Mechanism of insulin action

B. B. P. Gupta

Environmental Endocrinology Laboratory, Department of Zoology, North-Eastern Hill University, Shillong 793 022, India

Insulin acts on target cells via membrane-bound tetrameric receptors with tyrosine kinase activity. Binding of insulin to extracellular α-subunits of insulin receptor (IR) leads to autophosphorylation followed by increased tyrosine kinase activity in the cytoplasmic β-subunits of IR. Increased tyrosine kinase activity of IR phosphorylates and activates a protein called insulin receptor substrate-1 (IRS-1). Then activated IRS-1 interacts with a number of signalling proteins and transmits insulin signal through various signal transduction pathways. Activation of PI3-kinase by IRS-1 results in translocation of glucose transporters (GLUT 4) to the cell membrane and increased glucose influx. IRS-1 also activates $p21^{ras}$ via GRB2. Insulin action via $P21^{ras}$–MAPK–pp90 S6 kinase alters nuclear events and glycogenesis. Insulin-activated IR and IRS-1 also interact with several other signalling proteins. However, the details of insulin action via these proteins and resultant biological effects remain to be investigated.

In the last two decades, significant progress has been made in unravelling the facts regarding mechanism of action of a large number of hormones[1,2]. In general, while hydrophobic hormones (e.g., androgens, oestrogens, progestogens, corticosteroids, iodothyronines, etc.) produce their biological effects at target cell by increasing/decreasing DNA-dependent RNA synthesis, hydrophilic hormones (peptides/proteineceous hormones) act through their membrane-bound receptors and induce formation of second messengers (e.g., cAMP, cGMP, Ca^{++}, IP_3, DAG, etc.) which mimic actions of hormones[3]. However, unlike other hydrophilic hormones, insulin does not use any of the known second messengers involved in hormonal signal transduction[3–5]. Insulin, in addition to its major role in glucose homeostasis, also influences a number of other cellular processes such as glycolysis, glycogenesis, transport of ions and amino acids, lipid metabolism, DNA synthesis, gene transcription, mRNA turnover, protein synthesis and degradation, etc., and plays a major role in cellular growth and differentiation (Figure 1)[1]. The physiological importance of insulin becomes prominent in case of diabetes mellitus, related ketoacidosis and other complications[6,7]. Keeping in view the importance of insulin and to fully understand the events responsible for insulin resistance and the pathophysiology associated with insulin, it is necessary to know in detail the molecular mechanism of insulin action. This article incorporates a summary of the latest information on the mechanism of action of insulin with special reference to its receptor and major components of insulin-signalling pathway.

Insulin receptor

The insulin molecule is composed of two peptides which are called as α-chain and β-chain. These chains are composed of 21 and 30 amino acid residues, respectively[7,8]. The molecular weight of insulin ranges between 5700 Da and 6100 Da. Insulin is formed in β-cells of 'islets of Langerhans' that are scattered within the exocrine tissue of pancreas and comprise 1%–2% of the total pancreatic mass. Insulin is synthesized as a large but biologically inactive molecule called preproinsulin, which is composed of 108 amino acid residues and possesses an amino terminal 'signal sequence' of 24 amino acid residues. The signal sequence directs translocation of preproinsulin into the endoplasmic reticulum where it is packed into secretory vesicles. Proteolytic removal of the signal sequence and formation of three disulfide bonds result in the formation of a smaller molecule called proinsulin (molecular weight: 9000 Da), which is composed of 84 amino acid residues. Proinsulin contains α- and β-chains linked by a connecting sequence (C-peptide) composed of 33 amino acid residues. When elevated blood glucose triggers insulin secretion, proinsulin is instantly processed in the Golgi complex to remove C-peptide with the help of specific peptidases. Enzymatic removal of C-peptide leads to formation of biologically active insulin in which α- and β-chains are held together by two inter-chain disulfide bonds that are formed between the cystein residues at positions 7 and 20 of the α-chain and at positions 7 and 19 of the β-chain, respectively. An intra-chain disulfide bond is present between the cystein residues at positions 6 and

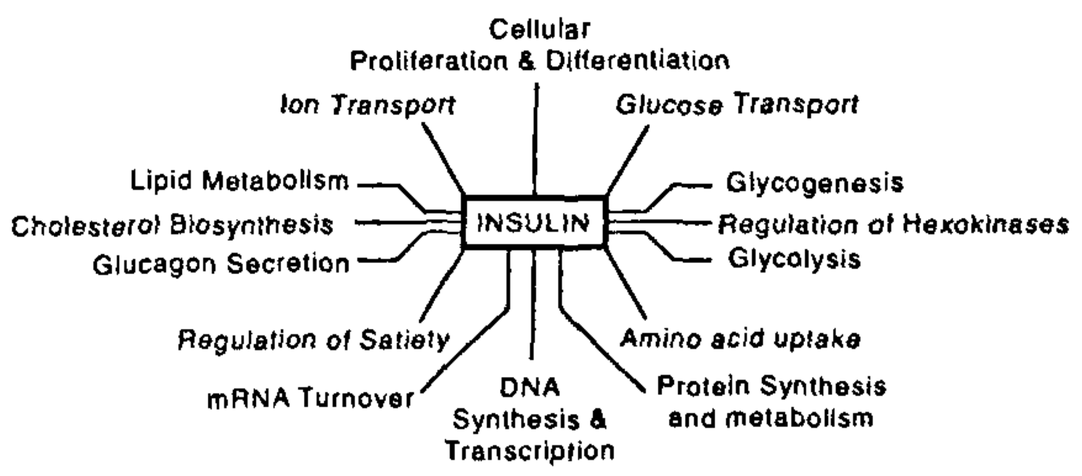

Figure 1. Insulin is involved in the regulation of a wide range of vital metabolic pathways and cellular functions. Its deficiency leads to diabetes and related disorders.

11 of the α-chain. It is important to mention that blood serum contains 'insulin-like' substances whose activities are not suppressed by antibodies which totally block the effects of insulin. These substances were first extracted with cold acid ethanol and separated into soluble and precipitable fractions. The two fractions were formerly termed as 'nonsuppresible insulin-like activity' (NSILA) and 'nonsuppressible insulin-like protein' (NSILP). Now they are called as 'insulin-like growth factor-I' (IGF-I) and 'insulin-like growth factor-II' (IGF-II), respectively[1]. Further, similar to proinsulin and insulin, IGF-I and IGF-II also contain three disulfide bonds, and 45% of the amino acids comprising IGF-I, IGF-II and insulin are identical. However, IGF-I, IGF-II and insulin act via their specific receptors[1–3].

Insulin acts on its target cells through its membrane-bound specific receptors. Insulin receptors are expressed in almost all mammalian tissues. The highest concentration of insulin receptor (> 300,000 receptors/cell) has been found in adipose tissue and liver – the major targets of insulin[9]. The native insulin receptor is composed of 2 α- and 2 β-subunits (Figure 2)[10]. The α- and β-subunits are covalently linked through disulphide bonds to form an $\alpha_2\beta_2$ hetero-tetramer[11]. The insulin receptor $\alpha_2\beta_2$ has a molecular weight of approximately 350,000 Da as estimated by reducing SDS-PAGE. The molecular weight, as estimated by reducing SDS-PAGE, of α-subunit and β-subunit has been found to be 135,000 Da and 95,000 Da, respectively[11,12].

α-subunit

The α-subunit, which is located entirely extra-cellularly, is responsible for insulin (ligand) binding[13–15]. *In vitro* mutagenesis and biochemical investigations indicate that the N-terminal domain (exons 1–3) is required for ligand recognition and also for high affinity ligand binding[16–18].

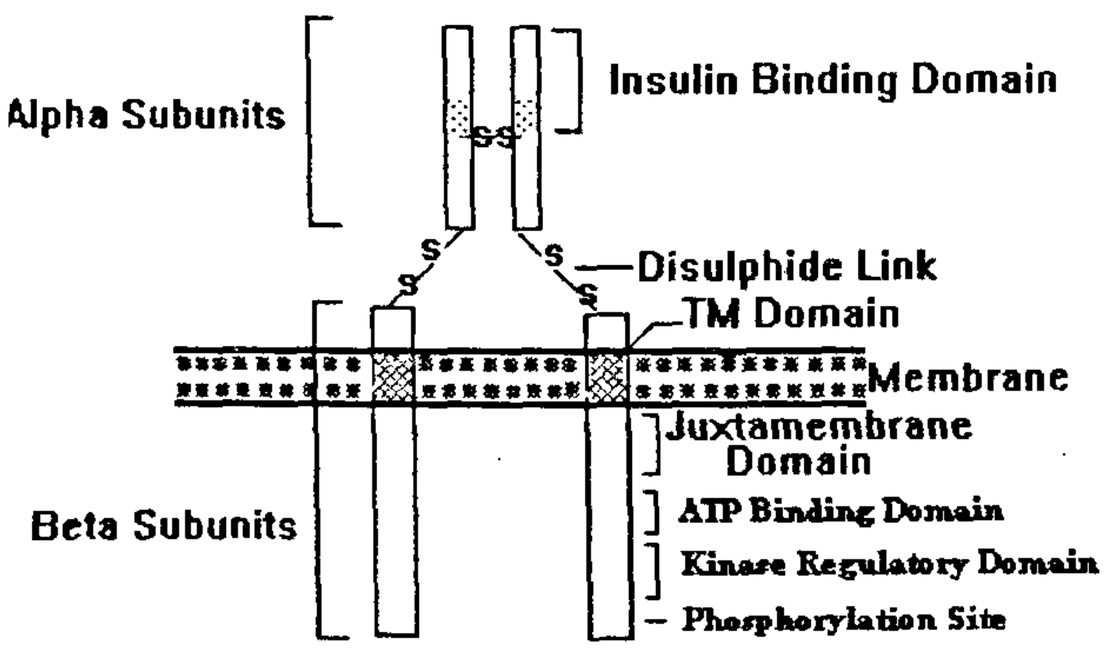

Figure 2. Schematic structure of insulin receptor. Insulin receptor is composed of two α-subunits and two β-subunits. The α-subunits interact with each other and form a single binding site for insulin. The cytoplasmic domains of β-subunits contain sites for autophosphorylation and possess tyrosine kinase activity.

