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Biochemical and cellular properties of insulin receptor signalling

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Abstract

The mechanism of insulin action is a central theme in biology and medicine. In addition to the rather rare condition of insulin deficiency caused by autoimmune destruction of pancreatic β -cells, genetic and acquired abnormalities of insulin action underlie the far more common conditions of type 2 diabetes, obesity and insulin resistance. The latter predisposes to diseases ranging from hypertension to Alzheimer disease and cancer. Hence, understanding the biochemical and cellular properties of insulin receptor signalling is arguably a priority in biomedical research. In the past decade, major progress has led to the delineation of mechanisms of glucose transport, lipid synthesis, storage and mobilization. In addition to direct effects of insulin on signalling kinases and metabolic enzymes, the discovery of mechanisms of insulin-regulated gene transcription has led to a reassessment of the general principles of insulin action. These advances will accelerate the discovery of new treatment modalities for diabetes.

After the discovery of insulin in 1921 (REF. ¹), its mechanism of action remained unclear for at least 30 years. In 1950, Rachmiel Levine² demonstrated that insulin increases membrane permeability to glucose, as a basis for its glucose-lowering effect, thus ending speculation as to whether insulin binds directly to glycolytic enzymes to modify their activity. In 1971, Jesse Roth and colleagues³ demonstrated the existence of a membrane receptor for insulin. However, the signalling mechanism elicited by insulin binding to the receptor did not become clear until Kasuga and Kahn⁴, using antibodies isolated from patients with autoimmune syndromes of insulin resistance to immunoprecipitate the receptor, demonstrated that it becomes phosphorylated on tyrosine residues in response to insulin binding — an unexpected finding, as tyrosine phosphorylation had until then been

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thought of as exclusive to oncogenes. Not until the insulin receptor (IR) cDNA was cloned by the Ullrich and Rutter groups in 1985 (REFS^{5,6}) did the notion of tyrosine phosphorylation as the basis for insulin signalling become widely adopted, effectively shifting attention from the cell surface to the intracellular pathways mediating the diverse actions of the receptor (FIG. 1).

In 1991, White and Kahn⁷ cloned the first of four IR substrate (IRS) proteins, a family of adaptor proteins whose main role is to convert the tyrosine phosphorylation signal into a lipid kinase signal by recruiting the catalytic subunit of the enzyme PI3K. The latter generates the triphosphorylated inositol required to activate the serine/threonine kinase AKT. The identification of AKT⁸ as an insulin-activated serine/threonine kinase solved the riddle of how the tyrosine phosphorylation signal is converted into a serine/threonine phosphorylation signal. In fact, while it had long been apparent that most actions of insulin required the phosphorylation of serine/threonine residues on various substrates, efforts to demonstrate direct effects of insulin on serine/threonine kinases had fallen short. AKT provided the missing link that enabled the clarification of the distal steps in insulin signalling, such as the modulation of glucose uptake by glucose transporter type 4 (GLUT4), glycogen synthesis by glycogen synthase kinase 3 (GSK3), protein and fat synthesis by mTOR and gene expression by forkhead family box O (FOXO) (FIG. 2).

Insulin has complex effects on metabolism, cell growth and differentiation. Virtually all mammalian cells possess IRs and hence respond to insulin. The major tissues targeted by insulin's effects on metabolism include: muscle, where insulin promotes glucose uptake and protein synthesis; adipose tissue, where insulin promotes glucose and fatty acid uptake and inhibits lipolysis; the liver, where insulin promotes glucose utilization, suppresses glucose production, and promotes triglyceride synthesis; and neurons, where it promotes anorexigenic and locomotor signals. Additional notable insulin target cells with a metabolic function include macrophages, endothelial cells and insulin-producing pancreatic β -cells. Moreover, some of the key mediators of insulin signalling — PI3K and AKT — are activated downstream of other growth factor receptors, and conversely some mitogenic pathways — growth factor receptor-bound protein 2 (GRB2) and ERK⁹ — are activated by insulin, creating a network of interacting signalling pathways¹⁰. Therefore, insulin's signalling mechanisms are complex, and this historical overview falls necessarily short of crediting additional signalling pathways that contribute to the hormone's diverse effects. Nonetheless, human genetics, animal models and cellular biology have built a consensus around the pathways described above as the main signalling nodes.

In this Review, we focus mainly on the cellular mechanisms of insulin signalling and on new insights into pathways of primary relevance to insulin's metabolic effects. We describe newly discovered elements of IR signal transduction and how they relate to the main metabolic actions of insulin. We focus on how the IR signal is modulated, including antagonistic proteins and combinatorial interactions among different isoforms, as well as temporal and spatial regulation of cellular insulin signalling. We also highlight key aspects of how these pathways fit into physiology and the pathophysiology of type 2 diabetes, with the general theme that increased insulin action is associated with increased insulin sensitivity, as defined by a leftward shift in the dose–response curve for insulin action on

glucose levels. However, work in this field has also identified multiple examples of insulin sensitivity being uncoupled from IR activity^{11–15} and other suggestions that insulin resistance is an adaptive trait^{16,17}. For recent reviews on insulin resistance, we refer the reader to REFS^{18–20}.

Antagonistic signalling

Every step in the insulin signalling cascade is a reversible enzymatic reaction. Thus, for each kinase activated by insulin, there are (often multiple) phosphatases that terminate its action. Moreover, in addition to the vast repertoire of serine/threonine kinases regulated by insulin, there is an even larger group of phosphatases that actively suppress the actions of the insulin-activated kinases. Furthermore, different post-translational modifications of the same target protein often result in different biological effects. These mechanisms are as important as signalling initiation but, because of their redundancy, have proved less amenable to cellular and genetic analyses. An overview of antagonistic signalling mechanisms is shown in FIG. 3a.

Insulin

The hormone itself is arguably the most important negative regulator of its own signalling. Similar to other receptor tyrosine kinases, IR undergoes ligand-induced internalization and lysosomal degradation or recycling back to the cell surface²¹ (FIG. 3b). This event requires a 12-amino-acid motif located on the intracellular part of the IR juxtamembrane domain²². This motif targets the receptor to clathrin-coated pits, and from there to lysosomes, whose acidic pH is thought to favour insulin release. Thus, as insulin levels rise, receptor down-regulation at the cell surface paradoxically decreases insulin signalling, contributing to its termination. This is the basis of the common observation that insulin resistance is associated with hyperinsulinaemia. However, because this phenomenon is strictly dependent on plasma insulin concentrations, it is promptly reversed when insulin levels fall, most notably after fasting in humans²³.

Inhibitory phosphorylation

As a general rule, tyrosine phosphorylation activates, and serine/threonine phosphorylation inactivates, IR and IRS proteins^{18,24}. At a more granular level, the effects are site-dependent, but serine/threonine phosphorylation is a major mechanism by which IR signalling is fine-tuned, antagonized or terminated.

One example that has been well-studied *in vitro* is the serine/threonine phosphorylation of IRS by the mTOR-ribosomal protein S6 kinase (S6K) pathway, which may link prolonged insulin signalling with decreased IRS protein levels. Unrestrained activation of S6K causes serine/threonine phosphorylation of IRS, which results in decreased IRS signalling and proteasomal degradation^{25,26} (FIG. 3c). Additional work has shown that mTOR and S6K phosphorylate IRS at multiple residues²⁴. Consistent with this concept, knocking out S6K1 from mice reduces weight gain and enhances insulin sensitivity²⁷. However, the importance of IRS serine/threonine phosphorylation in these phenotypes and in insulin action in general has been called into question. Mice with a knock-in allele of IRS1 in which the reported

S6K-directed phosphorylation site (Ser302) was mutated to alanine showed no alterations in insulin action²⁸. Thus, the mechanisms linking mTOR–S6K signalling to impaired insulin action may be independent of IRS.

