Molecular and Cellular Endocrinology 297 (2009) 41–49

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

The AMP-regulated kinase family: Enigmatic targets for diabetes therapy

Guy A. Rutter[a,*], Isabelle Leclerc[b]

[a] Section of Cell Biology, Division of Medicine, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, Exhibition Road, South Kensington, London SW7 2AZ, United Kingdom
[b] Section of Endocrinology and Metabolic Medicine, Division of Medicine, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, Exhibition Road, South Kensington, London SW7 2AZ, United Kingdom

ARTICLE INFO

Article history:
Received 28 April 2008
Received in revised form 12 May 2008
Accepted 28 May 2008

Keywords:
Glucose
Homeostasis
Diabetes
Insulin
Secretion
Hyperglycemia
Metformin
Thiazolidenedione
Kinase

ABSTRACT

AMP-activated protein kinase (AMPK) is a widely conserved Ser/Thr-specific protein kinase, homologous to *Saccharomyces cerevisiae* Snf1, and involved in nutrient sensing in lower organisms. In 2003, we reviewed the role of this enzyme in glucose homeostasis in mammals [Rutter, G.A., daSilvaXavier, G., Leclerc, I., 2003. Roles of 5′-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. Biochem. J. 375 (Pt 1), 1–16]. In the subsequent 5 years, dramatic strides have taken place in our understanding of the role of AMPK in the control of whole body metabolic homeostasis, the regulation of the enzyme by upstream kinases, and its molecular structure. These new studies and earlier work arguably propel AMPK, and perhaps related family members into a "super league" of potential therapeutic targets for maladies including diabetes, cancer, heart disease, and obesity. Here, we survey some of these recent advances, focussing on the role of this and related enzymes in the control of pancreatic β-cell function and glucose homeostasis.

Contents

1. Introduction

First described 35 years ago (Beg et al., 1973; Carlson and Kim, 1973), and named in 1987 (Carling et al., 1987), mammalian AMPK is homologous to the yeast sucrose non-fermenting (*Snf1*) gene product, involved in substrate selection in *Saccharomyces cerevisiae* (Celenza and Carlson, 1984), to the plant SNF-1 related kinase, SnRK (Alderson et al., 1991) and to *Caenorhabditis elegans* AAK-1 and AAK-2 (Apfeld et al., 2004). Considered principally as an "emergency response" enzyme in mammals, activated only during severe metabolic stress during which intracellular 5′AMP levels were raised as those of ATP fell, AMPK was substantially ignored by all but a very few groups for the next 20 years (Stapleton et al., 1996;

* Corresponding author. Tel.: +44 20 759 43340.
E-mail address: g.rutter@imperial.ac.uk (G.A. Rutter).
URL: http://www1.imperial.ac.uk/medicine/about/divisions/medicine/cellbiology/ (G.A. Rutter).

0303-7207/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2008.05.020

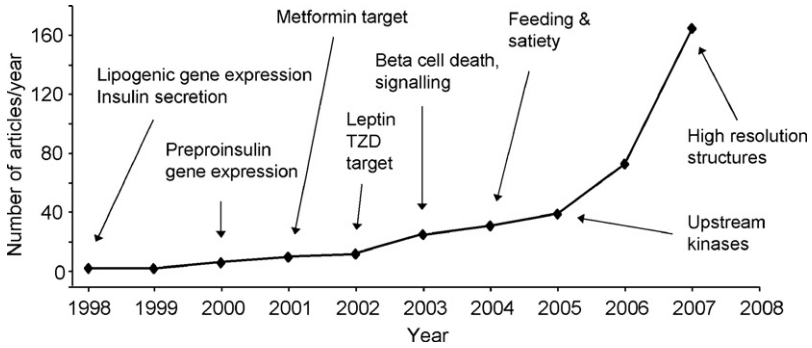

Fig. 1. Number of articles per year as reported by Pubmed and featuring "diabetes" and "AMPK" in the title or abstract.

Hardie et al., 1998). However, the cloning of the first mammalian catalytic subunit of the enzyme in 1994 (Beri et al., 1994; Carling et al., 1994) and the subsequent findings that AMPK may be a regulator of gene expression and a crucial target for antihyperglycemic agents, led to an explosion of interest in the enzyme in the last ~10 years as a potential therapeutic target for diabetes (Fig. 1) as well as for cancer (Luo et al., 2005) and obesity (Kola et al., 2006).

Whilst there is broad consensus that pharmacological activation of AMPK in liver and muscle may be a useful way of lowering blood glucose and lipid levels in diabetes (Long and Zierath, 2006), there is more debate as to how changes in the activity of this enzyme control fatty acid release from adipocytes, pancreatic β-cell function and hypothalamic satiety centres involved in the control of feeding. In the two latter cell types, changes in AMPK activity may be a crucial aspect of the normal signalling mechanisms through which nutrients including glucose are detected (Mountjoy and Rutter, 2007). We seek in this review both to outline the most exciting progress in understanding fundamental molecular aspects of AMPK biology and to address the latter, more controversial topics.

1.1. AMPK structure

As reviewed in detail elsewhere (Hardie, 2007), mammalian AMPK exists as a heterotrimeric complex of a catalytic, α-subunit (α1 or α2), a β-(scaffold) subunit (β1, β2 or β3) and a "regulatory" γ-subunit (γ1, γ2 or γ3). Recent X-ray diffraction studies on purified yeast (Townley and Shapiro, 2007) and mammalian (Xiao et al., 2007) core complexes have provided high-resolution structures and in the case of the mammalian enzyme (Xiao et al., 2007) have revealed that a phosphorylated residue in the α- or β-subunit binds preferentially to the AMP-complexed γ-subunit, likely both to increase the activity of the enzyme allosterically and to promote phosphorylation by upstream kinase(s) at the key regulatory site, Thr-172 of the α-subunit.