Exons 3–5 encode a cysteine-rich domain. This domain is involved in ligand binding as well as in the formation of α–α disulphide bonds which covalently link the α–β heterodimers[19–21]. Recently it has been shown that a disulphide bond is formed between Cys-524 of one α-subunit to Cys-524 of the adjacent α-subunit. Cys-435 and Cys-468 are reported to form an intra-subunit disulphide bridge[21]. The last few amino acid sequences of C-terminal of α-subunit contain the alternatively spliced exon 11. This domain also plays an important role in recognition and binding of insulin. Further, C-terminal is involved in covalent interactions with the *N*-terminal of β-subunit and in signal transmission from insulin-bound α-subunit to β-subunit[22]. Partial removal of α-subunit by digestion with trypsin or *in vitro* mutagenesis activates tyrosine kinase activity of the β-subunits[23]. It, thus, seems that in the absence of insulin the α-subunit maintains a constraint on the β-subunit which inhibits constitutively active tyrosine kinase.

β-subunit

The β-subunit of insulin receptor is composed of three domains, i.e. a short extra-cellular domain, a trans-membrane (TM)-domain and an intracellular domain. The extra-cellular domain contains sites for N- and O-linked glycosylation[24]. The trans-membrane domain of the β-subunit is composed of 23 amino acid residues. The intracellular (cytoplasmic) domain contains a tyrosine specific protein kinase (tyrosine kinase)[25]. The endogenous tyrosine kinase activity is a prerequisite for insulin action[26]. *In vitro* mutagenesis experiments indicate that replacement of a critical lysine residue (Lys-1030) located in the ATP-binding site by other amino acids inhibits autophosphorylation and completely inactivates the kinase activity without having any effect on insulin binding[27,28]. Substitution of Lys-1030 also blocks the metabolic and growth-promoting effects of insulin[27–30].

So far it is not exactly known how binding of insulin to extra-cellular domain of receptor activates the cytoplamic domain containing tyrosine kinase activity. The ligand-induced dimerization/oligomerization of tyrosine kinase receptors reportedly results in increased tyrosine kinase activity[31]. Insulin receptors also undergo dimerization or oligomerization during signalling[32,33]. Insulin-induced autophosphorylation first occurs in one β-subunit which phosphorylates the adjacent β-subunit within the holoreceptor by the process of trans-phosphorylation[34–36]. It seems that insulin binding induces dimerization/oligomerization of insulin receptors and thereby increases the autophosphorylation of β-subunits resulting in increased tyrosine kinase activity of the cytoplasmic domain of the receptor[34,37]. Attempts have been made to establish the role of the trans-membrane domain of insulin receptor in tyrosine kinase activation. Studies involving *in vitro*

site-directed mutations, substitution of trans-membrane domains from other membrane-bound receptors, alterations in length and orientation, and modification of the charged residues that flank the TM domain suggest that the TM domain of insulin receptor plays a passive role and can tolerate a wide range of changes without having any effect on the signal transduction process[38]. However, some mutations indicate that the TM domain might be playing a more complex role in trans-membrane signalling[39,40]. Recent reports suggest that the TM domain might be involved in regulation of ligand-induced internalization of the receptor[41]. It seems to decrease the rate of receptor internalization resulting in slower degradation of receptors and increased half-life of the active receptors. The increased receptor half-life prolongs the duration of insulin-induced signal transduction.

Binding of insulin to α-subunit triggers tyrosine phosphorylation in the β-subunit of the insulin receptors. The phosphorylation cascade involves 6 to 7 tyrosine residues[42,43]. Tyr-1158, Tyr-1162 and Tyr-1163 residues (+exon 11 form of the receptor) are found to be the major sites of autophosphorylation and constitute the regulatory domain of tyrosine kinase. When these residues are mutated to Phe (single or multiple), the autophosphorylation and kinase activation are significantly impaired[44]. Further, these mutations also reduce the ability of insulin receptors to phosphorylate insulin receptor substrate-1 (IRS-1) and modulation of insulin-associated biological functions[45–47]. The C-terminal of the β-subunit contains two additional sites of Tyr autophosphorylation (Tyr-1328 & Tyr-1334) which are not essential for activation of tyrosine kinase[48,49]. The probable function of these two additional sites of autophosphorylation remains to be established. The juxtamembrane region of the β-subunit contains at least one autophosphorylation site (Tyr-972)[50–52]. Phosphorylation of the kinase regulatory unit is not essential for the phosphorylation of Tyr-972 and phosphorylation of Tyr-972 is not important for the activation of tyrosine kinase[50]. However, studies involving point mutations directed at Tyr-965, Tyr-972 and Tyr-984 indicate that the juxtamembrane region of the receptor is also involved in insulin- induced internalization of receptors and phosphorylation of IRS-1 (refs 53–56). Insulin receptors mutated at ATP-binding site (Lys-1030) are unable to undergo autophosphorylation, do not possess tyrosine kinase activity and are incapable of transmitting signals which produce biological effects. It is, thus, clear that the juxtamembrane region of the β-subunit is a highly functional domain required for insulin-induced receptor internalization and signal transduction through IRS-1.

It is important to mention that the insulin receptor is synthesized as a proreceptor (molecular weight: 180,000 Da) encoded by a single mRNA transcribed by a single gene. The receptor gene is located on the short arm of the chromosome 19. It is composed of about 150 K base pairs and contains 22 exons and 21 introns[57–59]. The proreceptor is immediately processed in the rough endoplasmic reticulum where it is rapidly glycosylated followed by the formation of intra- and inter-subunit disulphide bonds. The post-transcriptional processing of the proreceptor occurs in the golgi complex where it is cleaved by proteolytic enzyme at the tetrabasic sequence (Arg-720–Lys-721–Arg-722–Arg-723) located at the junction of the α- and β-subunits[60–62]. The proreceptor seems to be hydrolysed/processed by furin enzyme which belongs to the Kex2-related protein convertase family[63]. After processing in the golgi complex, a functional heterotetramer receptor is formed which is transported and embedded in the plasma membrane.

Components in insulin-induced signal transduction

As mentioned earlier, binding of insulin to its receptor induces autophosphorylation and tyrosine kinase activity of the receptor. Interactions between phosphorylated insulin receptor and cytoplasmic proteins seem to be very important for expression of insulin action[24]. Interactions of phosphorylated receptors with signalling proteins directly or indirectly via insulin receptor substrates form a large signalling complex (Figure 3). Recent findings suggest that phosphorylated insulin receptor

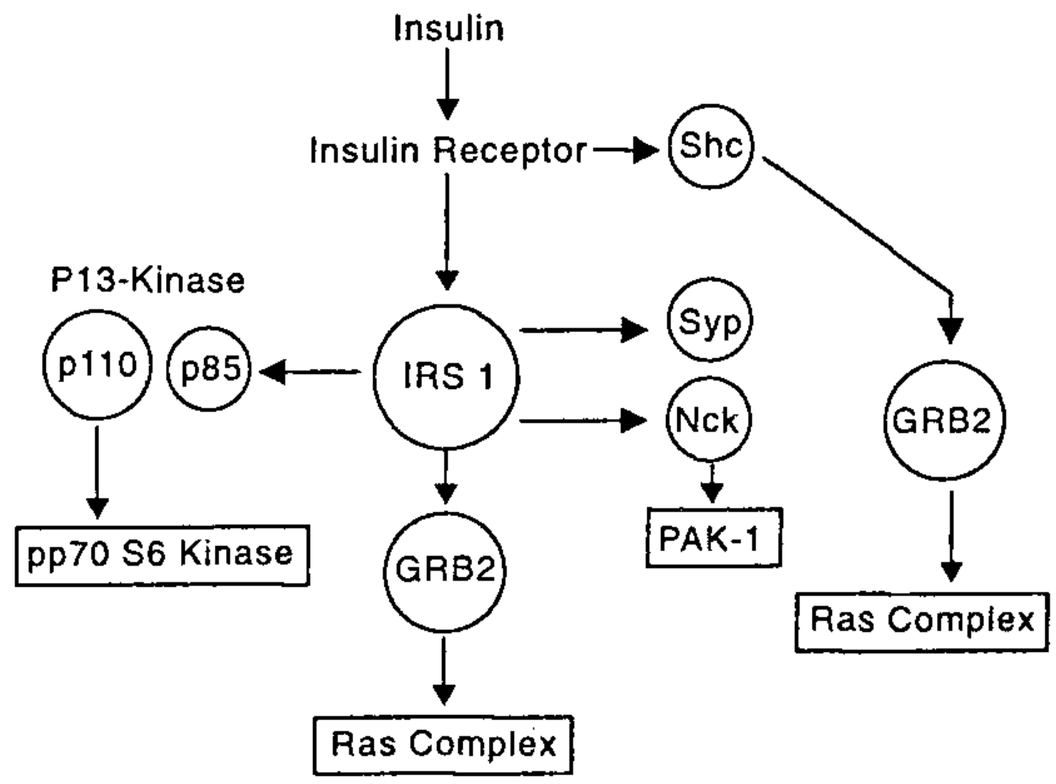

Figure 3. Major components of insulin-induced signal transduction. Binding of insulin to its receptor leads to autophosphorylation and increased tyrosine kinase activity in the β-subunits. The increased tyrosine kinase activity phosphorylates and activates insulin receptor substrate-1 (IRS-1) which acts as a docking molecule for other cytoplasmic signalling proteins (e.g., PI3-kinase, GRB2, Syp, Nck, etc.). IRS-1 transmits insulin-induced signals via signalling proteins which diversify the signal transduction pathways. GRB2 = growth factor receptor bound protein 2; IRS-1 = insulin receptor substrate-1; Nck = a 47 kD adapter protein; PAKs = p21-activated kinases; PI3-kinase = Phosphatidylinositol-3-kinase; pp70 S6 kinase = a ribosomal protein; Ras Complex = $p21^{ras}$ + mSOS + GAP with p62; Shc = a novel transforming protein; Syp = a protein tyrosine phosphatase.

transmits signal by phosphorylating a protein called insulin receptor substrate-1 (IRS-1)[64]. The phosphorylated IRS-1 acts as a docking protein and interacts with several other signalling proteins to complete the process of signal transduction leading to expression of biological effects of insulin[65]. In addition to this mechanism, the tyrosine kinase activity of insulin receptor might also be transmitting downstream signal by phosphorylating other cytoplasmic signalling proteins and transcription factors without involving IRS-1. There are also indications that insulin increases phosphorylation of cAMP responsive element binding protein (a transcription factor) and accelerates transcription activity associated with growth and proliferation of cells. A critical account of major components involved in the mechanism of insulin action through different signal transduction pathways is given below.