A second pathway leading to inhibitory phosphorylation is initiated by inflammatory cytokines²⁹. This pathway can be triggered by macrophage infiltration in adipose tissue during obesity^{30,31}. These macrophages secrete pro-inflammatory cytokines, including TNF α , IL-1 β , and IL-6, which act in a paracrine manner to activate serine kinases in adipocytes, including I κ B kinase β (IKK β), c-Jun N-terminal kinase (JNK), S6K and mTOR³² (FIG. 3). These kinases carry out inhibitory phosphorylation of IRS1, thus causing insulin resistance in adipocytes. Consistent with this, activation of inflammatory pathways can impair glucose metabolism and cause systemic insulin resistance^{32,33}. Recent evidence demonstrates that immune cell activation in liver, muscle, pancreas and brain can also impair local insulin signalling and lead to metabolic dysfunction³². However, the physiological relevance of IRS serine/threonine phosphorylation in these tissues has been questioned. Mice carrying an IRS1 allele in which the expected TNF α -dependent phosphorylation site (Ser307) is mutated to alanine showed impaired insulin action³⁴, the opposite of what would have been expected based on the paradigm established *in vitro*. This underscores our incomplete understanding of these pathways and the involvement of IRS serine/threonine phosphorylation in insulin action *in vivo*.

A third mechanism of IR antagonism is caused by lipotoxicity (FIG. 3). Diacylglycerol-dependent activation of protein kinase C ϵ (PKC ϵ) impairs IR autophosphorylation by phosphorylating Thr1160 in the activation loop of IR³⁵. Indeed, mutant mice in which this phosphorylation site has been mutated are protected against diet-induced impairment of insulin action in liver³⁵ but not in muscle, where the effects of diacylglycerol may instead be mediated by PKC θ ³⁶. In addition to diacylglycerol^{36,37}, ceramide accumulation can also impair AKT function by stimulating the activity of PKC ζ , which alters membrane localization of AKT, and protein phosphatase 2A (PP2A)^{38,39}, which is described in more detail below.

Lipid phosphatases

The plasma membrane concentrations of the lipid second messenger PtdIns(3,4,5)P₃ are controlled not only at the level of synthesis by PI3K but also at the level of localization (see below) and degradation. Two lipid phosphatases are known to dephosphorylate PtdIns(3,4,5)P₃ and thereby attenuate downstream insulin signalling: PTEN and SHIP2 (Src homology 2 (SH2)-containing inositol 5'-phosphatase 2)⁴⁰ (FIG. 3).

PTEN is a 3'-phosphatase that converts PtdIns(3,4,5)P₃ into its precursor, PtdIns(4,5)P₂, and terminates insulin signalling (FIG. 3d). This molecular function was uncovered in studies of DAF-18, the *Caenorhabditis elegans* homologue of PTEN^{40,41}. Moreover, PTEN has been long been recognized as a tumour suppressor that is frequently disrupted in human cancers^{42,43}. PTEN inhibition increases AKT activity, thus amplifying not only the effects of insulin on glucose metabolism^{44–50} but also cell growth and survival pathways⁴². PTEN haploinsufficiency in humans increases insulin sensitivity and results in weight gain⁵¹.

SHIP2 is a 5'-phosphatase that converts PtdIns(3,4,5)P₃ into PtdIns(3,4)P₂. *In vitro* studies established that SHIP2 negatively regulates insulin-stimulated rat Akt phosphorylation⁵². Subsequent *in vivo* studies demonstrated that knockout of *SHIP2* in mice protects against obesity and insulin resistance^{53–55}. Likewise, glycaemia in rodent models of insulin resistance and diabetes has been shown to be improved by liver-specific expression of a dominant-negative form of SHIP2, *SHIP* knockdown (using antisense oligonucleotides against *SHIP2*), or a small-molecule SHIP2 inhibitor^{56–59}.

Protein phosphatases

The discovery of the tyrosine kinase activity of IR and IRS triggered a search for tyrosine phosphatases that terminate their activation. However, the nearly 100 members of this gene family do not have selective substrates but appear to be able to dephosphorylate tyrosine residues rather promiscuously; moreover, their activity appears to be constitutive, suggesting that IR and IRS tyrosine phosphorylation is transient. Specificity is probably achieved via subcellular localization and physical proximity. Protein tyrosine phosphatase 1B (PTP1B) negatively regulates insulin signalling by dephosphorylating tyrosine residues on IR and IRS proteins, and PTP1B ablation in mice is associated with increased insulin sensitivity^{60–62}. This phosphatase appears to have a primary effect in the central nervous system^{63–66}, although increased IR and IRS phosphorylation in response to PTP1B depletion was shown in peripheral tissues as well^{67–69}. PTP1B also negatively regulates leptin signalling, by acting on Janus kinase 2 (JAK2). Because the functions of leptin partly overlap with those of insulin, PTP1B (like FOXO1, see below) has the potential to be an integrative node in signal transduction underlying shared biological effects between the two hormones.

Pleckstrin homology domain and leucine-rich repeat protein phosphatase 1 α (PHLPP1 α), PHLPP1 β , and PHLPP2 constitute a distinct family of serine/threonine protein phosphatases^{70,71} (FIG. 3e). They were first discovered as AKT Ser473 phosphatases^{72,73}, but other substrates include PKC and S6K^{74,75}. PHLPP is involved in two separate negative feedback loops with insulin signalling. First, insulin signalling promotes PHLPP protein translation via mTOR-dependent activation of S6K and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1)⁷⁶. Second, insulin signalling stabilizes PHLPP protein. This occurs due to AKT phosphorylation and inhibition of GSK3 β , which normally phosphorylates PHLPP, causing it to be degraded⁷⁷ (FIG. 3e).

Protein phosphatase 2A (PP2A) is another serine/threonine phosphatase that dephosphorylates Akt⁷⁸. The holoenzyme comprises three subunits: structural, regulatory and catalytic. Because each has multiple isoforms, it has been estimated that 75 different holoenzymes are possible⁷⁹. Certain components have been specifically linked with negative regulation of insulin signalling^{80,81}. However, in addition to AKT, there are numerous potential targets for PP2A-mediated dephosphorylation. This may underlie the paradoxical worsening of insulin resistance by PP2A inhibitors in mice⁸².

Pseudosubstrates of the IR kinase

One set of IR signalling inhibitors are the adaptor proteins GRB10 and GRB14 (FIG. 3b). These proteins contain two different domains that each bind to the IR (and the insulin-like

growth factor 1 (IGF1) receptor)⁸³. One is a unique peptide region referred to as BPS (between PH and SH2), which binds to the substrate-binding cleft of IR⁸⁴. The second domain is an SH2 domain, which binds the phosphorylated tyrosine residues on IR⁸⁵. Thus these GRBs act as pseudosubstrates and obstruct IRS binding and activation. Because the GRB SH2 domain protects IR phosphotyrosines from phosphatases, it potentiates IR phosphorylation, despite the overall function of these proteins as inhibitors^{86–88}. It was recently revealed that GRB10 is part of a negative feedback loop, as it is an IR inhibitor that is stabilized downstream of IR. Using phosphoproteomics, it was found that insulin signalling via Akt and mTOR complex 1 (mTORC1) causes direct phosphorylation of GRB10 (REFS^{89,90}). This stabilizes GRB10, thus perpetuating its inhibitory effect on IR (FIG. 3b).

The physiological importance of these proteins is demonstrated by the improved insulin sensitivity seen in mice lacking GRB10, GRB14 or both^{87,88,91,92}. Human genetic studies also link these proteins with insulin signalling. Single nucleotide polymorphisms (SNPs) located within or near *GRB10* correlate with glucose levels in the fasted state, glucose tolerance, glucose-stimulated insulin secretion and type 2 diabetes^{93–97}. SNPs near *GRB14* are associated with insulin levels, insulin sensitivity and type 2 diabetes^{93,97–99} as well as adiposity, body fat distribution and body mass index^{93,100–105}.

