TIMELINE

The twentieth century struggle to decipher insulin signalling

Philip Cohen

Abstract | Following the discovery of insulin, it took the rest of the twentieth century to understand how this hormone regulates intracellular metabolism. What are the main discoveries that led to our current understanding of this process? And how is this new knowledge being exploited in an attempt to develop improved drugs to treat the epidemic of type-2 diabetes?

The discovery of insulin in 1921 was one of the great biological and medical advances of the twentieth century. The near miraculous ability of insulin treatment to restore blood-glucose homeostasis in type-1 diabetics, who had previously been close to death, led Banting and Macleod to receive a Nobel Prize in 1923. Insulin also achieved fame as the first protein to have its amino-acid sequence determined, for which Fred Sanger was awarded the Nobel Prize for Chemistry in 1958. However, understanding how insulin induces its profound effects on blood-glucose homeostasis and metabolism has proved a more difficult 'nut to crack'; even 50 years after the discovery of insulin, little progress had been made in identifying its mode of action.

In this article, I recall the key discoveries that have led to a molecular understanding of the signalling pathways that function downstream of insulin (TIMELINE). In particular, I focus on the findings that led to the identification of phosphatidylinositol (PtdIns)-3,4,5-trisphosphate (PIP_3) as the main intracellular mediator of the metabolic actions of insulin, and the elucidation of the mechanisms through which PIP_3 exerts its effects on cells. I then discuss how proteins that participate in insulin signalling are being targeted in an attempt to develop new drugs for the treatment of type-2 diabetes. It is estimated that the number of diabetics will double to reach 300 million by 2020, and therefore effective treatments are needed urgently to deal with this epidemic.

Regulation of glucose homeostasis

The hallmark of insulin is its ability to lower the amounts of glucose in the blood. The theory that insulin exerted this effect by facilitating the transmembrane transport of sugar into certain extrahepatic tissues dates back to the work of Rachmiel Levene in 1949 (REF. 1). However, the biochemical details underlying this process were unknown and it was only decades later that insulin was shown to stimulate the uptake of glucose into adipose tissue by promoting the translocation of a glucose transporter from an intracellular store to the plasma membrane[2,3]. This transporter protein, which was shown to be expressed uniquely in insulin-sensitive muscle and adipose tissue[4], was cloned and characterized and called glucose transporter-4 (GLUT4)[5,6]. As discussed below, exactly how insulin stimulates the translocation of GLUT4 to the plasma membrane is only now being worked out (BOX 1); therefore, our present understanding of insulin signalling was gained largely by analysing other processes that are controlled by this hormone.

> "...the number of diabetics will double to reach 300 million by 2020, and therefore effective treatments are needed urgently..."

Activation of glycogen synthase by insulin. Glucose is converted to glucose 6-phosphate by hexokinase (in muscle and adipose tissue) or glucokinase (in the liver) and is then stored as glycogen (in muscle and the liver) or triglyceride (in adipose tissue). Glycogen storage is achieved in part by the activation of glycogen synthase (GS), which catalyses the final step in this process. In the early 1960s two mechanisms were identified by which GS could be activated: activation by glucose 6-phosphate and stimulation by insulin. In muscle, insulin was found to promote the conversion of GS from a highly phosphorylated low-activity form, which is dependent on glucose 6-phosphate for activity, to a relatively unphosphorylated high-activity species, which is less dependent on glucose 6-phosphate for activity[7,8]. This activation of GS occurs in minutes and was the first report of insulin affecting a specific enzyme[7]. These findings implied that insulin exerts its effects on GS by inhibiting a protein kinase and/or activating a protein phosphatase(s).