Insulin receptor substrate-I

In different types of insulin-sensitive cells (hepatoma cells, 3T3-L1 adipocytes, rat-1 fibroblasts, CHO cells, human epidermal carcinoma cells, rat adipocytes, etc.) insulin was reported to induce phosphorylation of a protein within seconds[66]. Since the phosphorylated protein was found to have a molecular weight of 185 kDa, it was termed as pp185 (phosphorylated protein 185). However, the precise role of pp185 in insulin-induced signal transduction remained unknown. Later on it was reported that the mutated receptors, in which Tyr-972 (IR Tyr-972) was substituted by Phe (IR-F972) in the juxtamembrane region, were normal for insulin binding, autophosphorylation and Tyr kinase activation, but were ineffective in inducing phosphorylation of pp185 (ref. 55). The mutated receptors were also unable to mediate insulin-induced synthesis of glycogen and DNA. These findings were the first indication that phosphorylation of pp185 is essential for insulin action and insulin-associated signal transduction.

The pp185 was purified and partially sequenced to obtain oligonucleotides for screening the rat liver complimentary DNA (cDNA) libraries[67]. As a result, a complete cDNA clone was obtained. The sequence analysis indicated that the cDNA encodes an unique new protein[68]. This protein was termed as insulin receptor substrate-I (IRS-1). IRS-1 has a molecular weight of 131,000 Da. However, unphosphorylated and phosphorylated IRS-1 migrate on SDS-PAGE around the molecular weights 160,000 Da and 180,000 Da, respectively. IRS-1 contains a consensus sequence for a nucleotide-binding site at *N*-terminus, a pleckstrin homology (PH) domain, a phosphotyrosine binding (PTB) domain, 20-22 potential Tyr phosphorylation sites and 40 potential Ser/Thr phosphorylation sites[69,70]. Though both PTB and PH domains link IRS-1 with the insulin receptor, the PH domain forms the main link between the insulin receptor and IRS-1 (ref. 71). Ser/Thr phosphorylation sites might be substrates for several kinases (e.g., cGMP-dependent protein kinase (PKG), casein kinase-II, calcium-dependent protein kinase (PKC), cAMP-dependent protein kinase (PKA), MAP kinase, etc.). The potential Tyr phosphorylation sites and also the immediate surrounding amino acid sequences are highly conserved in IRS-1 homologues. The immediate amino acid sequences around the Tyr phosphorylation sites seem to serve as potential binding sites for signalling proteins which contain SH2 domains[72]. In the absence of IRS-1, another substrate of insulin receptor called as IRS-2 (insulin receptor substrate-2) has been reported to interact with the autophosphorylated insulin receptor and to mediate the insulin-induced signal transduction[73–75].

IRS-1 signalling complex

After identification of IRS-1, attempts were made to identify other components of IRS-signalling complex. Studies conducted with the help of cloning and expression of IRS-1 have identified several signalling proteins which interact with IRS-1 and serve as essential components for insulin-signalling network. IRS-1 acts as a docking protein and transmits signals via different signalling proteins for different purposes. There are at least five proteins (PI3-kinase, GRB2, p85α and p85β, Syp, Nck) which interact with IRS-1 and transduce insulin-induced signals for various cellular functions[24]. A brief introduction of these proteins and their role in insulin signal transduction is given below.

PI3-kinase

PI3-kinase is a heterodimeric enzyme composed of two subunits, i.e. a regulatory subunit and a catalytic subunit. The regulatory subunit (molecular weight: 85,000 Da) is called as p85 and the catalytic subunit (molecular weight: 110,000 Da) as p110 (refs 76, 77). The p85 subunit contains two SH2 and one SH3 domains. Further, this unit also possesses regions homologous to 'breakpoint cluster region' (BCR) kinase and $p21^{ras}$-GTPase activating protein ($p21^{ras}$-GAP). PI3-kinase catalyses phosphorylation of phosphoinositides (PI), phosphatidylinositol-4-phosphate (PI-4-P) and phosphatidyl-inositol-4,5-bisphosphate (PI-4,5-P_2) leading to formation of phosphatidylinositol-3-phosphate (PI-3-P), phosphatidylinositol-3,4-bisphosphate (PI-3,4-P_2) and phosphatidylinositol-3,4,5-triphosphate (PI-3,4,5-P_3)[78]. Studies based on *in vitro* experiments using immunoprecipitated PI3-kinase and recombinant IRS-1 suggest that PI3-kinase has a high affinity for phosphorylated IRS-1. Addition

of phosphorylated IRS-1 activates PI3-kinase[64,65,68]. Further, the IR^{F972} receptor, which has normal kinase but fails to phosphorylate IRS-1, is unable to mediate insulin action and in activating PI3-kinase. Recently IRS-1 has been reported to be associated with PI3-kinase[79]. The association of PI3-kinase with IRS-1 acts as an activation step for the kinase[65]. When insulin binding stimulates Tyr kinase and phosphorylation of IRS-1, the SH2 domains of PI3-kinase bind preferentially to four phospho-peptide segments of IRS-1 surrounding Tyr-460, 608, 939 and 987 (ref. 80). These findings suggest that phosphorylated IRS-1 binds to p85 SH2 domain of PI3-kinase and activates several signalling pathways. PI3-kinase seems to diversify the insulin-induced signal transduction via pp70 S6 kinase, GLUT4 containing vesicles and p21-activated kinases (PAKs) (Figure 4). The p85 SH2 domain of PI3-kinase can also bind directly to the insulin receptor[81]. However, the significance of direct interaction between p85 and insulin receptor remains unknown.

Insulin promotes growth of mammalian cells and oocyte maturation of *Xenopus* by acting via IRS-1 – PI3-kinase pathway. Insulin stimulates tyrosine phosphorylation of IRS-1 by several folds in CHO cells overexpressing the insulin receptor (CHO/IR) or IRS-1 (CHO/IRS-1) or both (CHO/IR/IRS-1). The level of expression of IRS-1 has been found to be directly correlated with the level of association and activation of PI3-kinase[72]. CHO cells expressing an antisense mRNA and rat-1 fibroblasts microinjected with IRS-1 antibodies have recently provided evidence for insulin-induced cell growth via IRS-1 – PI3-kinase signalling pathway. Cells expressing the IRS-1 antisense mRNA exhibit significant decrease in insulin-stimulated Tyr-phosphorylated IRS-1 and PI3-kinase activity associated with simultaneous decrease in insulin-stimulated DNA synthesis[82]. Further, rat-1 fibroblasts microinjected with IRS-1 antibodies do not respond to insulin[83]. As mentioned earlier, insulin induces oocyte maturation in *Xenopus*. In *Xenopus* oocyte, microinjected IRS-1 is promptly phosphorylated after