A second set of IR inhibitory proteins are the SOCS proteins. Four members of this family (SOCS1, SOCS3, SOCS6 and SOCS7) have been shown to attenuate insulin signalling¹⁰⁶. There are two mechanisms for this inhibition: one is that a SOCS protein — through its SH2 domain — binds and occupies the phosphotyrosine site of the IR (FIG. 3b), and the second is that a SOCS protein — through its SOCS box domain — recruits a ubiquitin ligase to target IRS proteins for proteasomal degradation¹⁰⁶ (FIG. 3c). Consistent with these molecular mechanisms, a body of evidence has suggested that reducing or inhibiting SOCS proteins would benefit insulin sensitivity *in vivo*¹⁰⁶. Deleting SOCS3 specifically in muscle improves insulin signalling and insulin-stimulated glucose disposal during feeding with a high-fat diet¹⁰⁷. On the other hand, whole-body deletion of SOCS1 showed a trend towards improved glucose tolerance and was not protective against insulin resistance induced by a high-fat diet¹⁰⁸. The latter studies carry the caveat that *Socs1*^{-/-} mice die within three weeks of birth, and these studies were therefore carried out in mice with a double knockout of *Socs1* and a second gene, *Rag2*, which survive to adulthood¹⁰⁹. On balance, most studies indicate a role for SOCS proteins in the termination of insulin signalling, and further investigation will clarify the pathophysiological effects of this pathway.

An interesting counterpoint to these inhibitory pseudosubstrates is provided by SH2B1, which instead enhances insulin signalling^{83,110}. This protein binds IR phosphotyrosine residues through its SH2 domain, thus protecting these IR residues from dephosphorylation. However, SH2B1 does not block the active site of the IR kinase, thus potentiating insulin signalling^{83,110} (FIG. 3). The role of this protein in insulin signalling *in vivo* has been confirmed by studies in flies and mice deficient in SH2B1, which demonstrate impaired glucose metabolism and blunted insulin signalling in muscle, liver and fat^{111,112}. Notably, the mouse studies were carried out in mice that lack SH2B1 in peripheral tissues but retain its expression in neurons, as this protein also impacts body weight by modulating leptin

signalling^{83,110}. These pathways are validated by human genetic studies showing associations of SH2B1 polymorphisms with obesity and insulin resistance^{113–120}, although some findings indicate the gene is not directly linked with abnormal glucose homeostasis¹²¹.

Isoform specificity

Studies in humans, mice, and cell culture indicate that individual isoforms of the proteins involved in insulin signalling — and termination of insulin signalling — function distinctly. These findings support a combinatorial model of insulin signalling, whereby a single hormone can lead to a remarkable spectrum of consequences. This model has been previously reviewed¹⁰, and here, we highlight new findings.

IR

The IR itself has two isoforms, A and B. The latter is generated by alternative splicing and contains an additional exon encoding 11 amino acids¹²². IR-A is notable because it has comparable affinities for insulin and IGF-II. There appear to be small differences in the signalling properties of the two isoforms, such that IR-A is stronger than IR-B in its metabolic effects¹²³ and in promoting glycogen synthesis¹²⁴. In our view, IR-A functions primarily as a growth-promoting isoform during foetal life, whereas the apparent lower potency of IR-B, the predominant isoform in adult liver¹²⁵, is intended to reduce hepatocyte sensitivity to insulin, in view of the fact that liver cells are exposed to the high insulin concentrations of the portal vein.

IRS

There are four isoforms of IRS in mammals, although humans lack IRS3 (REF. 126). The isoforms differ with respect to tissue distribution and ability to engage a diverse subset of cellular substrates. Thus, the pleiotropy of insulin action can be explained by the ability of these adaptors to engage different cellular pathways. IRS1 and IRS2 are the main isoforms involved in metabolic homeostasis¹⁸. In the liver, ablation of IRS1 and IRS2 (REFS 127,128) phenocopies IR knockout, providing evidence that they are the main transducers of the insulin signal¹²⁹. Likewise, IRS1 and IRS2 double knockout in muscle impairs cellular metabolism as well as muscle growth¹³⁰, similar to double knockouts of IR and IFG1R in muscle¹³¹. In contrast to the overlapping cellular functions of IRS1 and IRS2, there are differences in their *in vivo* functions. Generalized ablation of IRS1 results in growth retardation and skeletal muscle insulin resistance, whereas IRS2 knockouts have strain-specific β -cell failure¹⁰.

Intensive investigation of the differences in the functions of these two IRS isoforms in the liver has been carried out over the past decade. Hepatocyte-specific knockout of either IRS1 or IRS2 caused moderate defects in glucose metabolism, with some evidence suggesting IRS1 is slightly more valuable for maintaining hepatic metabolism^{127,128,132,133}. There may also be temporal (fasting versus postprandial)¹²⁸ or spatial (pericentral versus periportal)¹³⁴ elements that lend specificity to IRS pathways, and those will be discussed in depth below.

AKT

There are three isoforms of AKT (1–3), and each may have some distinct effects^{135–138}. Although the two major forms of AKT (1 and 2) are not redundant *in vivo* — as shown by the relevant knockouts — in cells that express both, there appear to be two factors determining the specificity of insulin signalling: their relative abundance as well as their intracellular localization. A body of evidence has suggested that AKT2 is the most important isoform for glucose homeostasis, whereas AKT1 is important for growth and AKT3 for brain development¹³⁷. Indeed, mice lacking *Akt2* display impaired glucose homeostasis, while those lacking *Akt1* or *Akt3* do not¹³⁷. This was supported by *in vitro* evidence demonstrating a specific mechanism whereby AKT2 is specifically capable of promoting GLUT4 translocation, because it is enriched at the plasma membrane in response to insulin, an effect that is not seen for AKT1 (REFS^{139,140}). These findings emphasized the importance of the subcellular localization of insulin signalling components, and in-depth discussion about the spatial organization is provided below. However, advancements in research tools have allowed a reappraisal of the selectivity of AKT2 in glucose metabolism¹⁴¹. New data demonstrate that GLUT4 translocation is maintained after selective, acute inhibition of the kinase activity of either AKT1 or AKT2 (REF.¹⁴²). This suggests that either isoform is sufficient and that prior results were due to effects of long-term AKT2 depletion, independent of catalytic activity. On the other hand, there was a specific requirement for AKT1 in adipogenesis¹⁴², thus confirming that isoform specificity is important for some AKT functions.

Temporal regulation

Individual cellular effects of insulin occur with distinct temporal dynamics. These different dynamics depend on the kinetics of insulin secretion, the dynamic expression of insulin signalling proteins, the kinetics of kinase activities downstream of IR and the sensitivity of transcriptional mediators to insulin signalling. Therefore, different biological effects of IR signalling occur at different times after initiation of insulin signalling.

Dynamics of insulin secretion

Although the pattern of insulin secretion falls outside the scope of this review, IR signalling cannot be properly understood without considering that different biological responses by different insulin target cells are also the result of how insulin is released into the bloodstream¹⁴³. In the post-absorptive state, insulin is released — like most hormones — in pulses¹⁴⁴. In this state, insulin is important for maintaining active protein synthesis, preventing protein degradation and suppressing free fatty acid secretion, which might adversely affect hepatic glucose production¹⁴⁵. In anticipation of (cephalic phase) and in response to a meal, insulin is secreted in characteristic multi-phasic patterns. Glucose uptake in muscle and fat is likely the earliest response to a rise in plasma insulin, followed by suppression of hepatic glucose production and stimulation of lipogenesis. These dynamics become profoundly altered in insulin resistance and diabetes.

