TNFα) receptors are activated to stimulate a serine/threonine kinase that can phosphorylate IRS-1 and prevent its association with PI3-K, thus leading to an effective dampening of insulin signal. Presumably, a similar activity can also phosphorylate serine/threonine residues of the insulin receptor, and thus reduce the tyrosine kinase activity of the receptor toward intracellular substrates. Similarly, phorbol esters, cAMP-dependent serine kinase, and overexpression of PKC isoforms appear to increase serine/threonine phosphorylation of the insulin receptor. The serine/threonine kinases that phosphorylate the insulin receptor are not yet fully identified; however, it has been suggested that the insulin receptor is tightly associated with an intrinsic serine kinase activity.

Molecular Basis of Diabetes

Diabetes is among the most deadly diseases in the United States. The incidence of diabetes is increasing worldwide, affecting about 5–15% of the world population. Classically, diabetes is classified into two major types: 1 and 2. Diabetes is characterized by raised circulating glucose levels (hyperglycaemia). Chronic and uncontrolled hyperglycaemia are associated with abnormalities in blood vessels, eyes, kidneys, and the cardiovascular and nervous systems. Hypertension and abnormalities of lipoprotein metabolism are also detected in some patients with diabetes. Symptoms of diabetes include excessive urination, thirst and fatigue. Type 1 diabetes is associated with weight loss despite excessive food intake, whereas type 2 is associated with obesity. Blurred vision and infections in skin, genital and urinary tracts are present in some type 2 diabetics. On the other hand, some type 2 diabetics are asymptomatic, particularly at an early stage of the disease.

Human and animal studies have proposed that diabetes mellitus is a heterogeneous group of metabolic disorders caused by molecular and cellular defects of one or more of the events described above (insulin synthesis, secretion and degradation, insulin receptor synthesis and intracellular assembly, insulin action at the receptor and postreceptor levels). Additional factors may be linked to diabetes by indirectly affecting insulin levels and action. These include antibodies to circulating insulin and to insulin receptors, and raised levels of glucagon which counteracts insulin action (**Table 1**).

Table 1 Aetiology of diabetes mellitus

Destruction of islet tissues and of β cells of the endocrine pancreas
Defective biosynthesis of insulin precursor
Synthesis of genetically defective insulin precursor
Defective conversion of preproinsulin/proinsulin to active insulin
Increased rate of proinsulin exocytosis into the circulation
Decreased rate of insulin secretion into the circulation in response to stimulants
Abnormally high levels of insulin antibodies leading to insulin destruction
Overproduction of glucagon by the α cells of the endocrine pancreas
Tissue resistance to insulin:
• Genetically defective insulin receptors
• Decreased number of insulin receptors on the cell surface membrane: a role for antireceptor antibodies; association with obesity and hyperinsulinaemia
• Genetically defective substrates
• Decreased number of glucose transporters in target tissues
• Impaired insulin clearance from the blood

Type 1 diabetes, representing about 5% of diabetes cases, is usually diagnosed under the age of 20 years. It is generally considered an autoimmune disease characterized by β-cell destruction and absolute insulin deficiency. Studies on identical twins have revealed a moderate genetic basis for this type.

Type 2 diabetes, representing approximately 85% of diabetes cases, is usually diagnosed in adulthood (above 30 years of age). It is currently viewed as a complex metabolic disorder with multiple causes (Kahn *et al*., 1996), and is characterized by peripheral insulin resistance and impaired relative compensatory insulin secretion (Taylor, 1999). Insulin resistance is a complex polygenic trait, i.e. it is not inherited in a mendelian fashion. Thus, simple genetic models, such as studies of candidate genes, have failed to identify the gene(s) responsible for insulin resistance. Several aspects of insulin action appear to be impaired in type 2 diabetes: in skeletal muscle, there is a defect in nonoxidative glucose disposal, as well as abnormalities of glucose uptake and phosphorylation. Likewise, there appear to be defects in the ability of insulin to suppress free fatty acid release from adipocytes, and glucose production in liver. The nature of the fundamental defect remains unclear. It is widely held that a defect at the receptor and/or early postreceptor events in insulin action could provide a unifying mechanism for insulin resistance. For instance, in type A insulin resistance, leprechaunism and Rabson–Mendenhall syndrome, mutations in the insulin receptor gene have been shown to impair insulin action and lead to extreme insulin resistance (Taylor *et al*., 1994). In recent years, the pace of discovery of new molecules and novel signalling mechanisms downstream of the insulin receptor has quickened considerably (White, 1998). However, studies of genes encoding proteins that participate in the insulin signalling systems have failed to implicate one or more of these genes as a major candidate for the aetiology of type 2 diabetes. It is possible that, because of genetic heterogeneity, different genes are responsible for insulin resistance in different patients.

The identification of the insulin gene as the type 2 diabetes gene has led the way to define maturity-onset diabetes in the youth (MODY) as a new subtype of type 2 diabetes (Habener and Stoffers, 1998; Taylor, 1999). Mutations in the insulin gene impair processing of proinsulin to insulin and insulin binding to its receptor. Mutations in HNF4α, HNF1α, 1DX-1 and HNF1β transcriptional factors (see **Figure 1**) in MODY-1, 3, 4 and 5 respectively, impair β-cell function and lead to insulin deficiency. Mutations in IDX-1 lead to defective development of the pancreas. As expected, mutations in the glucokinase gene, which catalyses the rate-limiting step in glucose metabolism in β cells, lead to partial deficiency in insulin secretion in MODY-2. Because mutations in these genes are inherited in an autosomal dominant mendelian manner, MODY is considered a monogenic class of type 2 diabetes, as opposed to the polygenic basis of classical type 2 diabetes.

Another subtype of type 2 diabetes with genetic defects in β-cell function is the recently identified maternally inherited diabetes and deafness (MIDD). This disease is characterized by a specific A to G mutation in the mitochondrial transfer $RNA^{LEU(UUR)}$ gene at position 3243 (van den Ouweland *et al*., 1994). The A to G mutation in tRNA impairs mitochondrial oxidative phosphorylation, leading to defective insulin secretion from the β cell. Like other mitochondrial diseases, diabetes and deafness in MIDD are transmitted via the maternal lineage.

Summary

Our understanding of the basic mechanism of insulin signalling has greatly improved in recent years. However, identifying the molecular basis of a heterogeneous disease such as diabetes remains complicated by the continuous discovery of signalling molecules and their intertwined concerted involvement in mediating the multiple effects of insulin in the cell. Furthermore, the functional impairment caused by some genetic variations might be too subtle to be detected by the assays at our disposal, or may require the combined (epistatic) interactions of other mutant genes to give rise to the diabetic phenotype. With the development of more sophisticated genetic screening approaches, such as those based on analysis of affected siblings, it has become possible to overcome some of the obstacles posed by the nonmendelian inheritance of diabetes and insulin resistance. Important new information on the susceptibility to diabetes is likely to be gained from purely genetic-based studies, in which no assumption as to the pathogenetic disease mechanisms are made. At the same time, our increased ability to manipulate the murine genome (by gene knockout technology) will result in an increased armamentarium of animal models, in which different hypotheses can be tested.

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