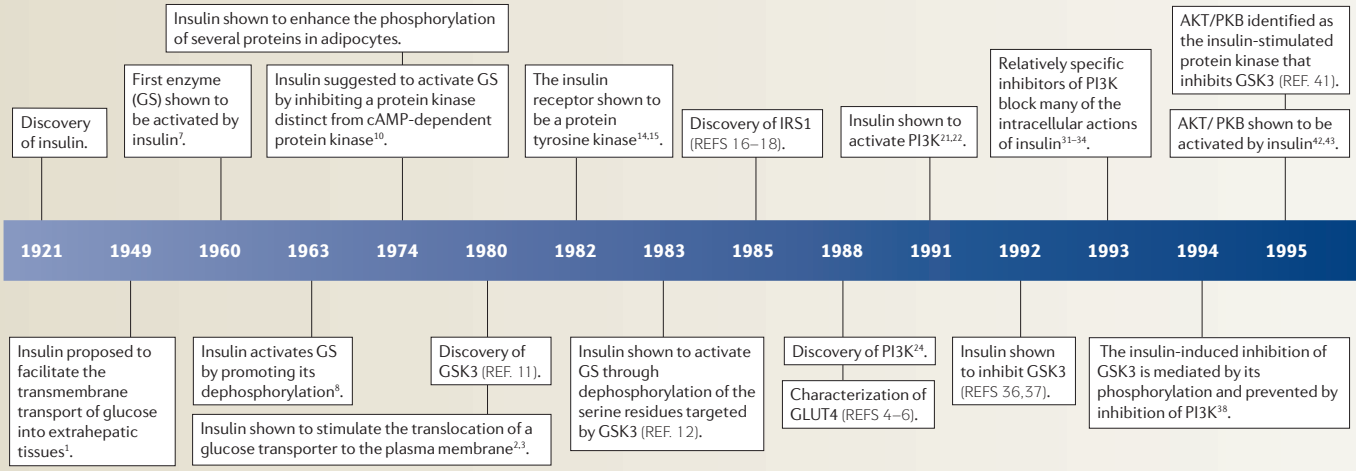
Inactivation of GS by protein kinases. In the late 1960s cyclic AMP (cAMP)-dependent protein kinase (PKA) was found to phosphorylate and inhibit GS, which indicated that insulin might exert its effect on GS by lowering the concentration of cAMP, thereby inhibiting PKA. However, in muscle, the activation of GS by insulin was not associated with a decrease in the intracellular concentration of cAMP[9]. This finding led to the hypothesis that insulin might generate a novel 'second messenger' or 'chemical mediator' that interacts with PKA to suppress the activation of PKA by cAMP. Over the years several molecules were proposed to be the insulin mediator, and were reported to mimic the activation of GS by insulin and several of the other known actions of insulin. However, these suggestions have not stood the test of time and it soon became clear that insulin activates GS by a mechanism that is independent of cAMP and PKA.

A new idea as to how insulin might activate GS was stimulated by the identification of protein kinases that inactivate GS by phosphorylating serine residues distinct from those targeted by PKA[10,11]. These studies culminated in the discovery of GS kinase-3 (GSK3)[11] and the finding that insulin activates GS primarily by inducing dephosphorylation of the serine residues that are targeted by GSK3, rather than the residues that are phosphorylated by PKA[12]. Therefore the action of insulin on GS had to result from the inhibition of GSK3 and/or the activation of a GS phosphatase. However, insulin was also shown to enhance the phosphorylation of several proteins in adipocytes[13], which implied that there must be one or more insulin-stimulated protein kinases.

Discovery of the second messenger

The recognition that the insulin receptor is a protein tyrosine kinase[14,15] was a landmark event that prefaced many subsequent discoveries. The insulin receptor is composed of two extracellular α-subunits and two transmembrane β-subunits that have protein tyrosine-kinase activity. Insulin binding activates an intramolecular autophosphorylation reaction in which one β-subunit phosphorylates the other at several sites. This reaction activates the tyrosine-kinase activity of the

Timeline | **Key events in the field of insulin signalling**



cAMP, cyclic AMP; *C. elegans*, *Caenorhabditis elegans*; *D. melanogaster*, *Drosophila melanogaster*; GLUT4, glucose transporter-4; GS, glycogen synthase; GSK3, glycogen synthase kinase-3; IRS1, insulin receptor substrate-1; PDK1, 3-phosphoinositide-dependent protein kinase-1; PI3K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; PKB, protein kinase B.

receptor enabling it to phosphorylate adaptor proteins that function as docking sites for effector proteins containing Src homology-2 (SH2) domains. The first evidence for such an effector protein emerged when insulin was shown to stimulate the tyrosine phosphorylation of a 185-kDa cytosolic protein[16]. The 185-kDa protein, now known as insulin receptor substrate-1 (IRS1), was characterized[17–19] and shown to be phosphorylated at multiple sites by the insulin receptor[20].

IRS1 was shown to associate with PtdIns 3-kinase (PI3K) activity following stimulation with insulin[21,22]. This PtdIns kinase had been identified previously through its association with several protein tyrosine kinases[23], and had been shown to catalyse the formation of a novel inositol phospholipid PtdIns 3-phosphate from PtdIns *in vitro*[24]. However, studies in neutrophils revealed that the physiological substrate of this enzyme was PtdIns-4,5-bisphosphate (PtdIns(4,5)P_2), implying that PI3K catalysed the formation of PIP_3 in cells[25,26]. The class 1 PI3Ks, as they came to be called, consist of a regulatory p85 and a catalytic p110 subunit[27–30], and the SH2 domain of the regulatory p85 subunit interacts with phosphotyrosine residues on IRS1. Through this interaction, PI3K is recruited to the plasma membrane where it converts PtdIns(4,5)P_2 to PIP_3 (FIG. 1).