1.2. Upstream regulators

One of the most important breakthroughs in the past 5 years has been the molecular identification of the principal upstream kinases responsible for AMPK phosphorylation in mammalian and other cells (Witters et al., 2006) (Fig. 2). Whilst biochemical approaches, including many attempts at the purification of an "AMPK kinase" from mammalian cell homogenates were tried over the past decade, none were successful, though calmodulin kinase I was shown to have some limited capacity to phosphorylate the enzyme, then thought unlikely to be physiologically relevant (Corton et al., 1995). Identification of three *S. cerivisiae* kinases able to phosphorylate the yeast AMPK homologue Snf1, namely Pak1p, Tos3p and Elm1p (Hong et al., 2003), subsequently allowed the identification of the mammalian homologue LKB1 (also termed STK11, mutated in Peutz-Jeghers syndrome) (Jenne et al., 1998), in a complex with MO25 and STRAD (Hawley et al., 2003; Woods et al., 2003; Shaw et al., 2004) as a physiologically important upstream kinase, likely to mediate the effects of changes in ATP/AMP ratio. Suggestively, in the pancreatic β-cell (Leclerc and Rutter, 2004) (see below), increases in cytosolic free Ca^{2+} likely to activate calmodulin-dependent kinases also increased AMPK phosphorylation in the absence of an increase in AMP, thus hinting at a physiological role for Ca^{2+}-calmodulin-dependent kinases as "AMPKKs". Subsequently, CaMKKα and CaMKKβ (Hawley et al., 2005; Woods et al., 2005) were shown to play a key role, particularly in neuronal tissues. Very recently, a third upstream kinase, transforming growth factor-1β-activated kinase or TAK1, has been identified in yeast (Momcilovic et al., 2006) and appears to be important in cardiac cells (Xie et al., 2006), but the role of this kinase in other cell types is still largely undefined (see below).

1.3. Role of AMPK activation in insulin-target tissues

Activation of AMPK in extrapancreatic tissues by antihyperglycemic agents including the biguanide metformin, and thiazolidenediones such as rosiglitazone and pioglitazone, is likely to exert several beneficial effects on glucose homeostasis in type 2 diabetes (Long and Zierath, 2006). Whilst there is substantial evidence implicating AMPK activation by these agents, as

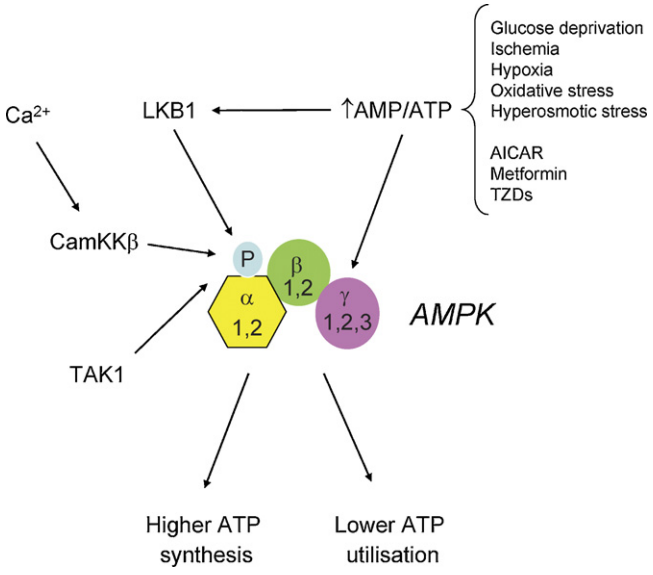

Fig. 2. Subunit structure and upstream regulators of AMPK.

well as during exercise, in the control of skeletal muscle glucose metabolism (Hardie, 2003) exciting recent advances have pointed to the importance of this enzyme in controlling glucose metabolism by the liver (Zhou et al., 2001), and have provided important new molecular insights.

Liver: The liver appears to be the principal site of action of the biguanides in man (Yu et al., 1999) and it is even conceivable that outside of the portal vein, systemic concentrations of metformin are sufficiently low (10–40 μM) (Wilcock and Bailey, 1990) to have little, or only a minor, effect on AMPK activity. By contrast, thiazolidendiones improve the insulin sensitivity of both the liver and extrapancreatic tissues including skeletal muscle (Yu et al., 1999). Studies with LKB1 knockout mice have revealed that metformin stimulates AMPK activity through LKB1-mediated phosphorylation (Shaw et al., 2005). Inhibition of gluconeogenesis is achieved at least to a large extent via the target of rapamycin2- (TORC2) and cyclic AMP-inducible factor CREB- (CRE binding protein) mediated action on the expression of phosho*enol*pyruvate carboxykinase and pyruvate carboxylase gene expression (Koo et al.,2005).

Skeletal muscle: Exercise-induced changes in intracellular ATP/AMP ratio, and possibly increases in free Ca^{2+} levels (see above), activate AMPK in skeletal muscle. In turn, and by mechanisms which have yet to be fully elucidated, this leads to the recruitment to the plasma membrane of the glucose transporter Glut4, through an insulin-independent process (KurthKraczek et al., 1999). Increased glucose uptake and enhanced glycolytic flux may then terminate the signal for transporter translocation by returning AMPK activity to prestimulatory levels.