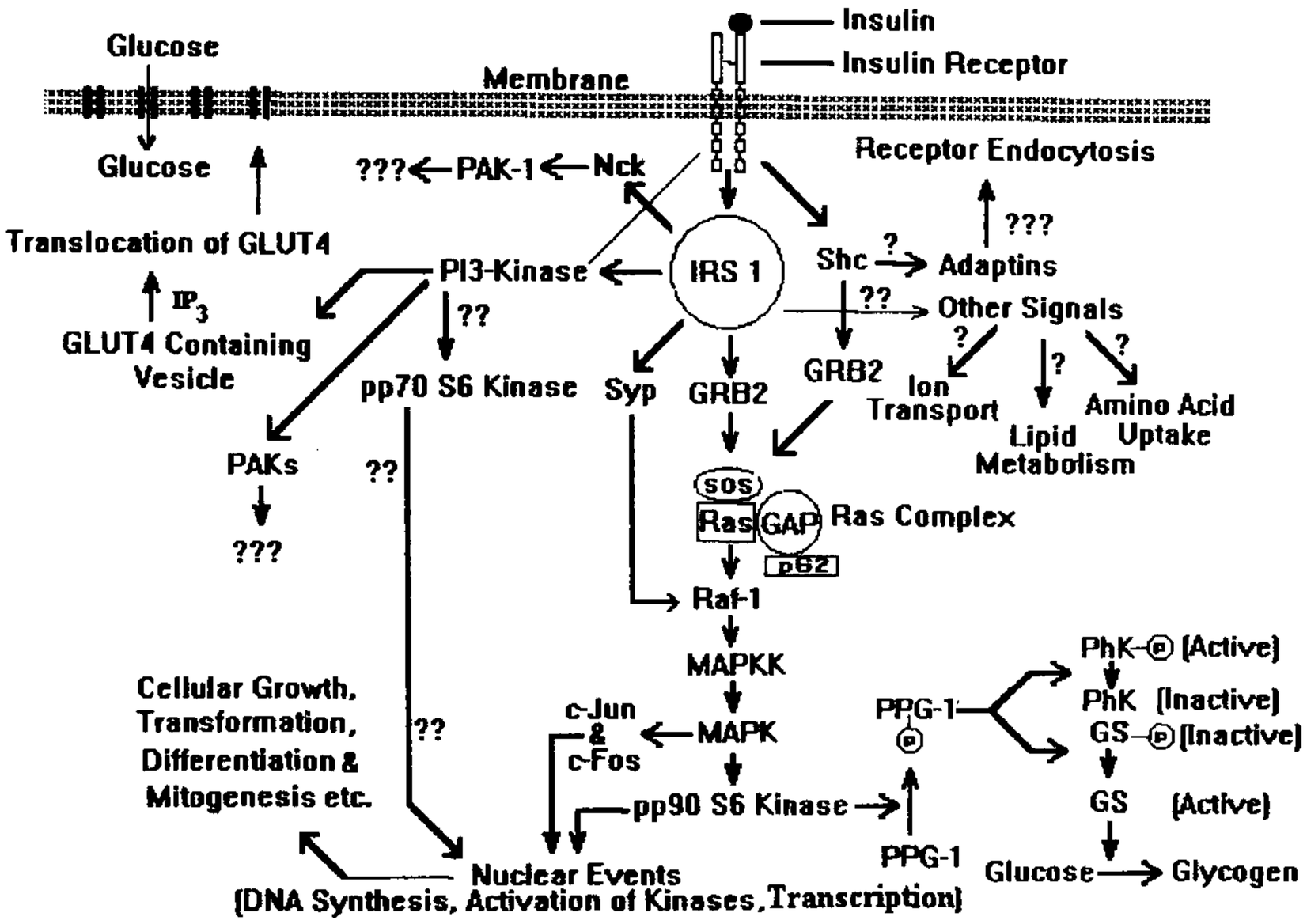

Figure 4. Schematic representation of mechanism of insulin action. Formation of insulin–receptor complex seems to transmit signals via two signalling proteins, IRS-1 and Shc. Insulin-activated IRS-1 stimulates PI3-kinase, Syp and GRB2. Activation of IP3-kinase stimulates translocation of GLUT4 to the cell membrane followed by glucose influx. Importance of PI3-kinase-induced activation of PAKs and pp70 S6 kinase remains to be established. Stimulation of GRB2 by active IRS-1 and/or Shc activates $p21^{ras}$–complex which transmits signals via Raf-1–MAPKK–MAPK pathway. Activated MAPK accelerates nuclear events and glycogen synthesis by stimulating transcription factors (c-Jun and c-Fos) and/or pp90 S6 kinase. GAP = GTPase activating protein; GLUT4 = insulin sensitive glucose transporter; GRB2 = growth factor receptor bound protein 2; GS = glycogen synthase; IP_3 = inositol trisphosphate; IRS-1 = insulin receptor substrate-1; MAPK = mitogen activated protein kinase; MAPKK = MAPK kinase; Nck = a 47 kD adapter protein; p62 = GAP-associated protein; PAKs = p21-activated kinases; PhK = Phosphorylase kinase; PI3-kinase = Phosphatidyl-inositol-3-kinase; pp70 S6 kinase = a ribosomal protein; pp90 S6 kinase = a ribosomal protein; PPG-1 = glycogen associated protein phosphatase-1; Raf-1 = Serine/threonine kinase; Ras Complex = $p21^{ras}$ + mSOS + GAP with p62; Shc = a novel transforming protein; mSOS = mammalian homologue to the Drosophila Son-of-sevenless protein; Syp = a protein tyrosine phosphatase

treatment with insulin. In this case there is about 8-fold increase in PI3-kinase activity associated with IRS-1 (ref. 84). These findings clearly suggest that insulin produces its effect on mammalian cell growth and *Xenopus* oocyte maturation by phosphorylating IRS-1. PI3-kinase binds to phosphorylated IRS-1 and gets activated. The activated PI3-kinase phosphorylates PI, PI-4-P, PI-4,5-P_2, leading to formation of PI-3-P, PI-3,4-P_2, PI-3,4,5-P_3 (IP_3), etc. These phosphorylated compounds (mainly IP_3) are supposed to act as insulin-induced signalling molecules and stimulate mammalian cell growth and *Xenopus* oocyte maturation[84]. Insulin has been recently reported to activate a p21-activated kinase (PAK) in muscle cells via PI3-kinase[85]. However, the PI3-kinase–PAK downstream signalling cascade and its importance remain to be established.

GRB2

Insulin-activated IRS-1 also binds to a small adapter protein called growth factor receptor bound protein 2 (GRB2). It contains SH2 and SH3 domains and binds to phosphorylated IRS-1 at Tyr-895 after stimulation by insulin[86–88]. GRB2 links IRS-1 and the $p21^{ras}$ signalling pathway. The SH3 domain of GRB2 non-covalently interacts with the $p21^{ras}$ GDP/GTP exchange factor, mSOS (mammalian homologue to the Drosophila Son-of-sevenless protein) leading to increased $p21^{ras}$ activity[89–91]. Ras ($p21^{ras}$: a ras protooncogene product) is a 21 kD membrane-bound GTP-binding protein (G-protein). It plays an important role in regulation of cell growth and tumour formation. It belongs to a super-family of small G-proteins which are actively involved in cell growth, protein transport, vesicular transport, etc[92–94]. The active form of $p21^{ras}$ is bound to GTP and inactive form to GDP. However, after stimulation by insulin, GDP bound to $p21^{ras}$ is replaced by GTP. Thus, $p21^{ras}$ shuttles between GDP- and GTP-bound forms. This cycle is regulated by GTPase-activating protein (GAP) and mSOS[95,96]. $p21^{ras}$ transforms cells by stimulating Ser/Thr phosphorylation cascade which leads to activation of the mitogen-activated protein kinase (MAPK) signal transduction pathways[96]. Microinjection of $p21^{ras}$ antibodies in cells inhibits insulin-induced cell growth. Insulin stimulates $p21^{ras}$ complex through IRS-1 and GRB2 (Figure 4). For inducing cellular transformation, IRS-1 interacts with both GRB2 and Syp (a phosphatase)[97]. The stimulated Ras complex has been reported to interact with and to activate Raf-1 kinase which phosphorylates and activates MAPK kinase (MAPKK)[98–100]. Then MAPKK induces phosphorylation and activation of MAPK[101]. The active MAPK phosphorylates ribosomal protein pp90 S6 kinase leading to its activation[102]. In turn, insulin-activated pp90 S6 kinase stimulates glycogen-associated protein phosphatase-1 (GPP-1). Thereafter, GPP-1 dephosphorylates the enzyme glycogen synthase and makes it active. Simultaneously, GPP-1 inactivates phosphorylase kinase (PRK) and glycogen phosphorylase (GPR) by catalysing their dephosphorylation. Thus, insulin-induced activation of glycogen synthase and inactivation of PRK and GPR via IRS-1–GRB2–Ras ($p21^{ras}$)–Raf-1–pp90 S6 kinase results in acceleration of glycogen synthesis[103].

Insulin can also stimulate $p21^{ras}$ complex via Shc without involving IRS-1. Shc is a newly discovered novel transforming protein which is supposed to provide an alternate pathway that links insulin-induced signals to $p21^{ras}$ signalling pathway involved in mitogenic signal transduction[104]. Shc contains a Src homology 2 (SH2) domain and a phosphotyrosine binding domain. In this pathway, the phosphorylated insulin receptor interacts with and phosphorylates Shc[105–107]. Interaction of phosphorylated insulin receptor with the PTB domain of Shc is necessary for phosphorylation and activation of Shc[108]. The residues which control the specific binding of Shc PTB domain to phosphotyrosine sites of insulin receptor have recently been identified[109]. Further, Shc PTB domain has been found to be essential for insulin-induced DNA synthesis and mitogenic signalling[110]. The amino terminal domain of Shc interacts with insulin receptor in phosphorylation-independent manner and its importance remains to be studied[111]. The SH2 domain phosphorylated Shc provides a binding site for GRB2 and results in the formation of a Shc–GRB2–mSOS complex and activation of $p21^{ras}$–Raf-1–MAPK–pp90 S6 kinase pathway[105,106]. Under the influence of insulin-like growth factor-I (IGF-I) and epidermal growth factor (EGF), Shc interacts with adapter proteins (adaptins) which regulate endocytosis of receptors[112]. However, the question whether insulin also induces interaction of Shc with adaptins leading to internalization of its receptors remains to be answered.

p85α, p85β, Syp and Nck

Since IRS-1 contains 22 potential sites (Tyr) for phosphorylation, it provides several binding sites for SH2 domain containing proteins[68,113]. There are at least three SH2 domain containing proteins other than PI3-kinase and GRB2 which are reported to interact with IRS-1. There are two isoforms of p85, namely p85α and p85β (ref. 114). Both the isoforms reportedly associate with the p110 catalytic subunit of PI3-kinase and phosphorylated IRS-1 (refs 115, 116). Thus, p85α and p85β can act as a chain between IRS-1 and PI3-kinase whereby they might be diversifying the insulin-induced signalling network.

