The role of ATF2 in insulin action

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1

Introduction and
Outline of the thesis

Chapter 1

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Regulation of glucose homeostasis

Glucose is the primary, and in the case of the brain, the essential source of energy for the cells in the body. The blood glucose level in the body is tightly regulated and maintained at approximately 5 mmol/l. Failure to maintain blood glucose in the normal range leads to chronically high (hyperglycemia) or low (hypoglycemia) glucose levels. In the absence of adequate treatment, hypoglycemia may result in lethargy, loss of consciousness and, in extreme cases, can lead to coma, brain damage and death. In case of persistent hyperglycemia, such as untreated diabetes mellitus, the high glucose level in the blood represents the main risk factor for development of diabetes-related complications, including retinopathy, nephropathy, diabetic neuropathy, and erectile dysfunction (1).

The blood glucose level is tightly controlled by the reciprocal actions of two hormones, insulin and glucagon. The peptide hormone insulin is produced in the pancreas in the β-cells of the islets of Langerhans. In response to high blood glucose levels, glucose enters the β-cells via the glucose transporter GLUT2. Within the β-cells, glucose is metabolized by glycolysis and citric acid cycle and converted into ATP via oxidative phosphorylation. The resulting increase in ATP-levels leads to closure of the ATP-dependent potassium channel at the cell surface and membrane depolarization. Upon membrane depolarization, the voltage-dependent calcium channel opens, calcium flows into the β-cell and triggers the secretion of insulin directly into the bloodstream. In the body, insulin exerts a pleiotropic and anabolic response, the most important effects being the suppression of endogenous glucose production by the liver, the stimulation of glucose uptake by skeletal muscle and white adipose tissue, the storage of glucose in the form of glycogen in liver and skeletal muscle, the stimulation of triglyceride synthesis and suppression of lipolysis in white adipose tissue and stimulation of amino acid uptake and protein synthesis. Glucagon, which is produced by the α-cells in the islets of Langerhans, counteracts the effects of insulin on glucose metabolism by stimulating the release of glucose from the liver via stimulation of hepatic gluconeogenesis and glycogenolysis.

Diabetes mellitus

Diabetes mellitus is a disease characterized by the inability to regulate blood glucose levels, resulting in chronically increased blood glucose levels, or ‘hyperglycemia’ (2). Multiple types of diabetes mellitus can be distinguished on the basis of the cause of the hyperglycemia, which either results from insufficient or even absence of insulin secretion by the β-cells, in combination with a suboptimal response of peripheral target tissues to insulin, a phenomenon referred to as insulin resistance.

In case of type 1 diabetes, dysregulation of the immune system results in immunological intolerance towards the insulin-producing β-cells. This leads to inflammation of the islets of Langerhans and selective destruction of the β-cells (3) Insulin synthesis and secretion are also affected in Maturity-onset diabetes of the young (MODY) (4) and Maternally inherited Diabetes and Deafness (MIDD), due to genetic factors impacting on β-cell development and mitochondrial function (5).

Chapter 1

Insulin resistance characterizes type 2 diabetes, the most prevalent type of diabetes, but is also found in gestational diabetes and steroid diabetes. In insulin resistance, the production of insulin is (initially) normal, but the response induced by insulin in peripheral tissues is blunted. When the production of insulin by the pancreas can no longer compensate for the peripheral insulin resistance due to β-cell dysfunction, the type 2 diabetes mellitus and hyperglycemia become overt.

Type 2 diabetes is often found as component of the metabolic syndrome, which is characterized by hypertension, central obesity, hyperlipidemia and insulin resistance that result in increased mortality due to cardiovascular incidents. The prevalence of both type 2 diabetes and the metabolic syndrome is reaching epidemic proportions, as the average age of onset of both diseases has markedly decreased over the past decades. In order to improve insulin action in patients with type 2 diabetes, a detailed understanding of the molecular mechanisms of insulin action and how this process is dysregulated under conditions of insulin resistance is required.