Subsequently, the crucial role of PI3K in insulin signalling was established in several ways. The identification of the microbial product wortmannin[31,32] and the compound LY294002 as potent and relatively specific inhibitors of class 1 PI3Ks, and the discovery that these compounds blocked many of the metabolic actions of insulin[33–35], were particularly important findings indicating that PIP_3 was the long sought after second messenger for insulin. However, how PIP_3 elicited the metabolic effects of insulin remained a mystery.

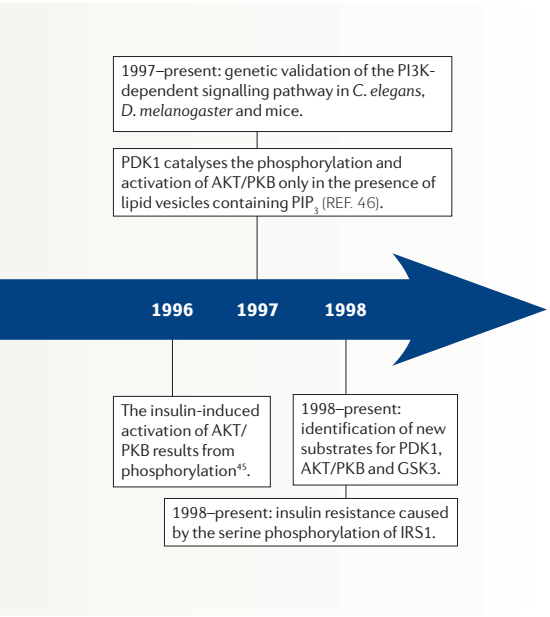
Box 1 | **Insulin-activated AKT/PKB has many functions**

AKT/protein kinase B (PKB) phosphorylates glycogen synthase kinase-3α (GSK3α) and GSK3β at serine residues that lie in Arg–Xaa–Arg–Xaa–Xaa–Ser/Thr motifs (where Xaa is any amino acid)[41]. AKT/PKB phosphorylates and regulates many proteins, and this motif is crucial for the specificity of AKT/PKB[75]. For example, AKT/PKB activates cyclic AMP phosphodiesterase-3B (PDE3B), which lowers the amounts of cyclic AMP and might underlie the antilipolytic actions of insulin in adipose tissue[76]. It also activates cardiac 6-phosphofructo 2-kinase, which might be responsible for insulin-induced activation of glycolysis in the heart[77]. AKT/PKB phosphorylates the tuberous sclerosis complex protein-2 (TSC2), which is thought to lead to the activation of the protein kinase mTORC1 and therefore the insulin-induced stimulation of protein synthesis[78]. There is also increasing evidence that phosphorylation of the Rab GTPase-activating protein AS160 by AKT/PKB underlies the insulin-stimulated translocation of the glucose transporter GLUT4 to the plasma membranes of muscle cells and adipocytes, a crucial event in promoting the uptake of glucose from the blood[79].

PIP_3-dependent signalling

By the early 1990s it was clear that GSK3 was indeed inhibited in minutes when cells were exposed to insulin[36,37]. The insulin-induced inhibition of GSK3 was blocked by wortmannin[38], which indicated that this inhibition was triggered by the formation of PIP_3. Moreover, inhibition was reversed by treatment with a serine/threonine-specific protein phosphatase[38]. These observations implied that GSK3 was inhibited by phosphorylation at a serine or threonine residue mediated by an insulin-stimulated protein kinase that was controlled by PIP_3.

Dissecting the kinase cascade. Two insulin-stimulated protein kinases, known as p90 ribosomal protein S6 kinase (p90RSK) and p70 ribosomal protein S6 kinase (p70S6K), were found to inhibit GSK3 *in vitro* by phosphorylating the α isoform of GSK3 (GSK3α) at Ser21 or the β isoform of GSK3 (GSK3β) at Ser9 (REFS 39,40). However, it soon became clear that neither p90RSK nor p70S6K was the relevant enzyme because drugs that suppressed the activation of both p90RSK and p70S6K had no effect on the insulin-induced inhibition of GSK3 (REF. 41). These studies led to the identification of a third insulin-stimulated protein kinase, AKT/protein kinase B (PKB), which was unaffected by the compounds that prevented the activation of p90RSK and p70S6K[41]. AKT/PKB also inhibited GSK3 by phosphorylating GSK3α at Ser21 and GSK3β at Ser9 (REF. 41), and was activated through PI3K[42,43]. The finding that GSK3 is inhibited by AKT/PKB helped



to explain how insulin could stimulate the phosphorylation of some proteins and the dephosphorylation of others.