A protein tyrosine phosphatase termed as Syp also binds to phosphorylated IRS-1 at Tyr-1172. Syp contains two SH2 domains[117]. Syp is the mammalian homologue

of a protein called cork screw (csw) found in *Drosophila*. It is located downstream of tyrosine kinase receptor and regulates *Drosophila* homologue of Raf-1 (refs 118, 119). Binding of Syp to IRS-1 results in its activation. So far there is scarcity of information on exact function of active Syp. It is supposed to be involved in the regulation of signal transduction via Raf-1. IRS-1–Syp interaction might be a pathway for the multiplication of insulin-signalling network and seems to be associated with down regulation of the signal. A recent study on insulin-signalling in mice expressing reduced levels of Syp indicates that the phosphatase might be involved in insulin-induced mitogenesis[120].

IRS-1 also binds to a 47 kD adapter protein called Nck which contains three SH3 and one SH2 domains[121,122]. Nck has recently been shown to bind IRS-1 (ref. 123). Overexpression of Nck in NIH 3T3 fibroblasts leads to cellular transformation and cell proliferation[124]. Due to the presence of SH2 and SH3 domains, Nck is supposed to link IRS-1 to downstream signal transduction pathway(s) involved in cell growth and proliferation.

There are several other proteins (e.g., PLC-γ, $p21^{ras}$-GAP, etc.) which contain SH2 domains but do not interact with IRS-1 (ref. 125). Thus, IRS-1 allows association of distinct group of proteins with SH2 domains. It seems to act as a potential signal distributor molecule for a wide range of insulin actions. However, the details of the mode of action of IRS-1 associated signalling proteins and their regulation in different tissues remain to be unearthed. At present the physiological role of IRS-1 in pathophysiology of diabetes also remains unknown[24].

Other substrates of insulin receptors

Insulin seems to produce most of its biological effects related to cell growth and proliferation and glucose homeostasis mainly via IRS-1 phosphorylation. However, recent studies indicate that there are other potential substrates for insulin receptors which might be involved in alternative pathways for insulin-induced signal transduction. For example, insulin-induced Tyr phosphorylation of Shc (a 62 kDa protein) seems to provide additional and/or alternative signalling pathway for activation of $p21^{ras}$ complex and downstream transduction of insulin signal independent of IRS-1. In addition to IRS-1 and Shc, insulin also induces appearance of a 120 kD phosphoprotein band which contains two proteins: an ecto-ATPase and an endogenous Tyr kinase (focal adhesion kinase)[126–128]. Insulin is also reported to phosphorylate a 15 kD protein termed as fatty acid-binding protein 422 (ap2), $p21^{ras}$–GTPase activating protein (GAP), GAP-associated 62 kD protein (p62) and p190, a new PI3-kinase associated ~60 kDa protein (p60), etc.[129–133]. It, thus, seems that there are many proteins which may act as substrates for insulin receptor, provide alternative IRS-1-independent signal transduction pathways and diversify the insulin-induced signals. Recently a protein called Ras-associated with diabetes (Rad) has been reported to be involved in insulin-signalling[134]. Rad is composed of 269 amino acid residues having a predicted molecular weight of 29,266 Da. It is necessary to mention that the precise functions of these alternative substrates remain to be explored.

Insulin action via nuclear pathway

In addition to its action via cytoplasmic proteins, insulin also produces biological effects by activating DNA synthesis and gene transcription. Insulin has been reported to stimulate transcription of a number of mRNAs. These mRNAs encode proteins which are involved in the regulation of cellular growth and metabolism. It seems that insulin somehow transmits its signal to nucleus, probably through transcription factors. Recently insulin has been reported to stimulate phosphorylation of a transcription factor called cAMP responsive element binding protein (CREB)[135] and a number of other DNA-binding proteins (pp34, pp40, pp48, pp62, pp64, pp66, pp72, pp94, etc.) in 3T3-442A adipocytes[136]. There is direct correlation between insulin-induced phosphorylation of pp94 (nucleolin) and increase in efflux of RNA from isolated nuclei[137]. CREB binds to cAMP responsive element (CRE) and stimulates the process of transcription. Insulin also induces rapid phophorylation of AP-1 family of transcription factors composed of c-Jun and c-Fos proteins in 3T3-442A cells[138]. These factors are involved in regulation of transcription of a number of genes associated with cellular growth and transformation. There are several kinases (e.g., MAP kinase, PKC, PKA, casein kinase-II, etc.) which can phosphorylate AP-1 transcription factors. Since insulin stimulates kinase-like activities in nuclear extracts[139], insulin can affect transcription via kinases – AP-1 pathway. Insulin stimulates phosphorylation of c-Jun and c-Fos related proteins which are capable of influencing the rate of transcription. Recent studies indicate that insulin influences gene transcription by modulating the activity of 'signal transducer and activator of transcription (STAT) 3 isoform' in liver cells[140,141]. These findings clearly indicate that insulin-induced signals are also transduced via nuclear pathway, particularly for actions associated with nuclear events.

Insulin and glucose transport

As mentioned earlier, insulin stimulates cellular uptake of glucose in its target tissues. It is important to mention

that there are two types of glucose transporters: Na^+-dependent glucose transporters and facilitative glucose transporters. The Na^+-dependent glucose transporters (which are found in the intestinal tract and kidney) are not influenced by insulin. In other tissues there are multiple types of facilitative glucose transporters. So far at least five types of facilitative glucose transporters are identified and are termed as GLUT1 (present in blood-brain barrier, muscle, RBC, colon, kidney, placenta, adipose tissue, etc.), GLUT2 (present in liver, kidney, intestine and pancreas), GLUT3 (present in neuronal cells, brain, placenta and kidney), GLUT4 (present in brown and white fat, skeletal and cardiac muscle and other insulin-sensitive tissues) and GLUT5 (present in the small intestine and spermatozoa)[142]. However, only GLUT4 has been found to be regulated by insulin. In the absence of insulin almost all GLUT4 is found in the cytoplasmic vesicular pool. Insulin induces a rapid increase (~ 10 fold) in translocation of GLUT4 from the cytoplasm to the plasma membrane which results in a 20–30 fold increase in the rate of glucose uptake[143–145].

At present it is not fully known how insulin stimulates translocation of GLUT4 to the membrane. There is a direct correlation between insulin-induced increase in PI3-kinase activity and the rate of GLUT4 translocation/glucose uptake[146]. In 3T3-L1 adipocytes, wortmannin and LY294002 (specific inhibitors of PI3-kinase) have been reported to inhibit insulin-induced PI3-kinase and glucose uptake without affecting autophosphorylation of the insulin receptor, phosphorylation of IRS-1 and association of PI3-kinase with IRS-1 (refs 147–149). LY294002 inhibits insulin-induced glucose uptake by inhibiting the translocation of GLUT4 containing vesicles[146,149]. Stimulation of PI3-kinase also leads to activation of pp70 S6 kinase. While inhibitors of PI3-kinase inhibit insulin-activated pp70 S6 kinase as well, selective inhibition of pp70 S6 kinase by rapamycin does not inhibit insulin-induced glucose uptake[149,150]. These findings strongly suggest that PI3-kinase plays a critical role in insulin-induced signal transduction and translocation of GLUT4 without involving pp70 S6 kinase. Insulin-activated pp70 S6 kinase has been reported to induce transcription and translation[151]. However, other growth factors are reported to stimulate PI3-kinase without having any effects on GLUT4 translocation[152]. It, thus, seems that insulin induced signals leading to activation of pp70 S6 kinase and translocation of GLUT4 diverge at some point between downstream of PI3-kinase and upstream of pp70 S6 kinase. As mentioned earlier, PI3-kinase catalyses the formation of PI-3-P, PI-3,4-P_2 and PI-3,4,5-P_3 (IP_3). Since these phosphoinositides do not act as substrates for any of the known phospholipases[153], they may act as messenger/signalling molecules for insulin action on glucose uptake. It is noteworthy that overexpression of the catalytic subunit p110α of PI3-kinase in 3T3 L1 adipocytes increases glucose transport and translocation of glucose transporters[154]. Further, inhibition of the regulatory subunit p85α of PI3-kinase reportedly inhibits the translocation of GLUT4[155]. Recently it has been found that isolated activation of PI3-kinase in 3T3 L1 adipocytes can initiate the signalling cascade leading to both actin arrangement and GLUT4 translocation[156]. Further, insulin induces PI3-kinase targeting to GLUT4-containing vesicles. In the rat adipocytes and 3T3 L1 adipocytes IRS-1–PI3-kinase complex associates with vesicles containing GLUT4 and induces translocation of GLUT4[157]. Moreover, this association of IRS-1–PI3-kinase with the vesicles results in accumulation of 3′-phosphoinositides which are supposed to play an important role in the membrane-associated events which accompany the translocation of GLUT4 to the cell surface. IP_3 has been reported to activate ζ-isoform of protein kinase C (PKC-ζ) *in vitro*[158]. Stimulation of PKC-ζ is essential for oocyte maturation and growth of fibroblasts[159]. It has also been found that increase in the concentration of the phosphorylated phosphoinositides is associated with increased cell proliferation[160]. However, the precise role of PI3-kinase products in insulin-induced translocation of GLUT4 and glucose uptake remains to be established.

In addition to its action via PI3-kinase, insulin might also be affecting glucose uptake by alternative pathway(s). While overexpression of $p21^{ras}$ in 3T3-L1 adipocytes results in increased translocation of GUT4 and glucose uptake[161], activation of $p21^{ras}$-pp90 S6 kinase pathway by epidermal growth factor (EGF) or thrombin does not lead to increased translocation of GLUT4 or glucose uptake[162]. Further, while $p21^{ras}$ is activated by both insulin and EGF, only insulin stimulates phosphorylation of IRS-1. In other words, activation of $p21^{ras}$ for stimulating glucose uptake needs signal transduction via IRS-1. However, the involvement of $p21^{ras}$ in insulin-induced glucose uptake seems to be independent of Raf-1 and MAPK[163], and may not be sufficient for mediating significant action of insulin on glucose uptake. It is noteworthy that small GTP-binding proteins of Rab family are found to be associated with GLUT4 containing vesicles[164]. However, the precise function of these G-proteins in insulin-induced GLUT4 translocation remains to be unknown.