Mechanism of insulin action

When reaching its target tissues, the extra-cellular insulin signal is relayed via the insulin receptor at the cell surface and the associated post-receptor insulin signal transduction pathways. Figure 1 summarizes the key events in the transduction of the insulin signal into the cells.

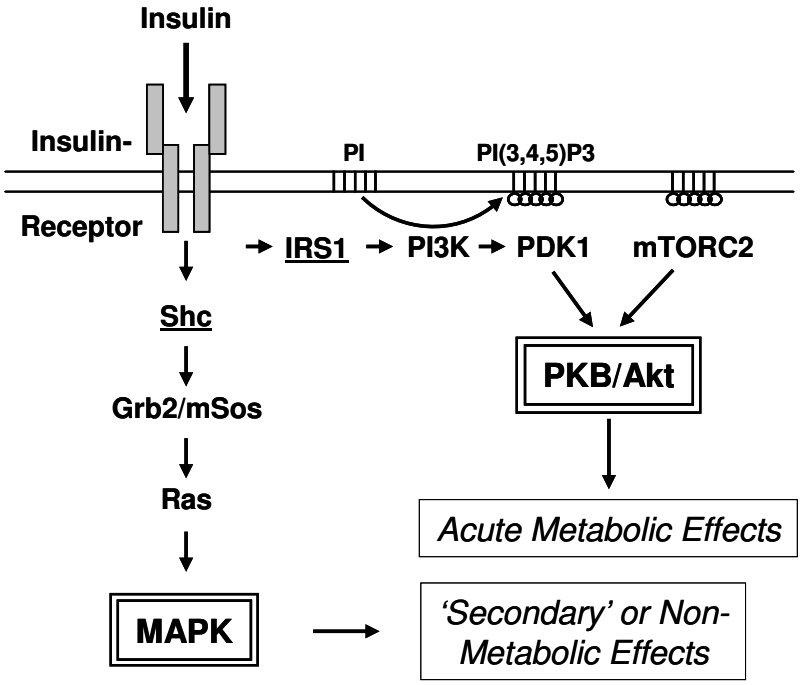

Figure 1. The two major insulin signaling pathways. For a detailed description see text.

The insulin receptor (IR) is a heterodimeric transmembrane protein, consisting of two extracellular α-chains responsible for insulin binding and two membrane spanning β-chains that contain intracellular tyrosine kinase domains. Insulin binds to the two α-chains of the IR on the outer surface of the plasma membrane. This interaction leads to a conformational change that induces activation of the intracellular kinase domains. These kinase domains subsequently trans-phosphorylate a number of tyrosine residues on the opposite β-chain (6). A subset of these phosphorylations stabilize the active conformation and further enhance the IR tyrosine kinase activity (amino acids (aa) 1146, 1150 and 1151), while other phospho-tyrosine (pY) residues (most notably aa 953, 960 and 972), function as docking sites for a number of IR substrates (7). Currently, over ten substrates of the IR have been identified, including isoforms of Src-homology-2-containing (Shc (8)), Grb2-associated binder 1 (Gab1 (9)), Cas-Br-M (murine) ecotropic retroviral transforming sequence homologue (Cbl (10)), the adaptor protein APS (11), and six members of the insulin receptor substrate (IRS1-6) family ((12-16), reviewed in (17)). The predominant substrates, however, are IRS1 and Shc. They define the two major insulin effector pathways: IRS1 and its downstream signaling pathway is responsible for most of the metabolic responses of insulin (18-20), while Shc regulates mostly non-metabolic processes induced by insulin, such as cell growth, survival and cellular differentiation (21). Both pathways will be discussed below.