Adipose tissue: In fat cells, activation of AMPK has been proposed to limit fatty acid efflux and to favour local fatty acid oxidation, thus lowering circulating free fatty acids levels (Daval et al., 2005). However, contrary findings (Moule and Denton, 1998; Yin et al., 2003) have suggested that β-adrenergic agonists act to increase the phosphorylation of AMPK at Thr-172 perhaps via cAMP-stimulated phosphorylation, an event which, from the use of a dominant-negative form of AMPK, implied that AMPK activation was required for maximal stimulation of lipolysis. In contrast to skeletal muscle, activation of AMPK has been reported to inhibit (Salt et al., 2000), to activate (Yamaguchi et al., 2005) or to have no effect on (Chavez et al., 2008) fat cell Glut4 translocation and glucose transport. The overall effects of AMPK activation on adipocyte glucose transport, lipolysis and lipogenesis thus remain to be defined categorically.

1.4. Role in the β-cell: control of secretion and cell survival

In 1998, Salt et al. showed that stimulation of AMPK with the 5-aminoimidazole-4-carboxamide-riboside (AICAR), an analogue of the non-phosphorylated precursor of AMP, ZMP, stimulated basal but inhibited glucose-stimulated insulin secretion (Salt et al., 1998). These findings were subsequently extended by ourselves in rodent β-cell lines as well as in rodent and human islets (daSilvaXavier et al., 2003; Leclerc et al., 2004c; Richards et al., 2006) using both AICAR and an adenovirus expressing a constitutively active form of AMPK (Woods et al., 2000) (Fig. 3); importantly, AMPK activity was shown to be inhibited as glucose concentrations rose over the physiological range in both MIN6 β-cells (daSilvaXavier et al., 2003) and in islets (Leclerc et al., 2004c) (Fig. 3). Which of the upstream AMPK kinases may play a role in β-cells remains to be established but the regulation of AMPK activity and phosphorylation by both glucose-dependent changes in AMP/ATP ratio (Salt et al., 1998; daSilvaXavier et al., 2000, 2003) and by depolarisation-stimulated increases in free Ca^{2+} concentration (Leclerc et al., 2004a) implies that both LKB1 and CaMKKα/β may be involved depending on the stimulus.

In 2000, we (daSilvaXavier et al., 2000) also demonstrated that AMPK activation, achieved using molecular means to over-express active or inactive (dominant-negative) forms of the enzyme, blocked the induction by glucose of the insulin and other glucose-regulated glycolytic genes (notably the liver-type pyruvate kinase gene). Subsequently, roles were demonstrated for AMPK in the control by glucose of PPARα gene expression in clonal INS-1E β-cells (Ravnskjaer et al., 2006), and several others as identified in a microarray study (Diraison et al., 2004). We later used the same molecular tools to demonstrate that AMPK activation suppressed glucose-induced insulin secretion (daSilvaXavier et al., 2003), in part by preventing the movement of secretory vesicles to the cell surface (Tsuboi et al., 2003), and by suppressing glucose induced increases in cytosolic-free ATP concentration and increases in intracellular-free Ca^{2+}. Importantly, AMPK activity and secretion were inversely correlated when changes in each parameter were measured in response to varying combinations of amino acids (Leclerc et al., 2004a). Broadly similar findings have been reported recently by others (Kim et al., 2008). Moreover, islets infected with an adenovirus expressing the constitutively active form of AMPK more poorly corrected streptozotocin-induced diabetes after transplantation into syngeneic mice, whilst dominant-negative AMPK-expressing islets achieved improved glycemic control compared to islets expressing an irrelevant gene (Richards et al., 2006). These differences seem likely to reflect both improved glucose stimulated insulin secretion, but also greater resistance to apoptosis of β-cells in which AMPK activity is reduced (Kefas et al., 2003b, 2004). On the other hand, in prediabetic Zucker diabetic fatty rats, stimulation of AMPK activity in both the periphery and in β-cells by systemic administration of AICAR prevented the development of hyperglycemia and preserved β-cell mass: however, treated animals displayed markedly improved insulin sensitivity and hence substantially decreased β-cell "stress" compared to controls (Pold et al., 2005). Activation of AMPK in isolated β-cells has been shown in several studies to be pro-apoptotic (Kefas et al., 2003a,b, 2004), acting in part via enhanced mitochondrial production of reactive oxygen species (ROS) (Cai et al., 2007; Riboulet-Chavey et al., 2008). This has been proposed (Cai et al., 2007) to be due to alterations in the $NADH/NAD^+$ couples and effects on the oxidation state of FMN in complex 1.

It should also be noted that active AMPK may also decrease cell survival by other means, including through the inhibition of proliferative pathways. Thus, AMPK directly phosphorylates the tuberous sclerosis complex 2 (TSC2) (Inoki et al., 2003). The TSC1–TSC2 complex acts as a GTP-activating protein (GAP) on the mammalian target of rapamycin mTOR activator, Rheb to transmit growth factor receptor tyrosine kinase-derived signals to the p70 S6 kinase (S6K) tumour suppressor (Inoki et al., 2003). The resulting inhibition of Rheb thus suppresses proliferative signals, favouring opposing, cell death pathways. Moreover, direct phosphorylation of the tumour suppressor p53 at Ser15 during persistent activation of AMPK leads to cellular senescence and eventually death (Jones et al., 2005). This may involve induction of $p21^{waf1/cip1}$, an inhibitor of cyclin-dependent kinases (CDKs). Of note, genes encoding both cell cycle regulators such as T-cell factor-7L2 (TCF7L2) (Grant et al., 2006), and CDK-associated proteins including CDKAL1 and CDKN2A and CDKN2B, are strongly associated with increased risk of type 2 diabetes (Sladek et al., 2007; Scott et al., 2007; Zeggini et al., 2007; Saxena et al., 2007), and appear to affect β-cell survival and or function. Interestingly, suppression of AMPK activity also reduced killing by proinflammatory cytokines and immunoreactive CD8+ T-cells (Riboulet-Chavey et al., 2008) suggesting that these agents may act at least in part during the immediate post-transplantation stage to destroy β-cells via AMPK activation.