Summary

Significant progress has been made towards understanding the structure of insulin receptor and mechanism of insulin action. The binding of insulin to the α-subunits of insulin receptor induces interactions among insulin receptors leading to their dimerization/oligomerization. As a result, the β-subunits are autophosphoryalted at Tyr residues. Autophosphorylation of β-subunits increases

the endogenous tyrosine kinase activity of the receptors. The autophosphorylated insulin receptors interact with other signalling proteins and form a multi-componential and multi-directional signalling complex (Figure 4). In general, the tyrosine kinase activity of the autophosphorylated insulin receptors phosphorylates and activates IRS-1. Then phosphorylated active IRS-1 acts as a docking protein which interacts with several cytosolic proteins (e.g., GRB2-Ras complex, PI3-kinase, Syp, Nck, etc.) and activates a number of signal transducing pathways. In addition, the autophosphorylated insulin receptor may also phosphorylate/interact with other alternative substrates and signalling proteins (e.g., Shc, p60, p62, etc.) directly and may transduce insulin signals via alternative pathways. Some major actions of insulin (e.g., DNA synthesis, gene transcription, cellular growth/proliferation/differentiation/transformation, etc.) are produced via events associated with the cell nucleus. Thus, insulin seems to produce its wide-ranging biological effects through a multi-componential and multi-directional signal transducing pathways. However, various aspects of insulin signal transduction via Shc, Syp, Nck, PAKs, adaptins, pp70 S6 kinase, STATs, transcription factors, Rad, Rab, etc. still remain to be unravelled.

1. Martin, C. R., *Endocrine Physiology*, Oxford University Press, Oxford, 1985.
2. Griffin, J. E. and Ojeda, S. R., *Textbook of Endocrine Physiology*, Oxford University Press, Oxford, 1992.
3. Bolander, F. F., *Molecular Endocrinology*, Academic Press, New York, 1989.
4. Kahn, C. R. and Crettaz, M., *Diabetes Metb. Rev.*, 1985, **1**, 5–32.
5. Rosen, O. M., *Science*, 1987, **237**, 1452–1458.
6. Unger, R. H. and Foster, D. W., in *Williams Textbook of Endocrinology* (eds Wilson, J. D. and Foster, D. W.), W.B. Saunders Company, London, 1985, pp. 1018–1080.
7. Norman, A. W. and Litwack, G., in *Hormones*, Academic Press, New York, London, 1987, pp. 264–322.
8. Gorbman, A., Dickhoff, W. W., Vigna, S. R., Clark, N. B. and Ralph, C. L., in *Comparative Endocrinology*, John Wiley & Sons, New York, 1983, pp. 349–371.
9. Kahn, C. R., Baird, K. 1., Flier, J. S., Grunfeld, C., Harmon, J. T., Harrison, L. C., Karlsson, F. A., Kasuga, M., King J. L., Lang, U., Podskalny, J. M. and Van Obberghen, E., *Recent Progr. Horm. Res.*, 1981, **37**, 447–538.
10. Kasuga, M., Hedo, J. A., Yamada, K. M. and Kahn, C. R., *J. Biol. Chem.*, 1982, **257**, 10392–10399.
11. Boyle, T. R., Campana, J., Sweet, L. J. and Pessin, J. E., *J. Biol. Chem.*, 1985, **260**, 8593–8600.
12. Massague J., Pilch, P. F. and Czech, M. P., *Proc. Natl. Acad. Sci. USA*, 1981, **77**, 7137–7141.
13. Ullrich, A., Bell, J. R., Chen, E. Y., Herrera, R., Petruzzelli, L. M., Dull, T. J., Gray, A., Coussens, L., Liao, Y. C., Tsubokawa, M., Mason, A., Seeburg, P. H., Grunfeld, C., Rosen, O. M. and Ramachandran, J., *Nature*, 1985, **313**, 756–761.
14. Ebina, Y., Ellis, L., Jarnagin, K., Edery, M. M., Graf, L., Clauser, E., Ou, J. H., Masiar, F., Kan, Y. W., Goldfine, I. D., Roth, R. A. and Rutter W. J., *Cell*, 1985, **40**, 747–758.
15. Yip, C. C., *J. Cell Biochem.*, 1992, **48**, 19–25.
16. Rafaeloff, R., Patel, R., Yip, C., Goldfine, I. D. and Hawley, D. M., *J. Biol. Chem.*, 1989, **264**, 15900–15904.
17. De Meyts, P., Gu, J. L., Shymko, R. M., Kaplan, B. E., Bell, G. I. and Whittaker, J., *Mol. Endocrinol.*, 1990, **4**, 409–416.
18. Tavare, J. M. and Siddle, K., *Biochim. Biophys. Acta*, 1993, **1178**, 21–39.
19. Gustafson, T. A. and Rutter, W. J., *J. Biol. Chem.*, 1990, **265**, 18663–18667.
20. Frias, I. and Waugh, S. M., *Diabetes*, 1987, **38**, 60–238.
21. Schaffer, L. and Ljungqvist, L., *Biochem. Biophys. Res. Commun.*, 1992, **189**, 650–653.
22. Cheatham, B. and Kahn, C. R., *J. Biol. Chem.*, 1992, **267**, 7108–7115.
23. Shoelson, S. E., White, M. F. and Kahn, C. R., *J. Biol. Chem.*, 1988, **263**, 4852–4860.
24. Cheatham, B. and Kahn, C. R., *Endocrine Rev.*, 1995, **16**, 117–142.
25. Kasuga, M., Karlsson, F. A. and Kahn, C. R., *Science*, 1982, **215**, 185–187.
26. Kahn, C. R. and White, M. F., *J. Clin. Invest.*, 1988, **82**, 1151–1156.
27. Chou, C. K., Dull, T. J., Russel, D. S., Gherzi, R., Lebwohl, D., Ullrich, A. and Rosen, O. M., *J. Biol. Chem.*, 1987, **262**, 1842–1847.
28. Elbina, Y. Araki, E., Taira, M., Shimada, F., Mori, M., Craik, C. S., Siddle, K., Pierce, S. B., Roth, R. A. and Rutter, W. J., *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 704–708.
29. McClain, D. A., Maegawa, H., Lee, J., Dull, T. J., Ullrich, A. and Olefsky, J. M., *J. Biol. Chem.*, 1987, **262**, 14663–14671.
30. Stumpo, D. J. and Blackshear, P. J., *J. Biol. Chem.*, 1991, **266**, 455–460.
31. Ullrich, A. and Schlessinger, J., *Cell*, 1990, **61**, 203–212.
32. Kahn, C. R., Baird, K. L., Jarett, D. B. and Flier, J. S., *Proc. Natl. Acad. Sci. USA*, 1976, **75**, 4209–4213.
33. Heffetz, D. and Zick, Y., *J. Biol. Chem.*, 1986, **261**, 889–894.
34. Cobb, M. H., Sang, B. C., Gonzales, R., Goldsmith, E. and Ellis, L., *J. Biol. Chem.*, 1989, **264**, 18701–18706.
35. Shoelson, S. E., Boni-Schnetzler, M., Pilch, P. F. and Kahn, C. R., *Biochemistry*, 1991, **30**, 7740–7746.
36. Lee, J., O'Hare, T., Pilch, P. F. and Shoelson, S. E., *J. Biol. Chem.*, 1993, **268**, 4092–4098.
37. Fratalli, A. L., Treadway, J. L. and Pessin, J. E., *J. Biol. Chem.*, 1992, **267**, 19521–19528.
38. Fratalli, A. L., Treadway, J. L. and Pessin, J. E., *J. Biol. Chem.*, 1991, **266**, 9829–9834.
39. Yamada, K., Gonclaves, E., Kahn, C. R. and Shoelson, S. E., *J. Biol. Chem.*, 1992, **267**, 12452–12461.
40. Cheatham, B., Shoelson, S. E., Yamada, K., Goncalves, E. and Kahn, C. R., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 7336–7340.
41. Goncalves, E., Yamada, K., Thatte, H. S., Backer, J. M., Golan, D. E., Kahn, C. R. and Shoelson, S. E., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 5762–5766.
42. Tornqvist, H. E., Pierce, M. W., Frackelton, A. R., Nemenoff, R. A. and Avruch, J., *J. Biol. Chem.*, 1987, **262**, 10212–10219.
43. White, M. F., Shoelson, S. E., Keutman, H. and Kahn, C. R., *J. Biol. Chem.*, 1988, **263**, 2969–2980.
44. Goren, H. J., White, M. F. and Kahn, C. R., *Biochemistry*, 1987, **26**, 2374–2381.
45. Ellis, L., Clauser, E., Morgan, D. O., Edery, M., Roth, R. A. and Rutter, W. J., *Cell*, 1986, **45**, 721–732.
46. Wilden, P. A., Siddle, K., Haring, E., Backer, J. M., White, M. F. and Kahn, C. R., *J. Biol. Chem.*, 1992, **267**, 13719–13727.
47. Wilden, P. A., Kahn, C. R., Siddle, K. and White, M. F., *J. Biol. Chem.*, 1992, **267**, 16660–16668.
48. Tavare, J. M., O'Brian, R. M., Siddle, K. and Denton, R. M., *Biochem. J.*, 1988, **253**, 783–788.
49. Tornqvist, H. E. and Avruch, J., *J. Biol. Chem.*, 1988, **263**, 4593–4601.
50. Feener, E. P., Backer, J. M., King, G. L., Wilden, P. A., Sun, X. J., Kahn, C. R. and White, M. F., *J. Biol. Chem.*, 1993, **268**, 11256–11264.
51. Tavare, J. M. and Denton, R. M., *Biochem J.*, 1988, **252**, 607–615.