AKT/PKB contains a pleckstrin homology (PH) domain that binds to PIP_3 with high affinity; however, this interaction does not activate AKT/PKB[44]. Instead, insulin-induced activation of AKT/PKB resulted from its phosphorylation at two residues, Thr308 and Ser473 (REF 45). Wortmannin prevented the insulin-induced activation of AKT/PKB and the phosphorylation of both sites, which implied that phosphorylation and activation of this protein is a PIP_3-dependent process.

This finding set the scene for the identification of a protein kinase that activated AKT/PKB *in vitro* by phosphorylating Thr308 (REF 46). Crucially, this protein kinase only activated AKT/PKB *in vitro* in the presence of lipid vesicles that contained PIP_3 (REFS 46,47) and was therefore called 3-phosphoinositide-dependent protein kinase-1 (PDK1)[46]. Similar to AKT/PKB, PDK1 has a PH domain[48] and PIP_3 binds to this domain with unusually high affinity[49], which indicates that a fraction of the cellular pool of PDK1 is associated with the plasma membrane and/or other PIP_3-containing membranes. The membrane fraction of PDK1 is thought to activate AKT/PKB when AKT/PKB is recruited from the cytosol to the membrane as a result of an insulin-mediated increase in the amounts of PIP_3. The binding of PIP_3 to the PH domain of AKT/PKB is also needed to induce a conformational change that makes Thr308 accessible to PDK1 (REF. 46).

The protein kinase that phosphorylates AKT/PKB at Ser473 was identified more recently and seems to be a specific form of the mammalian target of rapamycin (mTOR) bound to a regulatory subunit, known as RICTOR[50,51]. The PIP_3-dependent mechanism by which insulin activates the mTOR–RICTOR complex (called mTORC2) has yet to be elucidated.

The identification of PDK1 and mTORC2 provided the missing links in the chain of events through which insulin activates GS (FIG. 1). It soon became clear that AKT/PKB mediates many of the actions of insulin, not only the stimulation of glycogen synthesis, by phosphorylating and regulating many target proteins (BOX 1).

The pathway depicted in FIG. 1 was defined biochemically, but it was validated subsequently by genetic experiments using mammalian cells and tissues that do not express PDK1 (REF. 52) or AKT2/PKBβ[53], or cells that express GSK3 mutants that cannot be inactivated by AKT/PKB[54]. Genetic analysis of model organisms also revealed that PI3K and some of its downstream effectors are evolutionarily conserved components of the insulin-signalling pathway; in *Caenorhabditis elegans*, insulin regulates metabolic activity and longevity[55] and in *Drosophila melanogaster*, insulin controls cell size[56].

Recent insights from knockout studies. Further studies of the IRS family of proteins, the generation of IRS1- and IRS2-deficient mice, and studies of conditional insulin-receptor-knockout mice, in which the insulin receptor is absent in insulin-sensitive tissues, have been extremely informative. The analysis of these animals showed that the development of a diabetic phenotype requires defective function of the pancreatic islets as well as insulin resistance in one or more insulin-responsive tissues (reviewed in REF. 57). Therefore, it is pertinent to ask whether our increasing knowledge of insulin signalling can be exploited to develop improved treatments for type-2 diabetes.