Abundance of insulin signalling proteins

Insulin exerts homeostatic control over its own signalling pathway, turning it down when it is less needed (fasting), and amplifying it in response to nutrient influx. The IRS proteins provide an example. While IRS1 is expressed at a relatively consistent level, IRS2 is most abundant during fasting — because of transcriptional activation by FOXOs — and its levels are decreased by insulin^{146–148}. The importance of this *in vivo* was highlighted by studies showing that IRS2 carries out insulin signalling mainly during fasting and immediately after feeding, whereas IRS1 does so mainly in the fed state¹²⁸. Similarly, fasting induces FOXO expression in the liver, contributing to the termination of insulin action, while it decreases expression of the sterol regulatory element binding protein 1c (SREBP1c), resulting in inhibition of lipid synthesis.

Phosphorylation in response to insulin

The sequential phosphorylation of downstream targets is a cornerstone of insulin signalling (FIG. 4). The temporal dynamics of these sequences lends precision to phosphorylation-dependent protein interactions¹⁴⁹. Thus, the signalling to entire networks downstream of insulin is conceivably determined by the timing of such phosphorylation events¹⁵⁰.

Phosphoproteomic analyses have demonstrated tyrosine phosphorylation of dozens of proteins in response to insulin^{151–154}. As expected, tyrosine phosphorylation of IR and IRS proteins occurs within 1 minute of insulin treatment. Other downstream targets cluster into subcategories in which tyrosine phosphorylation is delayed, even by up to 45 minutes. Likewise, many sites remain phosphorylated over long periods, whereas others are transient. IRS1 provides an interesting example, as it has multiple phosphotyrosine sites, which display a range of different temporal patterns^{153,154}. Furthermore, there appear to be cell type-specific differences in the timing of IRS1 tyrosine phosphorylation^{151–154}.

In contrast to phosphotyrosine sites, the vast majority of insulin-regulated phosphorylation events are on serine residues. In 3T3-L1 adipocytes, the percentages have been quantified as 87.8% phosphoserine, 11.4% phosphothreonine and 0.8% phosphotyrosine¹⁵³. The serine and threonine sites regulated by insulin number in the thousands, and they can be subdivided into multiple clusters based on shared kinetics. For example, phosphorylation of Akt Thr308 occurs within 15 seconds and stabilizes, whereas that of Ser473 follows, peaking after one or more minutes^{153,154}. These specific temporal patterns of phosphorylation may underlie the differential responsiveness of each insulin signalling pathway.

Glucose uptake

The stimulation of cellular glucose uptake was one of the first recognized biological activities of insulin (FIG. 5a). Although insulin stimulates glucose uptake into many cell types, it has most pronounced effects on adipocytes and muscle cells, enhancing glucose uptake by approximately tenfold and fivefold, respectively¹⁵⁵. The phenomenon was first described in detail in studies of primary rat adipocytes. Insulin stimulated glucose uptake, reaching a plateau approximately ten times higher than that of unstimulated levels, within 15 minutes^{156,157}. The increased rate of glucose uptake was maintained with the continued presence of insulin, and it rapidly returned to pre-stimulated levels once insulin was

removed. Cells responded similarly to a second challenge of insulin. Once the glucose transporters had been identified, it could be shown that insulin controls glucose uptake by controlling the density of glucose transporters in the plasma membrane rather than by gating the transporters' activity^{158,159}. Adipocytes and myotubes express GLUT4, a member of the GLUT family of facilitative glucose transporters, and regulation of GLUT4 trafficking to and from the plasma membrane underlies insulin regulation of glucose uptake into these cells.

Insulin signal transduction intersects GLUT4 trafficking at a number of steps, including the biogenesis of vesicles that ferry GLUT4 to the plasma membrane, docking and fusion with the plasma membrane^{160,161}. These steps occur downstream of AKT activation. However, different effectors control different aspects of GLUT4 trafficking. The best described AKT substrate required for GLUT4 translocation is TBC1 domain family member 4 (TBC1D4; also known as AS160), a RABGAP^{162,163}. AKT phosphorylation of TBC1D4 functionally inactivates its GTPase-activating protein (GAP) activity, resulting in the activation of RAB proteins targeted by TBC1D4 (REFS^{161,164,165}) (FIG. 5b). In muscle cells, the primary isoform is RAB8a¹⁶⁶. In fat cells, it is RAB10, which is responsible for about half of the effect of insulin on GLUT4 (REFS^{167,168}). RAB10 functions at a step before vesicle docking, and recent evidence suggests it is involved in the biogenesis of GLUT4-containing transport vesicles¹⁶⁹. Thus, insulin signalling to GLUT4 that is independent of TBC1D4–RAB10 likely targets vesicle docking and fusion. Those effectors are yet to be discovered. Recently, a role for TBK1 kinase regulation of the *exocyst* in the docking and/or fusion of GLUT4-containing vesicle with the plasma membrane has been reported¹⁷⁰.

Transcriptional effects of insulin

The effects of insulin are mediated by several transcription factors, including FOXO, SREBP1c, CREB-regulated transcription co-activator 2 (CRTC2) and CREB binding protein (CBP)/p300-interacting transactivator 2 (CITED2). *In vivo*, evidence for a role of FOXO and SREBP1c is compelling. During fasting, hepatic FOXOs promote the expression of glucose-6-phosphatase and suppress that of glucokinase, thereby promoting net glucose output^{171–174}. Inactivation of FOXOs by insulin reverses these effects (FIG. 5c). In addition, FOXOs mediate the effects of insulin on adipocyte differentiation, neuropeptide transcription and processing, and β -cell health^{175–179}. SREBP1c promotes expression of the enzymes involved in fatty acid synthesis^{180,181}. Unlike FOXO, SREBP1c is activated by insulin both transcriptionally and post-translationally, wherein SREBP1c is processed to its mature form and translocates to the nucleus^{182–185} (FIG. 5c). The pathways of insulin signalling that regulate FOXOs and SREBP1c diverge, such that SREBP1c regulation is downstream of mTOR¹⁸⁶, whereas FOXOs are direct targets of AKT^{187–189}.

There are also substantial temporal differences in the effects of insulin on FOXO compared with those on SREBP1c. FOXOs are exquisitely and immediately sensitive to insulin, reaching maximal phosphorylation in the liver within 30 seconds of insulin administration *in vivo* and within 1 minute of insulin treatment in 3T3-L1 adipocytes *in vitro*^{153,154}. In fact, the peak of FOXO phosphorylation precedes peak activation of AKT¹⁵⁴. Consequently, the effects of inactivating FOXO to reduce glucose-6-phosphatase and increase glucokinase

occur rapidly¹⁷⁴. Conversely, SREBP1c activation occurs later and requires higher insulin doses. The increases in SREBP1c mRNA and in the mature form of SREBP1c protein peak approximately 12 hours after feeding^{190,191}. Accordingly, the transcriptional targets of SREBP1c also increase several hours after feeding^{174,192,193}. The slow increase in SREBP1c levels is consistent with SREBP1c maturation being downstream of mTOR¹⁸², one of the slower targets of insulin to be activated (minutes)¹⁵³. Though considering that SREBP1c is slower yet (hours), the molecular links between mTOR and SREBP1c may lend additional temporal control. One such link is the phosphatidic acid phosphatase lipin 1, which reduces nuclear SREBP1c¹⁹⁴. mTORC1 phosphorylates lipin 1, leading to the nuclear exclusion of lipin 1 and the accumulation of nuclear SREBP1c (FIG. 5c). A second link is CRTC2, which competes with SREBP1c for binding to SEC31A, a component of the COPII complex¹⁹⁵. Phosphorylation of CRTC2 by mTOR causes it to dissociate from Sec31A, thus enabling the trafficking and activation of SREBP1c (FIG. 5c). Thus, in addition to the bifurcation of insulin signalling to these two transcription factors, the temporal regulation of these two pathways plays a crucial role in insulin action.