52. Tornqvist, H. E., Gunsalus, J. R., Nemenoff, R. A., Frackelton, A. R., Pierce, M. W. and Avruch, J., *J. Biol. Chem.*, 1988, **263**, 350–359.
53. Backer, J. M., Kahn, C. R., Cahill, D. A., Ullrich, A. and White, M. F., *J. Biol. Chem.*, 1990, **265**, 16450–16454.
54. Backer, J. M., Shoelson, S. E. Weiss, M. A., Hua, X. Q., Cheatham, R. B., Haring, E., Cajill, D. C. and White, M. F., *J. Cell Biol.*, 1992, **118**, 831–839.
55. White, M. F., Livingstone, J. N., Backer, J. M., Lauris, V., Dull, T. J., Ullrich, A. and Kahn, C. R., *Cell*, 1988, **54**, 641–649.
56. Backer, J. M., Schroeder, G., Kahn, C. R., Myers Jr., M. G., Wilden, P. A., Cahill, D. A. and White, M. F., *J. Biol. Chem.*, 1992, **267**, 1367–1374.
57. Yang Feng, T. L., Franke, U. and Ullrich, A., *Science*, 1985, **228**, 728–731.
58. Muller-Wieland, D., Taub, R. A., Tewari, D. S., Kriauciunas, K. M., Reddy, S. S. K. and Kahn, C. R., *Diabetes*, 1989, **38**, 31–38.
59. Seino, S., Sieno, M. and Bell, G. I., *Diabetes*, 1990, **39**, 129–133.
60. Hedo, J. A., Kahn, C. R., Hayoshi, M., Yamada, K. M. and Kasuga, M., *J. Biol. Chem.*, 1983, **258**, 10020–10026.
61. Hedo, J. A. and Gorden, P., *Horm. Metab. Res.*, 1985, **17**, 487–490.
62. Hedo, J. A. and Simpson, I. A., *Biochem J.*, 1985, **232**, 71–78.
63. Alarcon, C., Cheatham, B., Lincoln, B., Kahn, C. R., Siddle, K. and Rhodes, C. J., *Biochem. J.*, 1994, **301**, 257–265.
64. Myers Jr., M. G., Backer, J. M., Sun, X. J., Shoelson, S. E., Hu, P., Schlessinger, J., Yoakim, M., Schaffhausen, B. and White, M. F., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 10350–10354.
65. Backer, J. M., Myers Jr., M. G., Shoelson, S. E., Chin, D. J., Sun, X. J., Mralpeix, M., Hu, P., Margolis, B., Skolnik, E. Y., Schlessinger, J. and White, M. F., *EMBO J.*, 1992, **11**, 3469–3479.
66. White, M. F., Maron, R. and Kahn, C. R., *Nature*, 1985, **318**, 183–186.
67. Rothenberg, P. L., Lane, W. S., Karasik, A., Backer, J., White, M. F. and Kahn, C. R., *J. Biol. Chem.*, 1991, **266**, 8302–8311.
68. Sun, X. J., Rithenberg, P., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Cahill, D. A., Goldstein, B. J. and White, M. F., *Nature*, 1991, **352**, 73–77.
69. Araki, E., Haag, B. L. and Kahn, C. R., *Biochim. Biophys. Acta*, 1994, **1221**, 353–356.
70. Mayer, B. J., Ren, R., Clark, C. K. and Baltimore, D., *Cell*, 1993, **73**, 629–630.
71. Yenush, L., Makali, K. J., Smith-Hall, J., Ishibashi, O., Myers, Jr. M. G. and White M. F., *J. Biol. Chem.*, 1993, **271**, 24300–24306.
72. Sun, X. J., Miralpeix, M., Myers Jr., M. G., Glasheen, E. M., Backer, J. M., Kahn, C. R. and White, M. F., *J. Biol. Chem.*, 1992, **267**, 22662–22672.
73. Wang, L. M., Keegan, A. D., Paul, W. E., Heideran, M. E., Gutkind, J. S. and Pierce, J. H., *EMBO J.*, 1992, **11**, 4899–4908.
74. Sun, X. J., Wang, L. M., Zhang, Y., Yenush, L., Myers, M. G. J., Glasheen, E., Lane, W. S., Pierce, J. H. and White, M. F., *Nature*, 1995, **377**, 173–177.
75. He, W., Crapora, A., Zhu, Y., O'Neill, T. J., Wang, L-M., Pierce, J. H. and Gustason, T. A., *J. Biol. Chem.*, 1996, **271**, 11641–11645.
76. Escobedo, J. A., Navankasattusas, S., Kavanaugh, W. M., Milfay, D., Fried, V. A. and Williams, L. T., *Cell*, 1991, **65**, 75–82.
77. Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A. and Schlessinger, J., *Cell*, 1991, **35**, 83–90.
78. Whiteman, M., Downes, C. P., Keeler, M., Keller, T. and Cantley, L., *Nature*, 1988, **332**, 644–646.
79. Pawson, T., *Nature*, 1995, **373**, 477–478.
80. Sun, X. J., Crimmins, D. L., Myers, Jr., M. G., Mirapleix, M. and White, M. F., *Mol. Cell Biol.*, 1993, **13**, 7418–7428.
81. Levy-Toledano, R., Taouis, M., Blaetter, D. H., Gordon, P. and Taylor, S. I., *J. Biol. Chem.*, 1994, **269**, 31178–31182.
82. Waters, S. B., Ymauchi, K. and Pessin, J. E., *J. Biol. Chem.*, 1993, **268**, 22231–22234.
83. Rose, D. W., Saltiel, A. R., Majumdar, M., Decker, S. J. and Olefsky, J. M., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 797–801.
84. Chuang, L. M., Myers, Jr., M. G., Backer, J. M., Shoelson, S. E., White, M. F., Birnbaum, M. J. and Kahn, C. R., *Mol. Cell Biol.*, 1993, **13**, 6653–6660.
85. Tsakardis, T., Taha, C., Grinstein, S. and Klip, A., *J. Biol. Chem.*, 1996, **271**, 19664–19667.
86. Skolnik, E. Y., Lee, C. H., Batzer, A. G., Vicentini, L. M., Zhou, M., Daly, R. J., Myers Jr., M. G., Backer, J. M., Ullrich, A., White, M. F. and Schlessinger, J., *EMBO J.*, 1993, **12**, 1929–1936.
87. Lowenstein, E. J., Daly, R. J., Batzer, A. G., Li, W., Margolis, B., Lammers, R., Ullrich, A., Skolnik, E. Y., Bar-Sagi, D. and Schlessinger, J., *Cell*, 1992, **70**, 431–442.
88. Skolnik, E. Y., Batzer, A., Li, N., Lee, C. H., Lowenstein, E., Mohammadi, M., Margolis, B. and Schlessinger, J., *Science*, 1993, **260**, 1953–1955.
89. Li, N., Batzer, A., Daly, R., Yajnik, V., Skolnik, E., Chardin, P., Bar-Sagi, B., Margolis, B. and Sclessinger, J., *Nature*, 1993, **363**, 85–88.
90. Egan, S. E., Giddings, B. W., Brooks, M. W., Buday, L., Sizeland, A. M. and Weinberg, R. A., *Nature*, 1993, **363**, 45–51.
91. Okada, S. and Pessin, J. E., *J. Biol. Chem.*, 1996, **271**, 25533–25538.
92. Der, C. J., *Cancer Treat. Res.*, 1989, **47**, 73–119.
93. Chardin, P., *Cancer Cells*, 1991, **3**, 117–126.
94. Macara, I. G., *Cell Signal*, 1991, **3**, 179–187.
95. Medema, R. H., De Vries-Smits, A. M. M., van der Zon, G. C. M., Maassen, J. A. and Bos, J. L., *Mol. Cell Biol.*, 1993, **13**, 155–162.
96. Medema, J. M. and Bos, J. L., *Crit. Rev. Oncogenesis*, 1993, **4**, 615–661.
97. Tanaka, S., Ito, T. and Wands, J. R., *J. Biol. Chem.*, 1996, **271**, 14610–14616.
98. Wood, K. W., Sarnecki, C., Roberts, T. M. and Blenis, J., *Cell*, 1992, **68**, 1041–1050.
99. Kyriakis, J. M., App, H., Zhang, X. F., Banerjee, P., Brautigan, D. L., Rapp, U. R. and Avruch, J., *Nature*, 1992, **358**, 417–421.
100. Schapp, D., van der Wal, J., Howe, L. R., Marshall, C. J. and Blitterswijk, W. J., *J. Biol. Chem.*, 1993, **268**, 20323–20336.
101. Ahn, N. G., Seger, R. and Krebs, E. G., *Curr. Opin. Cell Biol.*, 1993, **4**, 992–999.
102. Sturgill, T. W., Ray, L. B., Erikson, E. and Maller, J. L., *Nature*, 1988, **334**, 715–718.
103. Dent, P., Lavoinne, A., Nakielny, S., Caudwell, F. B., Watt, P. and Cohen, P., *Nature*, 1990, **348**, 302–307.
104. Pelicci, G. L., Lanfrancone, L., Grignani, F., McGlade, J., Cavallo, F., Forni, G., Nicoletti, I., Pawson, T. and Pelicci, P. G., *Cell*, 1992, **70**, 93–104.
105. Kovacina, K. S. and Roth, R. A., *Biochem. Biophys. Res. Commun.*, 1993, **192**, 1303–1311.
106. Pronk, G. J., McGlade, J., Pelicci, G., Pawson, T. and Bos, J. L., *J. Biol. Chem.*, 1993, **268**, 5748–5753.
107. Paz, K., Voliovitch, H., Hadari, Y. R., Roberts, C. T. Jr., LeRoith, D. and Zick, Y., *J. Biol. Chem.*, 1996, **271**, 6998–7003.
108. Isakoff, S. J., Yu, Y-P., Su, Y-C., Blaikie, P., Yajnik, V., Rose, E., Weidner, K. M., Sachs, M., Margolis, B. and Skolnik, E. Y., *J. Biol. Chem.*, 1996, **271**, 3959–3962.
109. van der Geer, P., Wiley, S., Gish, G. D., Lai, V. K., Stephens, R. and White M. F., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 963–968.
110. Ricketts, W. A., Rose, D. W., Shoelson, S. and Olefsky, J. M., *J. Biol. Chem.*, 1996, **271**, 26165–26169.
111. Sasaoka, T., Ishihara, H., Sawa, T., Ishiki, M., Morioka, H.,