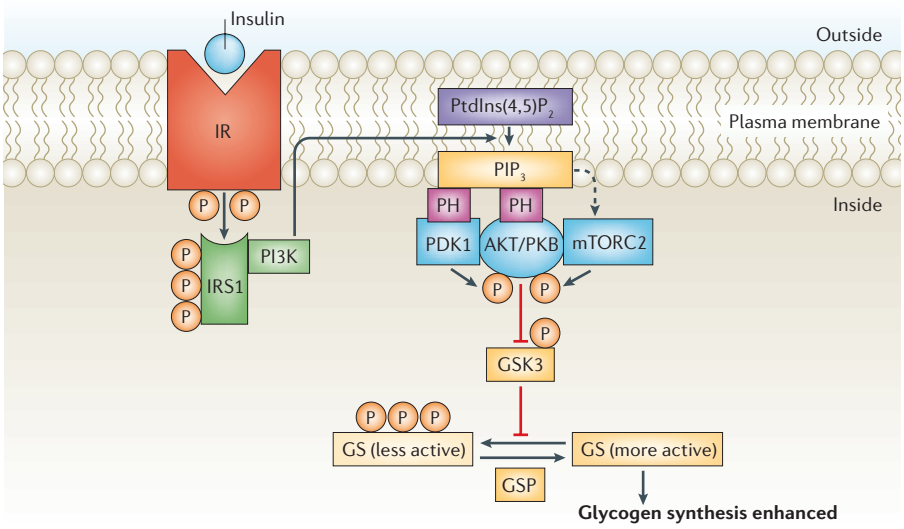


Figure 1 | **Activation of glycogen synthase by insulin in mammalian skeletal muscle.** The interaction of insulin with the insulin receptor (IR) on the outer surface of the plasma membrane activates the protein tyrosine-kinase activity that is associated with the β-subunit of the receptor. The receptor then phosphorylates itself and IR substrate-1 (IRS1). Phosphorylated IRS1 binds to phosphatidylinositol (PtdIns) 3-kinase (PI3K), which is recruited to the plasma membrane and converts the inositol phospholipid PtdIns-4,5-bisphosphate (PtdIns (4,5)P_2) to PtdIns-3,4,5-trisphosphate (PIP_3). The increased amounts of PIP_3 result in its interaction with the pleckstrin homology (PH) domain of AKT/protein kinase B (PKB), thereby recruiting AKT/PKB to the plasma membrane. At the plasma membrane, phosphorylation of AKT/PKB at Thr308 by PIP_3-bound 3-phosphoinositide-dependent protein kinase-1 (PDK1) results in AKT/PKB activation. PIP_3 also triggers the activation of the mammalian target of rapamycin complex-2 (mTORC2) by an unknown mechanism. mTORC2 then phosphorylates AKT/PKB at Ser473 for maximal activation. AKT/PKB phosphorylates the α-subunit of glycogen synthase kinase-3 (GSK3) at Ser21 and the β-subunit at Ser9; this modification reduces the activity of GSK3 and therefore promotes the dephosphorylation and activation of glycogen synthase (GS) by a GS phosphatase (GSP). The activation of GS, together with the increased rate of glucose transport, underlies the insulin-stimulated conversion of blood glucose to muscle glycogen.

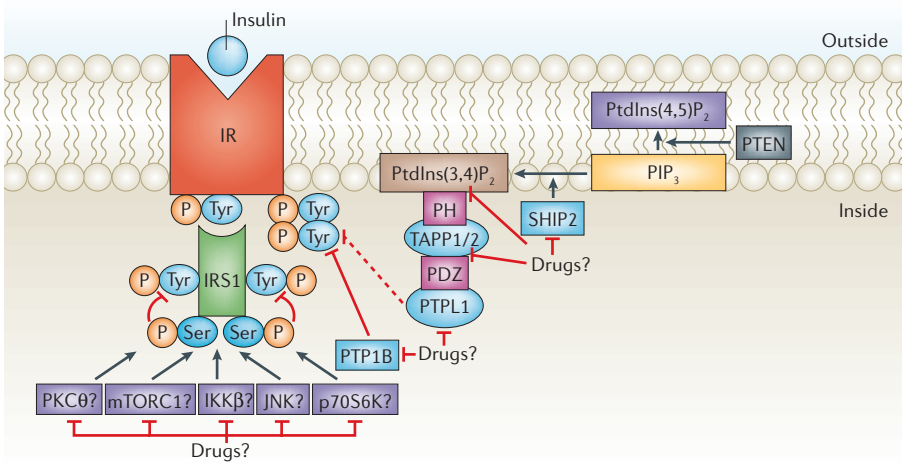


Figure 2 | **Inhibitors of insulin signalling are potential targets for the development of drugs for diabetes.** Insulin activates the protein tyrosine kinase (PTK) activity of the insulin receptor (IR), inducing receptor autophosphorylation, which results in further activation of the receptor and docking of the IR substrate-1 (IRS1). The receptor PTK then phosphorylates IRS1 at several tyrosine residues, which dock with phosphatidylinositol (PtdIns) 3-kinase (PI3K) triggering the conversion of PtdIns-4,5-bisphosphate (PtdIns(4,5)P_2) to PtdIns-3,4,5-trisphosphate (PIP_3) (as shown in FIG. 1). Insulin signalling through PIP_3 is reversed by several phosphatases: PTEN (phosphatase located on chromosome 10) converts PIP_3 to PtdIns(4,5)P_2; Src-homology-2 (SH2) domain-containing inositol phosphatase-2 (SHIP2) converts PIP_3 to PtdIns(3,4)P_2 and protein tyrosine phosphatase-1B (PTP1B) dephosphorylates the IR. PtdIns(3,4)P_2 binds to the pleckstrin homology (PH) domain of TAPP1/2 (tandem PH-domain-containing protein-1 and -2), which interact with the PDZ domains of the protein tyrosine phosphatase PTPL1. When the levels of PtdIns(3,4)P_2 increase in response to insulin, TAPP1/2 bound to PTPL1 is recruited to the membrane where PTPL1 might dephosphorylate the IR or IRS1. Kinases such as IκB kinase-β (IKKβ), Jun N-terminal kinase (JNK), the mammalian target of rapamycin complex-1 (mTORC1), p70 ribosomal S6 kinase (p70S6K) and protein kinase Cθ (PKCθ) phosphorylate IRS1 at serine residues. Serine phosphorylation of IRS1 inhibits its tyrosine phosphorylation and thereby inhibits its ability to recruit PI3K. Serine phosphorylation of IRS1 might underlie the inhibition of insulin signalling induced by cytokines, nutrients, free fatty acids and insulin, and the resistance to insulin in type-2 diabetes. Drugs that inhibit PTP1B, PTPL1, SHIP2, protein kinases that phosphorylate IRS1 at serine residues, or drugs that dissociate TAPP1/2 from PtdIns(3,4)P_2 or PTPL1, might sensitize cells to insulin.