Subcellular spatial regulation

Components of the IR pathway must colocalize in order to transmit downstream signalling. Archetypical examples of this are the recruitment of IRS, PI3K, PDK1, AKT and their cognate phosphatases to the plasma membrane for activation and inactivation, respectively. However, downstream of AKT, there are multiple subcellular sites at which IR signalling targets localize (FIG. 5).

Trafficking vesicles

After ligand binding, IR is internalized via clathrin-coated vesicles. From their location in endosomes, the IR kinase remains active for signalling, and downstream targets have been found to colocalize to these early endosomes^{196,197}. Evidence suggests that endosomal IR signalling is important for mitogenic but not for metabolic effects of IR¹⁹⁶. However, because insulin is a weak mitogen, the extent to which the mitogenic effects are the result of IR signalling as opposed to IGF1 receptor signalling remains conjectural¹⁹⁸. However, a second aspect of IR trafficking in vesicles that can affect metabolic signalling is the fate of the IR for recycling versus degradation. Recently, it was shown that the protein SORLA (sorting-related receptor with type A repeats, encoded by *SORL1*) directs the recycling of internalized IR back to the cell surface, thereby enhancing IR signalling¹⁹⁹. This may provide a molecular mechanism for the association of SNPs near *SORL1* with obesity traits^{200,201}.

A canonical target of insulin signalling that requires spatial regulation in trafficking vesicles is GLUT4. In unstimulated cells, GLUT4 is sequestered intracellularly. Insulin signalling induces its redistribution to the plasma membrane, increasing glucose uptake^{158,159}. Upon cessation of signalling, GLUT4 is retrieved from the plasma membrane, returning to the intracellular storage sites, poised to be re-recruited to the plasma membrane by another round of insulin signalling^{157,158} (FIG. 5b). Thus, the control of glucose flux, in both unstimulated and insulin-stimulated fat and muscle cells, requires the activities of a large

number of proteins that make the GLUT4 transport vesicles and regulate the fusion of the vesicles with the plasma membrane. This is one of the best characterized examples of regulation of biological activity by controlled compartmentalization.

Insulin stimulation of glucose uptake is most pronounced in fat and muscle cells, although insulin promotes a small (less than twofold) uptake into many cell types. This specialization is fat and muscle cell dependent. Ectopic expression of GLUT4 in other cell types does not by itself recapitulate its insulin-dependent translocation to the plasma membrane characteristic of fat and muscle cells²⁰². Insulin does not modify GLUT4 directly; instead, it acts on the trafficking pathway that transports GLUT4 to the plasma membrane. The insulin-regulated aminopeptidase (IRAP) is also translocated to the plasma membrane, with GLUT4 (REF. ²⁰³). IRAP is a component of vesicles that ferry GLUT4 to the plasma membrane, but it does not have a major role in glucose homeostasis²⁰⁴. Moreover, GLUT4-containing vesicles have not been reported to contain soluble proteins that are secreted concurrent with GLUT4 insertion into the membrane, suggesting that insulin-regulated trafficking of membrane proteins is restricted to a remodelling of the plasma membrane rather than being coupled to adipokine secretion. Overall, insulin control of GLUT4 translocation is a prime example of the regulation of insulin effects through compartmentalization, as well as of tyrosine kinase-dependent regulation of membrane trafficking.

Nuclear actions of insulin

The discovery that insulin causes the AKT-dependent nuclear exclusion of FOXOs shifted the focus on insulin signalling from the membrane to the nucleus. This mechanism is neither unique to FOXO nor unidirectional. The insulin-stimulated translocation of mature SREBP1c to the nucleus is one example. Another is the CREB co-activator CRTC2, which is phosphorylated by SIK2, an AKT substrate, and excluded from the nucleus^{205,206} (FIG. 5c).

FOXO subcellular localization is regulated by other signalling pathways that either function in conjunction with insulin to promote the nuclear exclusion of FOXOs, or counteract it. Fasting and glucagon²⁰⁷, cytokine- and other stress-activated pathways^{208,209} promote the import of FOXOS into the nucleus from the cytoplasm. Moreover, other nutritional cues can complement — or compete with — insulin and glucagon to influence FOXO localization through mechanisms other than phosphorylation. Oxidative stress causes FOXO deacetylation, which promotes nuclear retention and activation of FOXOs but also promotes rapid ubiquitylation and degradation^{179,210}. On the other hand, acetylation sensitizes FOXOs to insulin-induced phosphorylation while increasing stability^{179,211}. These modifications influence FOXO activity in multiple cell types including β -cells, hepatocytes, macrophages and endothelial cells^{179,212–214}.

Lysosome

The lysosome, where mTORC1 is activated²¹⁵, has emerged as an important site of cellular insulin signalling (FIG. 5d). mTORC1 translocates to the lysosome in response to amino acids, a process that is mediated by a protein complex termed the Ragulator²¹⁶. At the lysosomal membrane, mTORC1 interacts with its activator, RHEB. However, mTORC1

activation by RHEB only proceeds if insulin signalling is also activated. This is because in the absence of insulin signalling, RHEB is kept in its GDP-bound, inactive state by the GTPase-activating TSC protein complex²¹⁷. Following insulin stimulation, AKT phosphorylates and inactivates TSC2 (REFS^{218,219}). Recent work demonstrates that AKT-mediated phosphorylation causes TSC2 to dissociate from the lysosome²²⁰. This stabilizes the GTP-bound, active form of RHEB, thus activating mTORC1. These findings demonstrate spatial coordination of multiple signalling proteins at the lysosome as a mechanism to tightly control mTORC1 activation in response to both hormonal (insulin) and nutritional (amino acid) cues.

Regulation at endoplasmic reticulum–mitochondria contact sites

Mitochondria-associated endoplasmic reticulum membranes (MAMs) are endoplasmic reticulum membrane subdomains that interact physically with mitochondria. This juxtaposition facilitates the transfer of calcium and lipids between the two organelles and is thus considered a site where signalling pathways — particularly those relying on calcium flux — intersect with energy and lipid metabolism pathways²²¹.

Recent evidence suggests that MAMs are an important target site for insulin signalling²²². AKT and mTORC2 — the kinase complex that is responsible for carrying out the Ser473 phosphorylation of AKT — are localized to MAMs, and this localization is increased with insulin signalling^{223–226}. Evidence suggests that the localization of mTORC2 and of AKT-pS473 to MAMs contributes to the regulation of MAM function and calcium flux²²⁴. Conversely, the integrity of the MAMs contributes to insulin signalling, as disruption of MAMs dampens insulin signalling and impairs glucose homeostasis^{225,227}. Moreover, overabundance of MAMs worsens insulin signalling and glucose metabolism²²⁸. These findings indicate that the formation and number of MAMs both affect and are affected by insulin signalling in multiple ways.

Tissue microenvironment

In addition to the subcellular organization of IR targets, the spatial distribution of cells within a tissue can also impact insulin signalling (FIG. 5e). There are many neuronal, hormonal and metabolic signals that link distant tissues and affect insulin signalling, which are outside the scope of this review. Instead, this section will focus on the local effects of neighbouring cells within the same tissue. A seminal demonstration that neighbouring cells can affect insulin signalling is provided by the infiltration of macrophages into the adipose tissue during obesity, which can initiate inflammatory pathways that impair insulin signalling^{30–32}. Other cell–cell interactions that affect insulin signalling occur between the same or different cell types. This section highlights that insulin action in a given cell can be influenced by its spatial positioning in a tissue, via paracrine effects or differences in nutrient and/or hormone access.