IRS1 mediated signaling

The activated IR phosphorylates IRS1 on multiple tyrosine residues, which subsequently serve as docking sites for proteins containing Src-homology-2 (SH2) domains, the most important being the regulatory p85α subunit of class 1A phosphatidylinositol 3-kinase (PI-3K (18), reviewed in (22)). PI-3K consists of a p110 catalytic subunit and a p85 regulatory subunit. The binding of the p85 subunit via its two SH2-domains to pY residues on IRS1 leads to activation of the catalytic p110 subunit and recruitment of PI-3K to the plasma membrane. There, the p110 subunit catalyses the phosphorylation of specific phospholipids, phosphoinositides, on the 3-position to produce phosphatidylinositol-3-phosphates (PIP3), especially PI(3,4,5)P3. Signaling molecules that contain pleckstrin homology (PH) domains bind this type of lipid second messenger. The local insulin-induced increase in PIP3 results in the recruitment of the PH-domain containing kinases phosphoinositide-dependent kinase 1 (PDK1 (23)) and protein kinase B (PKB; also called Akt (24)) to the plasma membrane. PDK1 regulates the activity of members of the AGC family of protein kinases, which include protein kinase C (PKC), p70 ribosomal S6 kinase (p70S6K), serum glucocorticoid-induced kinase (SGK) and PKB/Akt, the latter being one of the most important signaling intermediates in metabolic insulin signaling. In case of PKB/Akt, binding to PIP3 facilitates the PDK1-mediated phosphorylation of Thr308, one of the sites critical for activation of the protein kinase (25). PIP3 is also required for phosphorylation of Ser473 on PKB/Akt by the mTORC2 complex, consisting of the protein kinase mammalian target of rapamycin (mTOR) bound to a regulatory subunit, known as rapamycin-insensitive companion of mTOR (rictor (26;27)).

PKB/Akt, when phosphorylated on Thr308 and Ser473 is active and directly regulates a number of multiple intracellular substrates important for glucose, protein and fat metabolism (see Figure 2, reviewed in (28) and (29)). For example, PKB/Akt regulates the activity of AS160 involved in translocation of glucose transporters (GLUT4) to the plasma membrane. In addition, PKB inhibits the enzyme glycogen synthase kinase 3 (GSK3),

thereby alleviating the repression of glycogen synthase (GS) and stimulating glycogen synthesis. PKB affects protein synthesis via phosphorylation of tuberous sclerosis complex 2 (TSC2; reviewed in (30)). This phosphorylation inhibits TSC2 activity. In complex with TSC1, TSC2 negatively regulates mTOR. Thus, the inhibition of TSC2 by PKB efficiently activates mTOR. As part of a larger protein complex activated mTOR then regulates protein synthesis by phosphorylating p70 S6K and eukaryotic translation initiation factor 4E binding protein-1 (4EBP1). PKB also regulates the expression of gluconeogenic and lipogenic enzymes by controlling the activity of several members of the forkhead box (FOXO) family of transcription factors (31;32). Hepatic gluconeogenesis is regulated via forkhead box other-1 (FOXO1), which activates gluconeogenic genes. FOXO1 is phosphorylated by PKB/Akt and subsequently exported from the nucleus, whereby transcription of gluconeogenic genes is terminated (33). The same principle applies to another forkhead box transcription factor, FOXA2, which is a crucial regulator of fasting lipid metabolism. PKB-mediated phosphorylation of FOXA2 prevents its nuclear localization and transcriptional activity (34).