Kinases as drug targets

An inspection of FIG. 1 indicates that several proteins could be targeted to develop drug treatments for diabetes. For example, one could envisage developing activators of PI3K, PDK1 or AKT/PKB, or compounds that enhance the activation of AKT/PKB by PDK1. However, the development of drugs that activate a target is, in general, even more challenging than developing inhibitors. Moreover, activators of PI3K, PDK1 or AKT/PKB might be oncogenic because mutations that activate PI3K or AKT/PKB, or that inactivate the tumour suppressor PTEN (which is a lipid phosphatase that converts PIP_3 to PtdIns(4,5)P_2), have been found in about 50% of human tumours. Indeed, pharmaceutical companies are developing inhibitors of PI3K, PDK1 and AKT/PKB as potential anti-cancer agents. Therefore, the development of drugs to combat diabetes has focused on components of the insulin-signalling pathway, the inhibition of which might mimic insulin action or sensitize cells to insulin (FIG. 2).

GSK3 as a potential target. GSK3 activity is inhibited by insulin (FIG. 1) and the amounts of GSK3 increase in diabetic muscle. Therefore, inhibitors of GSK3 might mimic the stimulation of GS by insulin and help to overcome the resistance to insulin in type-2 diabetes, which seems to occur mainly at the level of the IRS proteins.

Potent and highly specific inhibitors of GSK3 have been developed that induce the activation of GS, enhance the conversion of extracellular glucose to glycogen, inhibit gluconeogenesis in liver cells and normalize blood-glucose levels in animal models of type-2 diabetes (reviewed in REF. 58). These inhibitors normalize blood glucose predominantly through their effects on the liver because they have little or no effect on the uptake of glucose by muscle — a rate-determining step in the conversion of blood glucose to muscle glycogen but not to liver glycogen.

The studies with GSK3 inhibitors have shown that drugs that target a component of the insulin-signalling pathway can mimic the action of insulin to produce a beneficial effect (normalization of blood glucose). However, these studies are still at the preclinical stage, and whether GSK3 inhibitors can be used for long periods without unacceptable side effects remains to be seen. Although a 40% inhibition of GSK3 should be sufficient to mimic the action of insulin on GS, GSK3 has many physiological roles in the cell. For example, GSK3 inhibits the Wnt-signalling pathway, deregulation of which is associated with colon cancer, and it also suppresses the transcriptional activities of proto-oncogenes, such as Jun and Myc.

Targeting other protein kinases. The insulin-induced phosphorylation of IRS1 on tyrosine residues, which initiates insulin-signal transduction, is suppressed by the phosphorylation of IRS1 on serine residues; insulin resistance in type-2 diabetes might frequently occur through this mechanism (reviewed in REF. 57). Downregulation of insulin signalling involves phosphorylation of many serine residues on IRS1 by several protein kinases, including Jun N-terminal kinase (JNK)[59], IκB kinase-β (IKKβ)[60], p70S6K and its activator mTORC1 (REF. 61) and perhaps protein kinase Cθ ((PKCθ)[62]. The phosphorylation of IRS1 by mTORC1 and/or p70S6K might contribute to the feedback control of insulin signalling by glucose, amino acids and insulin itself. Indeed, sensitivity to insulin is enhanced in p70S6K-deficient or mTORC1-deficient cells or after exposure to rapamycin[61], a specific inhibitor of mTORC1. Activators of AMP-activated protein kinase (AMPK), including the drug metformin, which is used in the treatment of type-2 diabetes (BOX 2), suppress activation of the mTORC1–p70S6K pathway and might therefore enhance insulin signalling through PIP_3 (REF. 63); this effect could underlie some of the beneficial effects of metformin.

Rapamycin is a clinically approved immunosuppressant, whereas JNK and IKKβ regulate transcription factors, such as Jun, ATF2 and NF-κB, which control other physiological processes. Therefore, whether inhibitors of these protein kinases can be used to treat diabetes safely is unclear. Moreover, the regulation of IRS1 by serine/threonine phosphorylation remains a complex and evolving story, and other yet-to-be identified protein kinases could turn out to be the main players that mediate insulin resistance in type-2 diabetes.