Hepatic zonation

Hepatocytes are arranged in zones, described by their proximity to the portal vein (rich with nutrients and oxygen) or the central vein (relatively deprived of nutrients and oxygen). It has

long been known that certain metabolic pathways are preferentially localized to different zones, and interconnected pathways are localized together. Fatty acid oxidation and gluconeogenesis are localized to periportal hepatocytes, whereas glycolysis and fatty acid synthesis are localized to pericentral hepatocytes²²⁹. Recently, it was reported that while IR, IRS2 and AKT are equally distributed, IRS1 is twice as abundant in pericentral hepatocytes as in periportal hepatocytes¹³⁴. This may cause IRS1 to be preferentially involved in glycolytic and lipogenic pathways, potentially providing another layer of specificity for coordinated regulation of metabolic pathways.

Endothelial–parenchymal cell interactions

A neighbouring non-parenchymal cell type that can impact parenchymal cell insulin signalling is endothelial cells. One potential mechanism for this lies in their role in delivering insulin from plasma into the interstitium. This can occur through regulation of blood flow and through the transcytosis of insulin across the endothelium^{230,231}. The latter is thought to be rate-limiting for insulin delivery into tissues such as muscle and adipose tissue, where capillaries are lined with continuous endothelial cells²³⁰. However, the mechanisms of insulin delivery remain unclear. *In vitro* evidence suggests that the transcytosis is mediated by IR in endothelial cells^{232,233}. However, mice lacking IR specifically in endothelial cells maintain normal insulin sensitivity unless placed on a low-salt diet²³⁴. This finding is unexpected, but may be explained by the preservation of IGF1R in these mice. On the other hand, mice lacking IRS2 — or both IRS1 and IRS2 — in endothelial cells are insulin resistant, with impaired muscle glucose uptake²³⁵. This is due to poor insulin-stimulated capillary recruitment and insulin delivery to the interstitium, resulting in reduced AKT activation.

A second potential mechanism involves paracrine factors secreted from the endothelial cells that affect insulin signalling in parenchymal cells. Mice lacking FOXOs in endothelial cells display insulin resistance and impaired insulin signalling in hepatocytes²³⁶. This is associated with excessive release of nitric oxide from liver sinusoidal endothelial cells and concomitant tyrosine nitration of IR in hepatocytes. This highlights how insulin signalling in one cell type can influence insulin signalling in an adjacent cell type.

Other cell–cell interactions

Additional cell–cell interactions in the tissue microenvironment may also impact insulin signalling. One example is the unexpected finding that NOTCH receptors — best known for their role in development — are negative regulators of insulin signalling in mature cells^{237,238}. Notch receptors on the surface of a cell are activated by the binding of transmembrane protein ligands that are expressed on the surface of an adjacent cell²³⁹. This causes the intracellular domain of NOTCH to become cleaved and transported to the nucleus where it binds to the transcription factor recombining binding protein suppressor of hairless κ (RBPJ κ) and regulates gene expression. Mice that are heterozygous for NOTCH, or that lack RBPJ κ specifically in hepatocytes, have enhanced insulin signalling in liver including elevated AKT phosphorylation and improved suppression of hepatic glucose production²³⁷. This pathway is confirmed to be downstream of signalling from an adjacent cell, as expressing only the extracellular domain of NOTCH — which sequesters endogenous

ligands on adjacent cells — is sufficient to invoke the pathway and enhance AKT phosphorylation at Thr308 (REF. 238).

Conclusions and perspectives

As we approach the 100th anniversary of the discovery of insulin, the signalling pathways of this important hormone have largely been defined. Although there will always be gaps in our understanding, progress has been substantial. However, we should acknowledge the limits of a reductionist cellular and biochemical approach to insulin signalling. In the living organism, the integrated signals arising from insulin's pleiotropic actions in multiple tissues cannot be easily reconciled with intrinsic cellular pathways — to wit, the complexity of distinguishing between direct effects of insulin in the liver from indirect effects caused by substrate redistribution in other organs^{171,240}. A second conclusion relates to the clinical implications of these discoveries. The heterogeneity of insulin signalling has thus far defeated approaches to discover clinically useful insulin sensitizers: turning on the main path through IR, IRS, PI3K, AKT is fraught with risks, while turning off antagonistic signals, such as FOXO or the many serine/threonine kinase or phosphatases, appears unlikely to succeed in view of the built-in redundancy of these systems. Nonetheless, the body of knowledge developed in this field will probably contribute to the solution of the clinical problem of insulin resistance in its various clinical manifestations.

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Glossary

Anorexigenic

Appetite-suppressant

Paracrine

A mechanism by which neighbouring cells influence each other by way of a secreted factor

Diacylglycerol

A glycerol molecule with two fatty acid chains present in food or produced primarily in the liver, adipose tissue and intestine, diacylglycerol is best known for its signalling properties to activate protein kinase C but also functions as a precursor molecule in the synthesis of triglycerides and prostaglandins

Haploinsufficiency

A phenotype caused by a heterozygous loss-of-function mutation, or by a mutation partly affecting levels and/or activity of a gene product

Post-absorptive state

The state following food absorption by the gut, hence the fasting state

RABGAP

A protein activating the GTPase activity of RAB, a component of secretory vesicles involved in intracellular transport

Exocyst

An octamer assembled to promote intracellular vesicle transport

COPII complex

A complex of proteins assembled around coat protein II (COPII), required for anterograde transport from the endoplasmic reticulum to the Golgi

Transcytosis

The transport of proteins across the interior of a cell

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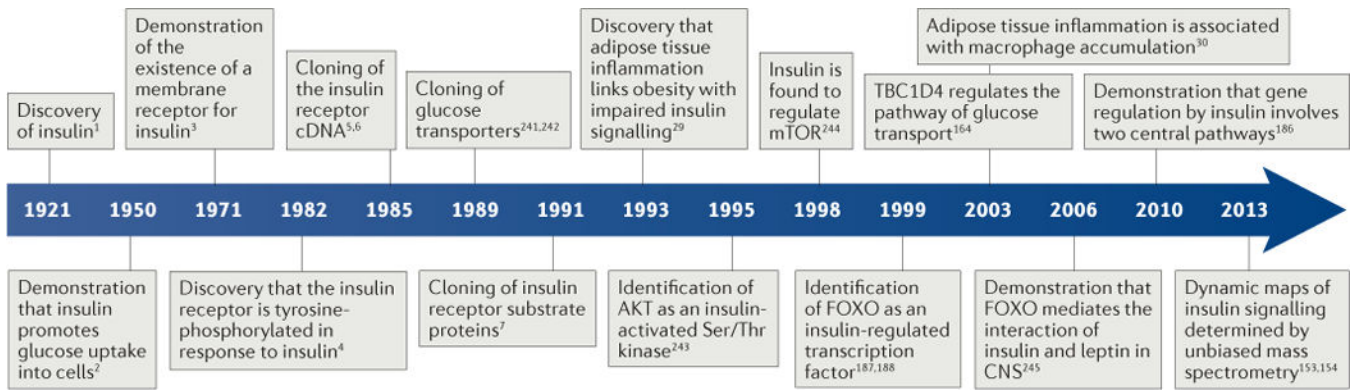


Figure 1. Timeline of the major discoveries in insulin signalling

CNS, central nervous system; FOXO, forkhead family box O; TBC1D4, TBC1 domain family member 4.