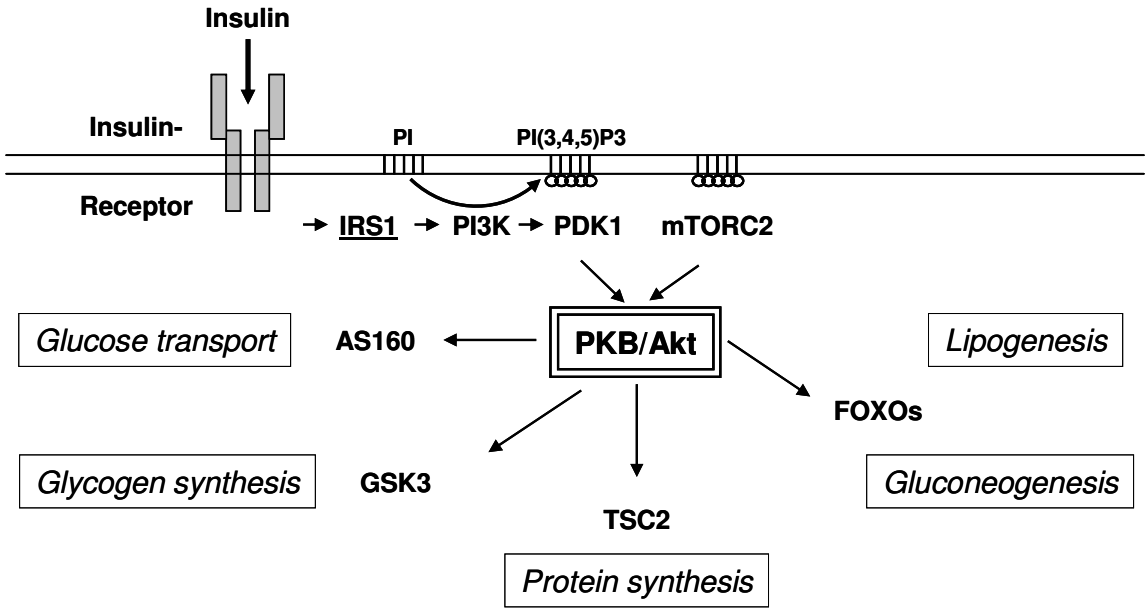

Figure 2. The IRS-dependent insulin signaling pathway. For a detailed description see text.

Shc-mediated signaling

The other major IR-pathway signals via Shc ((17); Figure 3). The activated IR phosphorylates Shc on tyrosines, which facilitates the binding of the adaptor protein growth factor receptor bound 2 (Grb2) via its SH2-domain. Via its SH3 domain, Grb2 is bound to the nucleotide exchange factor mammalian son-of sevenless (mSos). The recruitment of Grb-2-mSos to receptor-associated Shc brings mSos in the vicinity of the small GTP-binding protein Ras that is localized at the plasma membrane. mSos activates Ras by exchanging Ras-bound GDP for GTP. Ras then functions as a molecular switch triggering activation of multiple effectors, including PI-3K, Raf and RalGDS.

The p110 subunit of PI-3K has been shown to interact with active Ras (35) and this interaction modestly increases the PI-3K activity (36). However, in cells expressing

normal untransformed Ras, the contribution of mitogen-induced Ras-dependent PI-3K activity to the total PI-3K activity is only minor (24;37). Raf-activation is complex (for a detailed review see (38)), but Ras-dependent recruitment of Raf to the plasma-membrane seems sufficient for its activation. The active Raf kinase then triggers a kinase cascade that results in the phosphorylation and activation of MAPK and ERK kinase (MEK1/2). Subsequently, MEK1/2 activates the mitogen activated protein kinase (MAPK) family member extracellular signal regulated kinase (ERK1/2) via phosphorylation. ERK1/2 targets include p90 ribosomal protein S6 kinase (p90RSK) and transcription factors such as c-Myc, TCF and Elk1, thereby promoting gene expression (39;40). It has been shown that ERK does not play a role in mediating the acute metabolic effects of insulin (41;42). However its role in the regulation of insulin-induced gene expression has not been thoroughly investigated.

Insulin-induced Ras activation also leads to the activation of Ras-like small GTPase Ral via the Ral-guanine exchange factor Ral-GDS. Via still unknown mechanisms, presumably involving Src kinase, Ral then induces activation of the stress-activated protein kinases (SAPKs) p38 and JNK (43;44). Targets of these kinases include ATF2 and the members of the Jun transcription factor family, in addition to SAP-1, Elk1 and MAPKAPK-2 and -3 (40;45;46). The insulin-induced activation of p38 has further been described to play a role in insulin-induced glucose transport in a number of differentiated cell types (47;48). The role of JNK-activation in insulin-induced responses is still largely unknown.