Box 2 | **The most common treatment for type-2 diabetes**

The discovery of insulin had a decisive impact on the treatment of type-1 diabetes, but did not eradicate diabetes: >90% of diabetic patients have type-2 diabetes, which is characterized by the failure of insulin to regulate the metabolism of its target tissues (known as insulin resistance). Indeed, far from being eradicated, type-2 diabetes has become an epidemic, with the number of affected people expected to double to 300 million by 2020. Moreover, because of the many long-term complications of diabetes, such as kidney and heart disease, impotence, limb amputations and blindness, treatment for diabetes accounts for 10% of health-care expenditure in Europe (25 billion € per annum). In the United States, mortality from diabetes increased by 30% from 1988–1998, whereas mortality from cardiovascular disease declined by 30% over this period.

Metformin (known as glucophage (Bristol–Myers Squibb) in the United States), the most common drug for type-2 diabetes, has been used clinically for over 50 years. Metformin exerts its beneficial effects by activating the AMP-activated protein kinase (AMPK), the main sensor of energy changes in the cell. AMPK is activated by increased amounts of 5′-AMP when the amounts of cellular ATP fall. Activated AMPK shuts down the main energy-using cellular systems and switches on the energy-generating systems to replenish ATP levels. For example, AMPK stimulates glucose uptake into muscle during exercise[80] and inhibits gluconeogenesis in the liver[81], explaining how metformin can help to normalize blood glucose levels[82]. Recent work has shown that metformin is a weak inhibitor of the respiratory chain[83], indicating that it might activate AMPK by slightly increasing the levels of 5′-AMP. However, metformin has side effects, which often include unpleasant gastrointestinal problems.

Phosphatases as drug targets

Insulin signalling is terminated by the dephosphorylation of tyrosine residues on the insulin receptor and/or IRS proteins, which is catalysed by protein tyrosine phosphatases (PTPs) such as PTP1B. Therefore, drugs that inhibit PTP1B would be expected to sensitize cells to insulin. Indeed, PTP1B-deficient mice are hypersensitive to insulin and normalize blood-glucose levels at half the normal circulating insulin concentration[64]. These mice do not become obese when fed on a high fat, high carbohydrate diet, making PTP1B an attractive target for the development of drugs to treat obesity as well as diabetes. However, although potent and specific inhibitors of this enzyme have been developed[65], the hydrophilic nature of the catalytic centre has made it extremely difficult so far to develop inhibitors that can be used as therapeutic drugs.

The SH2-domain-containing inositol 5-phosphatase-2 (SHIP2) converts the PIP_3 that is generated in response to insulin to PtdIns(3,4)P_2, which probably has a distinct second messenger role(s), because it interacts specifically with TAPP1 (tandem PH-domain-containing protein-1) and the closely related TAPP2 (REF. 66). The C termini of TAPP1/TAPP2 interact with the PDZ domains of the protein tyrosine phosphatase PTPL1 (REF. 67); the insulin-stimulated formation of PtdIns(3,4)P_2 induces the recruitment of TAPP1/TAPP2 and PTPL1 to the plasma membrane[67]. At the plasma membrane, PTPL1 probably dephosphorylates phosphotyrosine-containing proteins, such as the insulin receptor[68]; therefore this phosphatase could be another potential target for drug therapy. An alternative approach would be to develop a drug that prevented the interaction of PtdIns(3,4)P_2 with TAPP1/TAPP2 or the interaction of TAPP1/TAPP2 with PTPL1 (FIG. 2). However, the identification of drug-like molecules that disrupt protein–protein interactions is still challenging.

Other potential drug targets include PTEN and SHIP2, the lipid phosphatases that metabolize PIP_3 (FIG. 2). The inhibition of these enzymes might mimic insulin signalling by increasing the amounts of PIP_3 (reviewed in REF. 69). However, PTEN is a tumour suppressor, and its inhibition might be oncogenic unless PTEN-inhibitory drugs could be delivered to specific tissues. Moreover, SHIP2 inhibitors would need to be selective because the related SHIP1 isoform is crucial for immune function.

PP1-G_L is an attractive target. In liver cells, GS is dephosphorylated and activated by a glycogen-associated form of protein phosphatase-1 (PP1) in which the PP1 catalytic subunit is complexed to a glycogen targeting subunit G_L[70], the synthesis of which is induced by insulin and glucocorticoids[71].