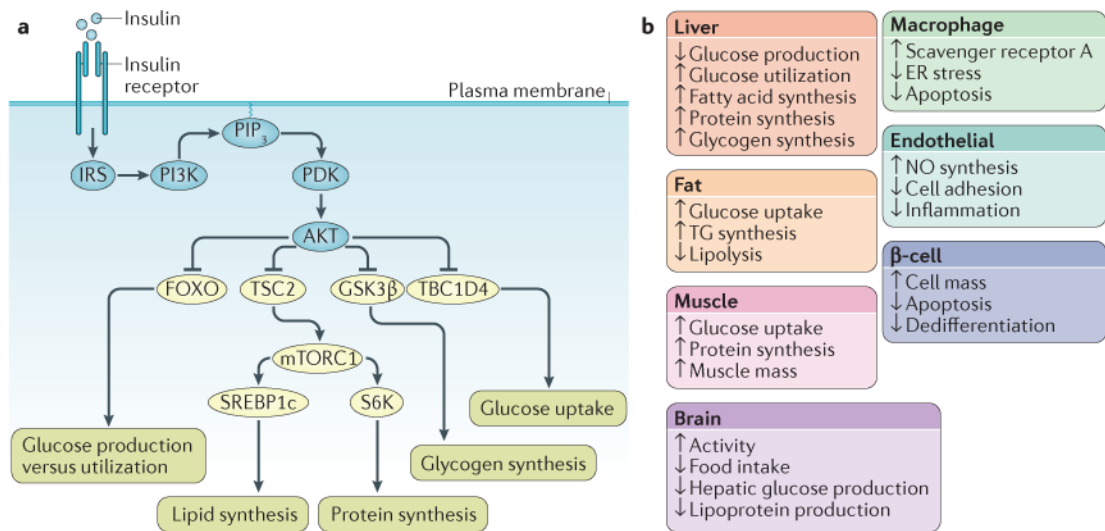


Figure 2. Activation of insulin signalling

a | Following insulin binding, the insulin receptor (IR) tyrosine kinase is activated, causing tyrosine phosphorylation of IR and of the IR substrate (IRS) proteins. Phosphotyrosine sites on IRS allow binding of the lipid kinase PI3K, which synthesizes PtdIns(3,4,5)P₃ (PIP₃) at the plasma membrane. This recruits the phosphoinositide-dependent kinase (PDK), which directly phosphorylates the Thr308 residue of AKT. A second phosphorylation of AKT, at the Ser473 residue, is carried out by mTOR complex 2 (mTORC2) (not shown). Activated AKT goes on to phosphorylate a number of substrates at Ser/Thr residues. These include: the forkhead family box O (FOXO) transcription factors; the protein tuberous sclerosis 2 (TSC2), which permits activation of mTORC1 and its downstream targets ribosomal protein S6 kinase (S6K) and sterol regulatory element binding protein 1c (SREBP1c); glycogen synthase kinase 3β (GSK3β) and the RabGAP TBC1 domain family member 4 (TBC1D4). These effector proteins mediate the effects of insulin on glucose production, utilization and uptake, as well as the synthesis of glycogen, protein and lipid. **b** | Effects of insulin signalling in various tissues and cell types. ER, endoplasmic reticulum; NO, nitric oxide; TG, triglyceride.

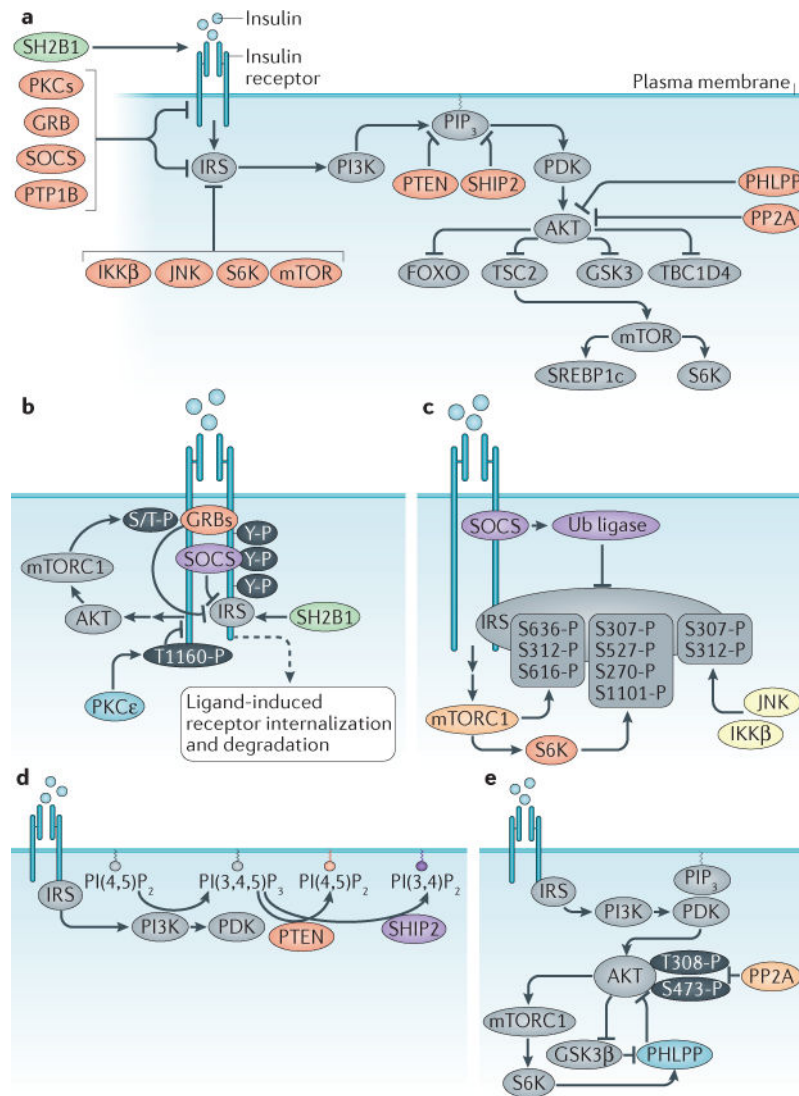


Figure 3. Modification of insulin signalling

Insulin signalling is antagonized through numerous mechanisms. **a** | Overview of regulatory mechanisms; these include ligand-induced insulin receptor (IR) internalization and degradation (not shown in this panel), inhibitory phosphorylation of IR substrate (IRS) (by kinases such as I κ B kinase β (IKK β), c-Jun N-terminal kinase (JNK), ribosomal protein S6 kinase (S6K) and mTOR), depletion of PtdIns(3,4,5)P₃ (PIP₃) by lipid phosphatases (PTEN and SHIP2), removal of activating phosphorylation groups by protein phosphatases (PTP1B, PHLPPs and PP2A), and inhibition by alternative substrates of the IR kinase (such as GRB10, GRB14 and SOCS proteins). In contrast, a different alternative substrate of IR (SH2B1) potentiates insulin signalling. Detailed mechanisms focused on different proteins of the insulin signalling cascade are shown in panels **b–d**. **b** | IR. GRB10, GRB14 and SOCS proteins are pseudosubstrates of the IR kinase that block IRS binding. The inhibitory activity of GRB10 is potentiated downstream of insulin signalling, due to mTOR complex 1 (mTORC1)mediated phosphorylation and stabilization. Protein kinase C ϵ (PKC ϵ) phosphorylates IR, thus blocking IR autophosphorylation. **c** | IRS proteins. SOCS proteins

antagonize insulin signalling in a second manner by recruiting a ubiquitin ligase (Ub ligase) that targets IRS proteins for degradation. Inhibitory Ser/Thr phosphorylation of IRS is carried out by multiple kinases, including mTORC1, S6K, JNK, and IKK β . **d** | PIP₃. The levels of this lipid mediator are reduced by lipid phosphatases PTEN and SHIP2. **e** | AKT. Dephosphorylation and inactivation of AKT is carried out by PP2A and PHLPP proteins. The latter are activated by AKT signalling itself, through mechanisms involving mTORC1 and glycogen synthase kinase 3 β (GSK3 β). GRB, growth factor receptor-bound protein; PHLPP, pleckstrin homology domain and leucine-rich repeat protein phosphatase; PP2A, protein phosphatase 2A; PTP1B, tyrosine-protein phosphatase non-receptor type 1B; SH2B1, SH2B adapter protein 1; SHIP2, Src homology 2 (SH2)-containing inositol 5'-phosphatase 2; SOCS, suppressor of cytokine signalling.