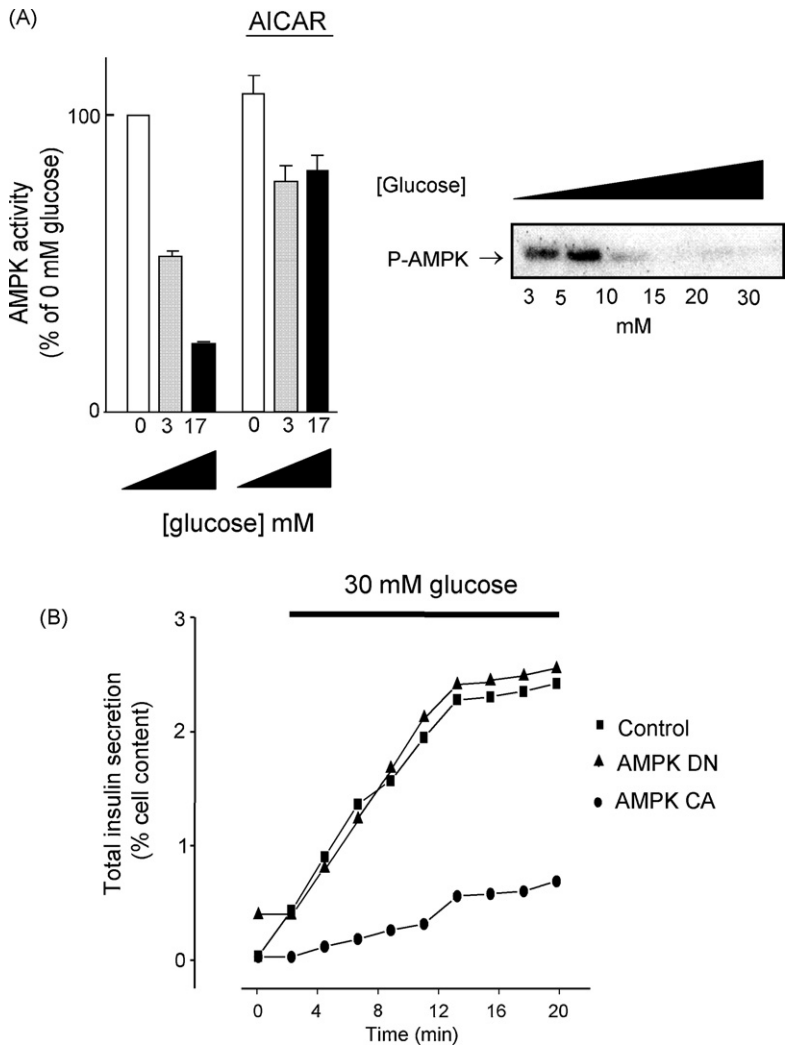

Fig. 3. Regulation of AMPK in β-cells by physiological changes in glucose concentration (A) and impact of adenovirus-mediated over-expression of dominant-negative (DN) or activated (CA) forms of AMPK on insulin secretion from MIN6 cells (B). Data from (daSilvaXavier et al., 2003) and (Tsuboi et al., 2003).

Clearly, then, activation of β-cell AMPK would seem to be contraindicated for the treatment of diabetes: note that, in contrast to approaches to β-cell "resting" such as the use of diazoxide (Hansen et al., 2004), AMPK activation in response to metabolic inhibition by metformin and similar agents, may also inhibit β-cell survival. Interestingly, troglitazone activates AMPK and inhibits insulin secretion from clonal MIN6 β-cells (Wang et al., 2007), whilst α-lipoic acid exerts the same effects on both islets and clonal cells, in part through mitochondrial depolarisation (Targonsky et al., 2006) and the production of reactive oxygen species. On the other hand, the effects of metformin on insulin secretion are more controversial; Marchetti and coworkers (Lupi et al., 1997) have provided evidence that insulin secretion is *stimulated* by metformin in islets from type 2 diabetics, perhaps reflecting respiratory chain inhibition and suppressed reactive oxygen species production. By contrast, our own findings using islets from both rodent and human (Leclerc et al., 2004b) demonstrated a clear activation of AMPK with metformin matched by a decrease in insulin secretion. It should be emphasised, however, that supra-physiological concentrations of metformin were used in the latter studies (see above for discussion of physiological metformin concentrations), given that the effects on intracellular metabolism are strongly time and concentration dependent (Owen et al., 2000).