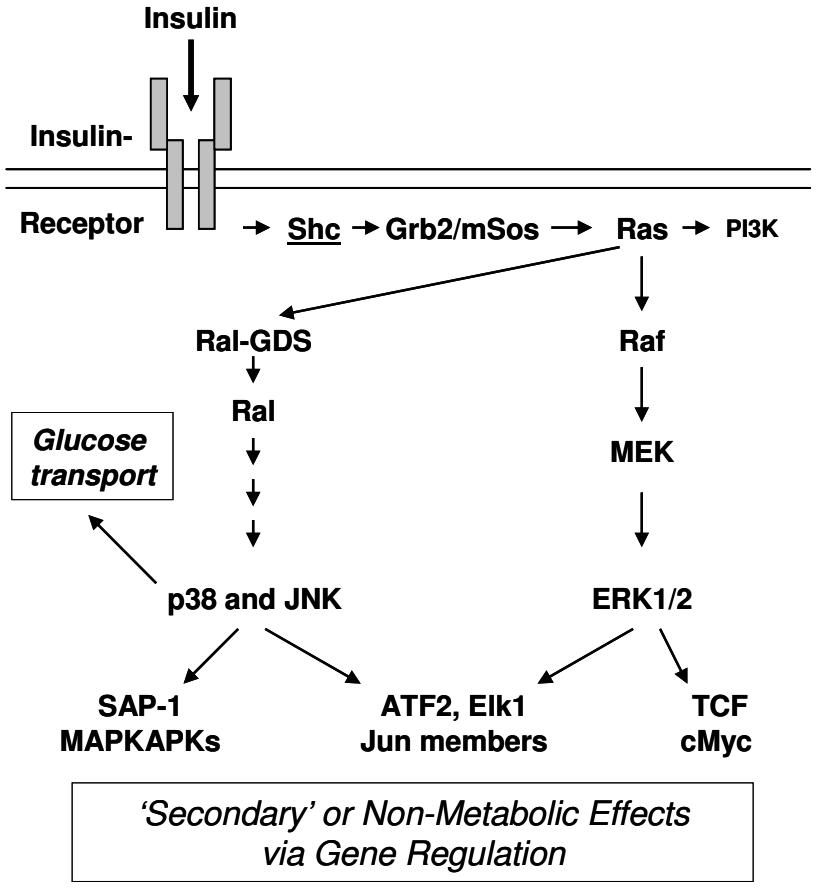

Figure 3. The Shc-dependent insulin signaling pathway. For a detailed description see text.

Previous studies performed in our research group identified ATF2 as a novel component of the insulin signaling system in cultured cells (44). The ATF2-phosphorylation in response to insulin was found to be dependent on a two-step mechanism which required cooperation of the ERK1/2-pathway with one of the SAPK-pathways ((44) and this thesis).

The SAPKs p38 and JNK, which are both capable of activating ATF2 on their own (e.g. not in cooperation with other kinases) are known to be activated by insulin stimulation (47;49). However, increasing evidence suggests that JNK, but also p38, play a key role in the development of insulin resistance in a number of tissues (50-53). Therefore, ATF2 can function as a potential regulator of insulin-induced gene expression, but can also be involved in development of insulin resistance and possible, it can do both.

Outline of this thesis

The research described in this thesis is aimed at further characterization of the role of ATF2 in insulin action. Chapter 2 is an introduction to the ATF2 protein, with particular focus on its possible functions in metabolic control and insulin action. Chapters 3 and 4 address the mechanism of insulin-induced ATF2 phosphorylation in JNK-deficient and JNK-containing cultured cells, respectively. In chapter 5, data on the identification of insulin-induced ATF2-dependent genes in cultured cells is presented. Chapter 6 describes our findings on the *in vivo* ATF2 regulation by insulin and the effects of high fat diet-induced insulin resistance thereon. In chapter 7 these results are summarized and discussed.

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