Imamura, T., Usui, I., Takada, Y. and Kobayashi, M., *J. Biol. Chem.*, 1996, **271**, 20082–20087.
112. Okabayashi, Y., Sugimoto, Y., Totty, N. F., Hsuan, J., Kido, Y., Sakaguchi, K., Gout, I., Waterfield, M. D. and Kasuga, M., *J. Biol. Chem.*, 1996, **271**, 5265–5269.
113. Araki, E., Sun, X. J., Haag, B. L., Zhang, Y., Chuang, L. M., Zhang, Y., Yang-Feng, T., White, M. F. and Kahn, C. R., *Diabetes*, 1993, **42**, 1041–1054.
114. Ofsu, M., Hiles, I., Gout, I., Fry, M. J., Ruis-Larrea, F., Panayotou, G., Thompson, A., Dhand, R., Hsuan, J., Totty, N., Smith, A. D., Morgan, S. J., Courtneidge, S. A., Parker, P. J. and Waterfield, M. D., *Cell*, 1991, **65**, 91–104.
115. Hiles, I. D., Ofsu, M., Volinia, S., Fry, M. J., Gout, I., Dhand, R., Panayotou, G., Ruiz-Larrea, F., Thompson, A., Totty, N. F., Hsuan, J. J., Courtneidge, S. A., Parker, P. J. and Waterfield, M. D., *Cell*, 1992, **70**, 419–429.
116. Reif, K., Gout, I., Waterfield, M. D. and Cantrell, D. A., *J. Biol. Chem.*, 1993, **268**, 10780–10788.
117. Kuhne, M. R., Pawson, T., Lienhard, G. E. and Feng, G. S., 1993, *J. Biol. Chem.*, 1993, **268**, 11479–11481.
118. Perkins, L. A., Larsen, I. and Perrimon, N., *Cell*, 1992, **12**, 225–236.
119. Lechleider, R. J., Freeman, R. M. and Neel, B. G., *J. Biol. Chem.*, 1993, **268**, 13434–13438.
120. Arrandale, J. M., Gore-Willse, A., Rocks, S., Ren, J-M., Zhu, J., Davis, A., Livingston, J. N. and Rabin, D. U., *J. Biol. Chem.*, 1996, **271**, 21353–21358.
121. Park, D. and Rhee, S. G., *Mol. Cell Biol.*, 1992, **12**, 5816–5823.
122. Li, W., Hu, P., Skolnik, E. Y., Ullrich, A. and Schlessinger, J., *Mol. Cell Biol.*, 1992, **12**, 5824–5833.
123. Li, C. H., Li, W., Nishimura, R., Zhou, M., Batzer, A., Myers Jr., M. G., White, M. F., Schlessinger, J. and Skolnik, E. Y., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 11713–11717.
124. Chou, M. M., Fajardo, J. E. and Hanafusa, H., *Mol. Cell Biol.*, 1992, **12**, 5834–5842.
125. Anderson, D., Koch, C. A., Grey, L., Ellis, C., Moran, M. F. and Pawson, T., *Nature*, 1990, **250**, 979–982.
126. Margolis, R. N., Taylor, S. I., Seminara, D. and Hubbard, A. L., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 7256–7259.
127. Margolis, R. N., Schell, M. J., Taylor, S. I. and Hubbard, A. L., *Biochem. Biophys. Res. Commun.*, 1990, **166**, 562–566.
128. Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. R., Reynolds, A. B. and Parsons, J. T., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 5192–5196.
129. Hresco, R. C. Bernier, M., Hoffman, R. D., Flores-Riveros, J. R., Liao, K., Laird, D. M. and Lane, M. D., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 8835–8839
130. Pronk, G. J., Medema, R. H., Burgering, B. M., Clarc, R., McCoemick, F. and Bos, J. L., *J. Biol. Chem.*, 1993, **267**, 24058–24063.
131. Porras, A., Nebreda, A. R., Benito, M. and Santos, E., *J. Biol. Chem.*, 1992, **267**, 21124–21131.
132. Ellis, C., Moran, M., McCormick, F. and Pawson, T., *Nature*, 1990, **343**, 377–381.
133. Sung, C. K., Sanchez-Margalet, V., Truitt, K. and Imboden, J., *J. Cell Biol. (Suppl.)*, 1994, **18A**, 143.
134. Reynet, C. and Kahn, C. R., *Science*, 1993, **262**, 1441–1444.
135. Holcomb, B., Hsieh, P., Hoeffler, J. and Draznin, B., *Clin. Res.*, 1993, **41**, 210A.
136. Csermely, P. and Kahn, C. R., *Biochemistry*, 1992, **31**, 9940–9952.
137. Csermely, P., Schnaider, T., Cheatham, B., Olson, M. O. J. and Kahn, C. R., *J. Biol. Chem.*, 1993, **268**, 9747–9752.
138. Kim, S. J. and Kahn, C. R., *J. Biol. Chem.*, 1994, **269**, 11887–11892.
139. Kim, S. J. and Kahn, C. R., *J. Cell Physiol.*, 1993, **157**, 217–228.
140. Campos, S. P., Wang, Y. and Baumann, H., *J. Biol. Chem.*, 1996, **271**, 24418–24424.
141. Ceresa, B. P. and Pessin, J. E., *J. Biol. Chem.*, 1996, **271**, 12121–12124.
142. Stephens, J. M. and Pilch, P. F., *Endocrine Rev.*, 1995, **16**, 529–546.
143. Cushman, S. W. and Wardzala, L. J., *J. Biol. Chem.*, 1980, **255**, 4758–4762.
144. Birnbaum, M. J., *Int. Rev. Cytol.*, 1992, **137**, 239–297.
145. Smith, R. M., Charron, M. J., Shah, N., Lodish, H. F. and Jarett, L., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 6893–6897.
146. Okada, T., Kawano, Y., Sakakibara, T., Hazeki, O. and Ui, M., *J. Biol. Chem.*, 1994, **269**, 3568–3573.
147. Kakanishi, N., Catt, K. J. and Balla, T., *J. Biol. Chem.*, 1994, **269**, 6528–6535.
148. Vlahos, C. J., Matter, W. F., Hui, K. Y. and Brown, R. F., *J. Biol. Chem.*, 1994, **269**, 5241–5248.
149. Cheatham, B., Vlahos, C. J., Cheatham, I., Wang, L., Blenis, J. and Kahn, C. R., *Mol. Cell Biol.*, 1994, **14**, 4902–4911.
150. Fingar, D. C., Hausdorff, S. F., Blenis, J. and Birnbaum, M. J., *J. Biol. Chem.*, 1993, **268**, 3005–3008.
151. Proud, C. G., *TIBS*, 1996, **21**, 181–185.
152. Blenis, J., Chung, J., Erikson, E., Alcota, D. A. and Erikson, R. L., *Cell Growth Differ.*, 1991, **2**, 279–285.
153. Serunian, L. A., Haber, M. T., Fukui, T., Kim, J. W., Rhee, S. G., Lowenstein, J. M. and Cantley, L. C., *J. Biol. Chem.*, 1989, **264**, 17809–17815.
154. Katagiri, H., Asano, T., Ishihara, H., Inukai, K., Shibasaki, Y., Kikuchi, M., Yazaki, Y. and Oka, Y., *J. Biol. Chem.*, 1996, **271**, 16987–16990.
155. Haruta, T., Morris, A. J., Rose, D. W., Nelson, J. G., Mueckler, M. and Olefsky, J. M., *J. Biol. Chem.*, 1995, **270**, 27991–27994.
156. Martin, S. S., Haruta, T., Morris, A. J., Kippel, A., Williams, L. T. and Olefsky, J. M., *J. Biol. Chem.*, 1996, **271**, 17605–17608.
157. Heller-Harrison, R. A., Morin, M., Guilherme, A. and Czech, M. P., *J. Biol. Chem.*, 1996, **271**, 10200–10204.
158. Nakanishi, H., Brewer, K. A. and Exton, J. H., *J. Biol. Chem.*, 1993, **268**, 13–16.
159. Berra, E., Diaz-Meco, M. T., Dominguez, I., Municio, M. M., Sanz, L., Lozano, J., Chapkin, R. S. and Moscat, J., *Cell*, 1993, **74**, 555–563.
160. Parker, P. J. and Waterfield, M. D., *Cell Growth Differ.*, 1992, **3**, 747–752.
161. Kozma, L., Baltensperger, K., Klarlund, J., Porras, A., Santos, E. and Czech, M. P., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 4460–4464.
162. Robinson, L. J., Razzack, Z. F., Lawrence, J. C. and James D. E., *J. Biol. Chem.*, 1993, **268**, 26422–26427.
163. Fingar, D. C. and Birnbaum, M. J., *J. Biol. Chem.*, 1994, **269**, 10127–10132.
164. Cormont, M., Tanti, J. F., Zahraoui, A., Van Oberghen, E. Tavitian, A. and Le Marchand-Brustel, Y., *J. Biol. Chem.*, 1993, **268**, 19491–19497.

Received 26 February 1997; revised accepted 23 October 1997