It should also be mentioned that two other groups (Wang et al., 2005; Gleason et al., 2007) have provided evidence which argues against a role for AMPK in the control of insulin secretion. In the first of these studies, AICAR was shown to stimulate secretion from isolated islets; it must be stressed, however, that this riboside may have complex effects, including, after triple phosphorylation to generate an ATP analogue (ZTP), closure of ATP-sensitive K^+ channels, likely to activate insulin release. On the other hand, Gleason et al. (2007), using the strongly glucose-responsive INS-1(832/13) cell line, as well as MIN6 cells, demonstrated that expression of an inactivated form of AMPK ($\alpha 2K^{45}R$) exerted no effect on basal or glucose-stimulated insulin secretion, arguing against a controlling role for AMPK. Differences between the sublines of cells used, in the metabolism and transport of AICAR (particularly its conversion to more highly phosphorylated products which may close ATP-sensitive K^+ channels), and the use in the latter studies of a different dominant-negative form of AMPK with differing substrate selectively/specificity compared to the $\alpha 2D^{157}A$ used in our own studies, may be involved in these discrepancies: note that in neither of the latter studies was a constitutively active form of AMPK deployed. In our view, definitive answers will come only with the generation of mice expressing activated AMPK as a transgene selectively in the β-cell, or inactivated for *both* AMPK isoforms in these cells. (Note that mice inactivated for AMPKα2 throughout the body

show defective glucose tolerance as a result of altered sympathetic tone, but insulin secretion from isolated islets is normal (Viollet et al., 2003b); mice inactivated for AMPKα1 displayed no obvious defects in glucose homeostasis (Viollet et al., 2003a). Mice lacking both catalytic isoforms die *in utero*.)

1.5. AMPK isoform expression in β-cells

From the discussion above it follows that for AMPK to provide a useful therapeutic target, one would require that the patterns of isoform expression be distinct in β-cell type *versus* other cell types. Interestingly, AMPKα1 is the predominant catalytic isoform in MIN6 β-cells (daSilvaXavier et al., 2000) as well as in INS-1 β-cells and rat islets (Sun et al., 2008). This is a radically different scenario from that in insulin sensitive tissues such as skeletal muscle (Chen et al., 2000) and liver (Leclerc et al., 1998), where α2 subunits predominate, but similar to the situation in pulmonary artery smooth muscle and carotid body cells (Evans et al., 2005) and, less dramatically, adipose tissue (Daval et al., 2005). Pulmonary smooth muscle cells respond to hypoxia, and the conserved profile of AMPK catalytic subunit isoform expression in these cell types may reflect common signalling modalities in response to metabolic stresses. In MIN6 β-cells, α1 immunoreactivity was restricted to the cytosol, whereas α2 immunoreactivity was present in both the cytosol and nucleus, and blockade of α2 activity (but not α1) by antibody microinjection mimicked the effects of glucose to enhance transcription of the preproinsulin and other glucose-regulated genes (daSilvaXavier et al., 2000). These observations may be consistent with a role for the α1 isoform in controlling electrical activity or Ca^{2+} homeostasis in β-cell type as well as other cell types (though note that activation of AMPK is associated with decreased Ca^{2+} influx and insulin secretion in β-cells) (daSilvaXavier et al., 2003; Tsuboi et al., 2003). Of interest, leptin stimulates AMPK activity in skeletal muscle (Minokoshi and Kahn, 2003), expressing predominantly the α2 subunit, but neither this adipokine (Leclerc et al., 2004c) nor adiponectin (Okamoto et al., 2008) (GAR, Wheeler, MB, unpublished results) was reported to have any effect on AMPK activity in primary β-cells. However, and in contradictory findings, others have reported the activation of AMPK by adiponectin in MIN6 cells (Huypens et al., 2005) and in rat islets (Gu et al., 2006). The reason for this discrepancy remains unclear, but the complex behaviour of adiponectin in solution (i.e. the equilibrium between globular and fibrous forms) may well be involved. Whether the predominance of AMPK α1 *versus* AMPK α2 isoforms in β-cells is also related to the unusual metabolic configuration of these cells (Sekine et al., 1994; Liang et al., 1996; Schuit et al., 1997; Zhao and Rutter, 1998; Zhao et al., 2001), in which anaerobic glycolysis is "disallowed" thanks to the absence of detectable lactate dehydrogenase and lactate/monocarboxylate (MCT-1) transport activity (Otonkoski et al., 2007) remains to be examined.

Interestingly, culture with fatty acids (palmitate) has been reported to cause decreases in AMPKα1 expression and a fall in glucose-induced insulin secretion (Sun et al., 2008). By contrast, acute exposure to palmitate increased AMPK phosphorylation and insulin secretion from MIN6 cells in parallel (Wang et al., 2007). However, conclusions as to the relationship between AMPK activity and insulin secretion cannot be drawn easily from such experiments, given the likely lipotoxic effects of these agents in the longer term, and direct stimulatory effects as secretagogues in the short term.

1.6. AMPK homologues and the β-cell

Other, monomeric members of the AMPK family include QSK kinase, salt-induced kinase (SIK), Qin-induced kinase (QIK), microtubule-affinity-regulating kinase (MARK) 1, MARK2, MARK3/Par-1A/C-TAK [TGF (transforming growth factor)-β-activated kinase]/MAP3K7 1, MARK4, AMPK-related kinase 5 (NUAK1/ARK5), SNF1/AMPK-related kinase (NUAK2/SNARK), brain-specific kinase 1 (BRSK1/SAD-A), BRSK2/SAD-B and (sucrose-non-fermenting-related kinase (SNRK) are each targets for the upstream regulator LKB1 (Lizcano et al., 2004) (see below and Fig. 4). Whether these play any role in the β-cell is unclear. Interestingly, SNARK is regulated by AICAR in keratinocytes (Lefebvre et al., 2001) as well as INS-1 insulinoma (Lefebvre and Rosen, 2005) cells and is highly abundant in islets (L. Parton, GAR, unpublished); mRNA encoding ARK5 is also detectable but at substantially lower levels.