G_L also interacts with the activated form of glycogen phosphorylase (called phosphorylase *a*), which suppresses the activation of GS by PP1-G_L[72]. When the levels of blood

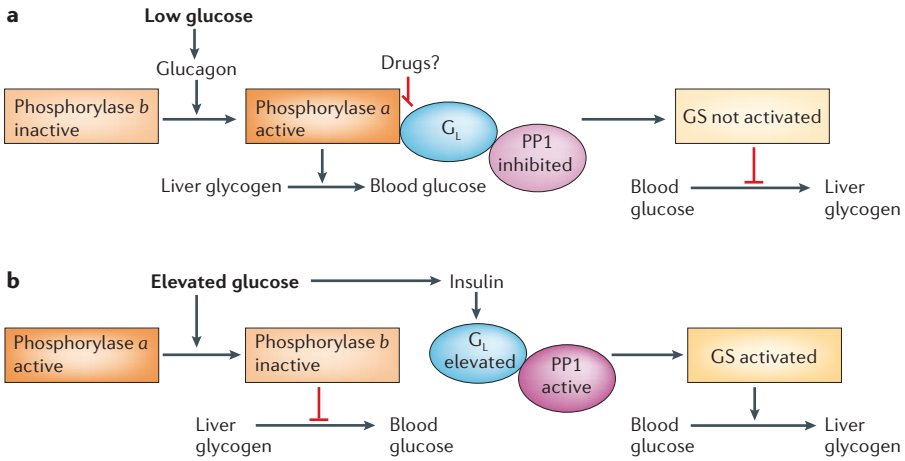


Figure 3 | **Drugs that dissociate phosphorylase *a* from PP1-G_L might stimulate glycogen synthesis in the liver. a** | When blood-glucose levels are low or there is an increased energy demand for glucose, the hormones glucagon or adrenaline trigger the conversion of the unphosphorylated, inactive form of glycogen phosphorylase (phosphorylase *b*) to the phosphorylated, active form (phosphorylase *a*). Phosphorylase *a* not only catalyses the rate-limiting step in the conversion of liver glycogen to blood glucose, but also binds to the C terminus of the liver glycogen-targeting subunit G_L of protein phosphatase-1 (PP1). This interaction prevents PP1 from dephosphorylating and activating glycogen synthase (GS), and thereby inhibits glycogen synthesis. **b** | When the blood-glucose levels are elevated, glucose enters liver cells and binds to phosphorylase *a*. This interaction transforms phosphorylase *a* into a more effective substrate for the protein phosphatase that converts phosphorylase *a* to phosphorylase *b*. This conversion not only inhibits glycogenolysis, but also stops the inhibition of PP1-G_L, allowing PP1-G_L to dephosphorylate and activate GS, which promotes the conversion of blood glucose to liver glycogen. The level of expression of G_L is also enhanced by insulin. Drugs that prevent the interaction of phosphorylase *a* with G_L might mimic the ability of insulin to stimulate the conversion of blood glucose to liver glycogen and help to normalize the high blood-glucose levels that are the hallmark of diabetes. The development of inhibitors of phosphorylase *a* or activators of GS would be alternative strategies to stimulate liver glycogen synthesis; however, inhibitors of phosphorylase *a* are not proving effective in clinical trials.

glucose are elevated, glucose binds to liver phosphorylase *a* and induces a conformational change that promotes the inactivation of the enzyme. Therefore, glycogenolysis is switched off and the inhibition of PP1-G_L is blocked, allowing this phosphatase to activate GS and promote the conversion of blood glucose to liver glycogen[73] (FIG. 3).

Phosphorylase *a* binds to the C terminus of G_L[74] and a drug that prevents the interaction of phosphorylase *a* with G_L could lower blood-glucose levels by inducing the dephosphorylation and activation of GS (FIG. 3). Because PP1-G_L dephosphorylates sites on GS that are targeted by GSK3, a drug that prevents the interaction of phosphorylase *a* with G_L could synergize with GSK3 inhibitors. A combination of drugs might allow the reduction of the effective therapeutic concentration of each drug and therefore potential side effects of each drug might be minimized.

Concluding remarks

After 50 years with little progress, the dissection of the insulin-signalling pathway finally took off in the early 1980s and has continued at an ever-accelerating pace. Although many details remain unknown, and undoubtedly a few surprises lie in store, the outline had already become clear by the late 1990s. The elucidation of the PIP_3-signalling pathway has focused attention on components that might be targets for the development of improved drugs to treat type-2 diabetes. However, the challenge of developing a safe and effective oral drug to treat this chronic disease cannot be underestimated. There are potential concerns that drugs that inhibit several of the proteins involved in insulin signalling might be oncogenic. Indeed, the elucidation of this signalling pathway might well lead to the development of several anti-cancer drugs. Over the next 20 years we shall see whether our expanding knowledge of the insulin-signalling network results in treatments to combat the imminent epidemic of diabetes.

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Competing interests statement

The author declares no competing financial interests.

DATABASES

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