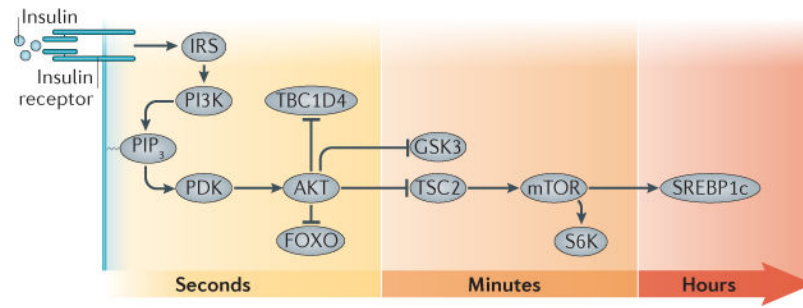


Figure 4. Temporal regulation of insulin signalling

The transmission of the insulin receptor (IR) signal to its various mediators occurs over varying lengths of time, from seconds (AKT, TBC1D4 and FOXO), to minutes (GSK3, TSC2, mTOR and S6K), to hours (SREBP1c). FOXO, forkhead family box O; GSK3, glycogen synthase kinase 3; IRS, IR substrate; PDK, phosphoinositide-dependent kinase; PIP₃, PtdIns(3,4,5)P₃; S6K, ribosomal protein S6 kinase; SREBP1c, sterol regulatory element binding protein 1c; TBC1D4, TBC1 domain family member 4; TSC2, tuberous sclerosis 2.

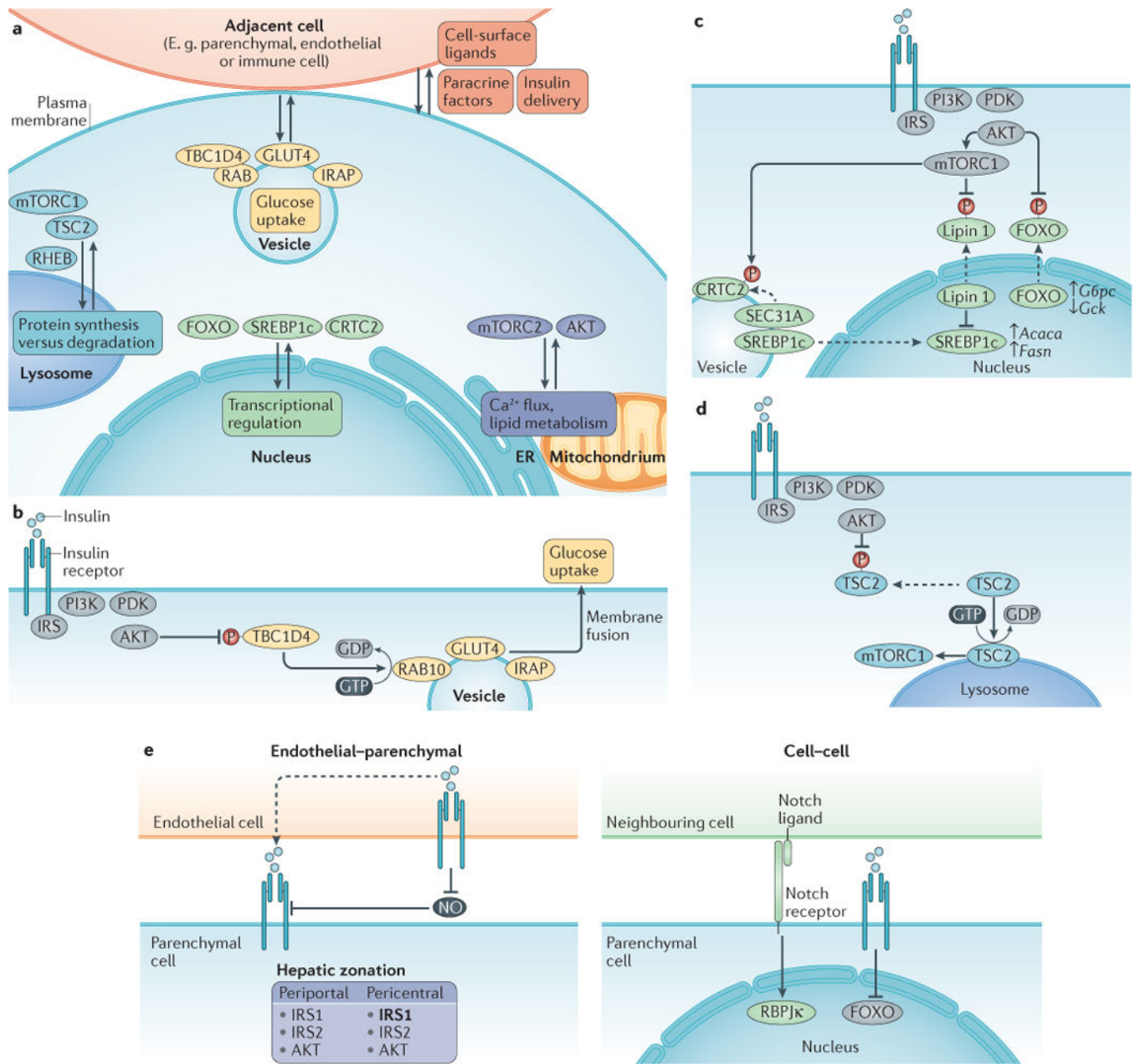


Figure 5. Spatial regulation of insulin signalling

a Overview of the compartmentalization of the downstream targets of insulin signalling is a mode of regulation. Recent findings have uncovered the importance of lysosomal localization for mTOR complex 1 (mTORC1) activation, the endoplasmic reticulum (ER) and/or mitochondria-associated ER membrane (MAM) for calcium flux and lipid metabolism, the nucleus for transcriptional control of metabolic processes and vesicle trafficking for the regulation of glucose uptake. Cell–cell interactions also contribute to insulin signalling regulation by modulating insulin delivery, paracrine factors and cell surface ligands. Detailed mechanisms focused on key targets of insulin signalling are shown in panels **b–d**. **b** | Glucose uptake. TBC1 domain family member 4 (TBC1D4) is a

RABGAP that typically stimulates the conversion of active RAB-GTP into inactive RAB-GDP. AKT phosphorylates and inactivates TBC1D4, which allows active RAB to promote the movement of GLUT4-containing vesicles to the cell surface. **c** | Transcriptional effects. Forkhead family box O (FOXO) transcription factors are phosphorylated by AKT, causing their nuclear exclusion and inactivation. Sterol regulatory element binding protein 1c (SREBP1c) is activated downstream of mTORC1 through two mechanisms. Nuclear lipin 1 causes nuclear remodelling that reduces nuclear SREBP1c; AKT phosphorylates and inhibits lipin 1. SEC31A is a vesicle transport protein that interacts with the transcription factor CREB-regulated co-activator 2 (CRTC2). After mTOR phosphorylates CRTC2, SEC31A becomes available to instead bind and transport SREBP1c to the nucleus. **d** | Lysosomal regulation of protein synthesis and degradation. TSC2 promotes the inactive, GDP-bound form of the lysosomally localized protein RHEB. AKT phosphorylates tuberous sclerosis 2 (TSC2), causing its dissociation from the lysosome, thus allowing RHEB to activate its target, mTORC1. **e** | Tissue microenvironment and cell–cell interactions. Endothelial cells influence parenchymal cell insulin signalling by physically delivering insulin through transcytosis and by suppressing the production of detrimental nitric oxide (NO). Insulin receptor substrate 1 (IRS1) is preferentially expressed in pericentral hepatic cells, as indicated in bold. Notch receptor is activated by a ligand expressed in an adjacent cell. It affects insulin signalling, as its target transcription factor recombining binding protein suppressor of hairless κ (RBPJ κ) interacts with FOXO proteins at gene promoters. IRAP, insulin-regulated aminopeptidase.