By contrast, per-arnt-sim domain or PAS kinase (also termed PASKIN) (Borter et al., 2007), is another Ser/Thr kinase, more distantly related to AMPK (it is phylogenetically closer to LKB1) (Fig. 4) that has been shown to have an important role in the β-cell, controlling preproinsulin gene expression (daSilvaXavier et al., 2004). PAS domains are widespread in the prokaryotic kingdom and act as sensors to detect metabolic changes (Ponting and Aravind, 1997; Gu et al., 2000; Rutter et al., 2001). PASK was rapidly activated in clonal β-cells and rat islets in response to elevated glucose concentrations both by reversible phosphorylation at Thr-152 in the "T-loop" (daSilvaXavier et al., 2004), and through enhanced expression of the *PASK* gene. Blockade of PASK function with a dominant-negative form of the enzyme, with short interfering RNAs or by antibody microinjection into single cells blocked the induction of preproinsulin and *pdx*-1 and other genes by glucose (daSilvaXavier et al., 2004). More recently, Jared Rutter and coworkers (Hao et al., 2007) demonstrated that islets from $PASK^{-/-}$ mice display defective glucose-stimulated insulin secretion although expression of preproinsulin was normal. The molecular mechanisms through which glucose regulates PASK activity, and by which activated PASK regulates β-cell function, remain undefined. However, these mice also displayed improved insulin sensitivity after a high-fat diet, demonstrating that lowered levels of PASK in peripheral tissues may be protective against insulin resistance. Whilst a recent report (Borter et al., 2007) has suggested that PASK/PASKIN expression is weak and insensitive to regulation by glucose in islets, our own unpublished screen of expression in islets from a variety of tissues, including human, demonstrate the clear presence of both mRNA and protein, albeit at levels which are substantially lower than those in haemopoetic cells or testis, the main sites of PASK expression (GAR, G. daSilvaXavier, unpublished).

1.7. Role of AMPK in other pancreatic islet cell types

The impact of changes in AMPK activity on the survival or function of other islet cell types is still largely unexplored, and represents an important area for future research. AMPK activity is present in clonal αTC1-9-cells and is stimulated at low glucose concentrations and by phenformin, but not by AICAR (Sun, G, IL and GAR, unpublished). All of the above changes are reversed by the AMPK inhibitor compound C. Contrary to the situation in β-cells, over-expression of dominant-negative AMPK *suppresses* glucagon secretion stimulated by low glucose concentrations (Leclerc et al., 2007).

1.8. Mechanisms of AMPK actions in the β-cell: downstream effectors

The identification of molecular targets for regulation by AMPK represents a challenging prospect not least since close to 30% of all gene products in the human genome contain consensus sites for phosphorylation by the enzyme (Rutter et al., 2003). At present,

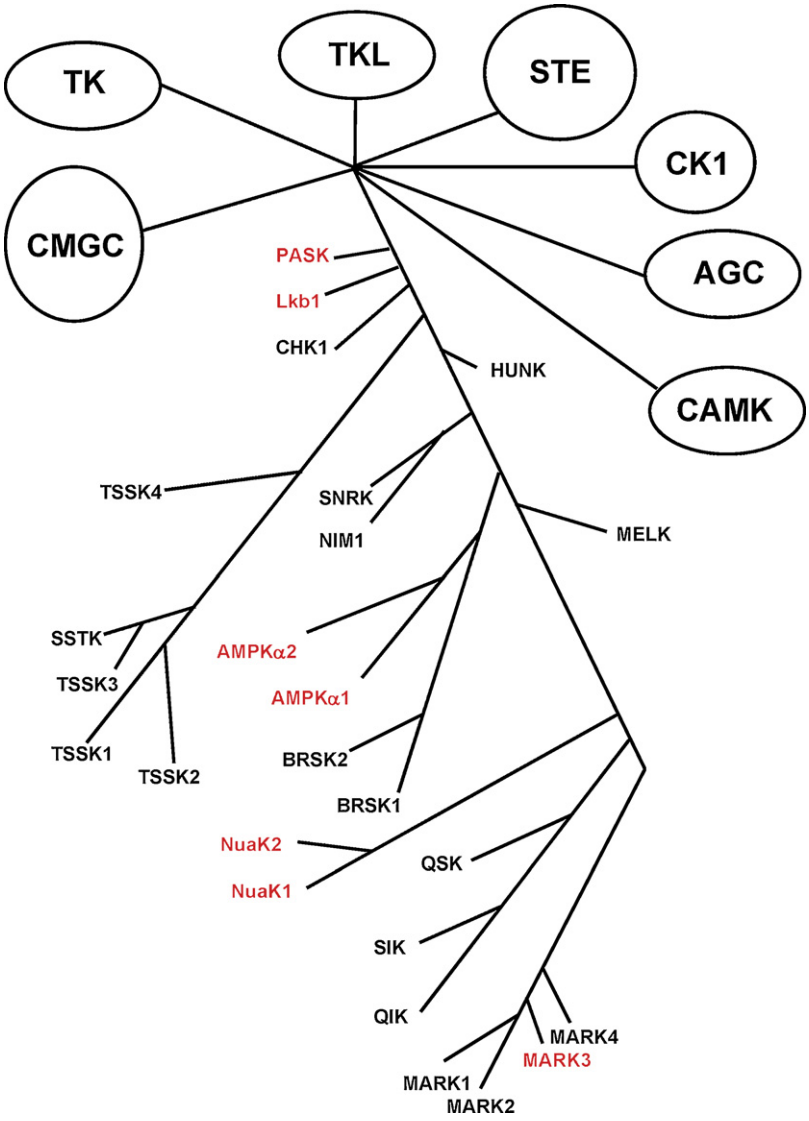

Fig. 4. Modified dendogram illustrating the position of AMPK and other family members in the protein "kinome". Family members with a demonstrated or suggested role in β-cell function are in red. Modified from (Manning et al., 2002). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the search for important targets has largely involved candidate gene approaches. Phosphorylation and inhibition of acetyl-CoA carboxylase-1 (ACC1), and the consequent decease in malonyl-CoA synthesis, represent one of the likeliest mechanisms through which active AMPK may exert its effects on the beta and other cells. Indeed, there is substantial evidence for a role for consequent changes in intracellular acyl-CoA levels (decreased when carnitine palmitoyl transferase I is deinhibited by a fall in malonyl-CoA, leading to enhanced mitochondrial β-oxidation; Corkey et al., 1989).

Phosphorylation of components of the cytoskeletal machinery involved in secretory granule translocation may also be involved. Thus, AMPK activation suppresses granule movement (Tsuboi et al., 2003) whilst exerting no discernible effect on vesicle fusion at the plasma membrane; an attractive potential target for AMPK-mediated phosphorylation is the microtubule and secretory granule-associated motor protein, kinesin-1 (Rutter and Hill, 2006). Such a hypothesis would be consistent with a role for the *Drosophila melonagaster* AMPK in the phosphorylation of the homologous myosin regulatory light chain 2 (MLC2) (Lee et al., 2007), and abnormalities in cell polarity and mitosis in AMPK-null flies, for the *C. elegans* AMPKα2 homologue AAK-2 in locomotion (Lee et al., 2008) and for mammalian AMPK in tight junction assembly and cell polarity (Zheng and Cantley, 2007).

Activated AMPK also suppresses glucose metabolism and the generation of Ca^{2+} signals, by as yet undefined mechanisms; note that this represents a very different role for AMPK in the β-cell type *versus* other (e.g. muscle) cell types where, in the latter case, AMPK activation is involved in *enhancing* glycolysis for ATP generation during anoxia (Hue et al., 2003). Whether AMPK phosphorylates ion channels such as the cystic fibrosis transconductance regulator, CFTR (Hallows et al., 2000, 2003a,b), the two pore calcium channel TASK (Wyatt et al., 2007) or large conductance Ca^{2+}-regulated K^+ channels (BKCa) remains to be explored, as does the role of AMPK in the control of intracellular calcium channels including inositol 1,4,5-*tris*phosphate and ryanodine receptors.

1.9. Role in the hypothalamus: control of food intake and body weight?

A role for AMPK in feeding centres of the ventromedial hypothalamus was first suggested by studies from Minokoshi et al. (2004) and Andersson et al. (2004) each of whom used stereoactive injec-

tion of adenoviruses expressing active or inactive AMPK into the rat hypothalamus *in vivo*. These groups demonstrated that hypothalamic AMPK was inhibited by intracerebrovascular (ICV) leptin, but activated by the orexigenic agent ghrelin. Recent data suggest that changes in ACC activity (Gao et al., 2007) as well as endogenous cannabinoid levels (Kola et al., 2008) may be involved in these actions, implicating AMPK in the control of body weight. Studies (Claret et al., 2007) using mice delete for AMPKα1 or α2 in specific hypothalamic nuclei largely support these findings, though the unexpected responses of Agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons to glucose reported complicate the interpretation of the latter study. Interestingly, AMPK activation is also required for the normal sympathetic response to hypoglycaemia (McCrimmon et al., 2008), indicating a role for AMPK in the control of glucose sensing neurons in the arcuate nucleus and paraventricular hypothalamus. We have shown that NPY/AgRP neurons, but not pro-opiomelanocortin (POMC) neurons, appear to mediate the effects of glucose via changes in AMPK activity, at least in *ex vivo* cultures (Mountjoy et al., 2007).

1.10. Conclusions: prospects for therapy

AMPK activation in muscle and liver is now established beyond reasonable doubt as a useful approach for the treatment of hyperglycemia and diabetes. AMPK and other members of the same family of stress-activated protein kinases, including PASK (Fig. 4), are critically involved in the recognition of glucose by the β-cell and thus represent more challenging, but still exciting, targets for therapy based on the control of β-cell mass or function, as well as targets for the control of obesity. However, in both contexts it seems possible that inhibition, rather than activation of AMPK, would be desirable. On the other hand, PASK activation would be required in the β-cell but inhibition in skeletal muscle. Such complexity imposes evident constraints on the therapeutic targeting of these enzymes in man. Nevertheless, the development of agents capable of selectively inhibiting α1-containing AMPK complexes, which predominate in the β-cell (and to a lesser extent in carotid body, pulmonary smooth muscle and fat cells), provides one intriguing possibility. Conversely, the use of adipokines, or potentiators of adipokine signalling, may allow selective stimulation of AMPK in extrapancreatic targets for insulin action.

Acknowledgements

Funded by grants to GAR from the Wellcome Trust (Programme grants nos. 067081/Z/02/Z; 081958/Z/07/Z), Medical Research Council (UK) (G0401641), National Institutes of Health, U.S. (ROI DKO71962-01), European Union FP6 (Savebeta), and to IL from Diabetes UK (BDA04/0002895) and the Wellcome Trust (WT082366MA). We thank Dr. Francesca Semplici for assistance in the preparation of the figures.

References

Alderson, A., Sabelli, P.A., Dickinson, J.R., Cole, D., Richardson, M., Kreis, M., Shewry, P.R., Halford, N.G., 1991. Complementation of snf1, a mutation affecting global regulation of carbon metabolism in yeast, by a plant protein kinase cDNA. Proc. Natl. Acad. Sci. U.S.A. 88, 8602–8605.

Andersson, U., Filipsson, K., Abbott, C.R., Woods, A., Smith, K., Bloom, S.R., Carling, D., Small, C.J., 2004. AMP-activated protein kinase plays a role in the control of food intake. J. Biol. Chem. 279, 12005–12008.

Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P.S., Curtis, R., 2004. The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in *C. elegans*. Genes Dev. 18, 3004–3009.

Beg, Z.H., Allmann, D.W., Gibson, D.M., 1973. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity with cAMP and with protein fractions of rat liver cytosol. Biochem. Biophys. Res. Commun. 54, 1362–1369.

Beri, R.K., Marley, A.E., See, C.G., Sopwith, W.F., Aguan, K., Carling, D., Scott, J., Carey, F., 1994. Molecular cloning, expression and chromosomal localisation of human AMP-activated protein kinase. FEBS Lett. 356, 117–121.

Borter, E., Niessen, M., Zuellig, R., Spinas, G.A., Spielmann, P., Camenisch, G., Wenger, R.H., 2007. Glucose-stimulated insulin production in mice deficient for the PAS kinase PASKIN. Diabetes 56, 113–117.

Cai, Y., Martens, G.A., Hinke, S.A., Heimberg, H., Pipeleers, D., Van de, C.M., 2007. Increased oxygen radical formation and mitochondrial dysfunction mediate beta cell apoptosis under conditions of AMP-activated protein kinase stimulation. Free Radic. Biol. Med. 42, 64–78.

Carling, D., Aguan, K., Woods, A., Verhoeven, A.J., Beri, R.K., Brennan, C.H., Sidebottom, C., Davison, M.D., Scott, J., 1994. Mammalian AMP-activated protein kinase is homologous to yeast and plant protein kinases involved in the regulation of carbon metabolism. J. Biol. Chem. 269, 11442–11448.

Carling, D., Zammit, V.A., Hardie, D.G., 1987. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. FEBS Lett. 223, 217–222.

Carlson, C.A., Kim, K.H., 1973. Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. J. Biol. Chem. 248, 378–380.

Celenza, J.L., Carlson, M., 1984. Cloning and genetic mapping of SNF1, a gene required for expression of glucose-repressible genes in Saccharomyces cerevisiae. Mol. Cell. Biol. 4, 49–53.

Chavez, J.A., Roach, W.G., Keller, S.R., Lane, W.S., Lienhard, G.E., 2008. Inhibition of GLUT4 translocation by Tbc1d1, a Rab GTPase-activating protein abundant in skeletal muscle, is partially relieved by AMP-activated protein kinase activation. J. Biol. Chem. 283, 9187–9195.

Chen, Z.P., McConell, G.K., Michell, B.J., Snow, R.J., Canny, B.J., Kemp, B.E., 2000. AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation. Am. J. Physiol. Endocrinol. Metab. 279, E1202–E1206.

Claret, M., Smith, M.A., Batterham, R.L., Selman, C., Choudhury, A.I., Fryer, L.G., Clements, M., Al-Qassab, H., Heffron, H., Xu, A.W., Speakman, J.R., Barsh, G.S., Viollet, B., Vaulont, S., Ashford, M.L., Carling, D., Withers, D.J., 2007. AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J. Clin. Invest. 117, 2325–2336.

Corkey, B.E., Glennon, M.C., Chen, K.S., Deeney, J.T., Matschinsky, F.M., Prentki, M., 1989. A role for malonyl-CoA in glucose-stimulated insulin secretion from clonal pancreatic beta-cells. J. Biol. Chem. 264, 21608–21612.

Corton, J.M., Gillespie, J.G., Hawley, S.A., Hardie, D.G., 1995. 5-Aminoimidazole-4-carboxamide ribonucleoside: a specific method for activating AMP-activated protein kinase in intact cells? Eur. J. Biochem. 229, 558–565.

daSilvaXavier, G., Leclerc, I., Salt, I.P., Doiron, B., Hardie, D.G., Kahn, A., Rutter, G.A., 2000. Role of AMP-activated protein kinase in the regulation by glucose of islet beta-cell gene expression. Proc. Natl. Acad. Sci. U.S.A. 97, 4023–4028.

daSilvaXavier, G., Leclerc, I., Varadi, A., Tsuboi, T., Moule, S.K., Rutter, G.A., 2003. Role for AMP-activated protein kinase in glucose-stimulated insulin secretion and preproinsulin gene expression. Biochem. J. 371, 761–774.

daSilvaXavier, G., Rutter, J., Rutter, G.A., 2004. Involvement of Per-Arnt-Sim (PAS) kinase in the stimulation of preproinsulin and pancreatic duodenum homeobox 1 gene expression by glucose. Proc. Natl. Acad. Sci. U.S.A. 101, 8319–8324.

Daval, M., Diot-Dupuy, F., Bazin, R., Hainault, I., Viollet, B., Vaulont, S., Hajduch, E., Ferre, P., Foufelle, F., 2005. Anti-lipolytic action of AMP-activated Protein kinase in rodent adipocytes. J. Biol. Chem., 280.

Diraison, F., Leclerc, I., Motakis, E., Parton, L., Rutter, G.A., 2004. Impact of sterol response element binding protein-1c and AMP-activated protein kinase on pancreatic islet β-cell gene expression profile. Diabetes 53, S84–S91.

Evans, A.M., Mustard, K.J., Wyatt, C.N., Peers, C., Dipp, M., Kumar, P., Kinnear, N.P., Hardie, D.G., 2005. Does AMP-activated protein kinase couple inhibition of mitochondrial oxidative phosphorylation by hypoxia to calcium signaling in O_2-sensing cells? J. Biol. Chem. 280, 41504–41511.

Gao, S., Kinzig, K.P., Aja, S., Scott, K.A., Keung, W., Kelly, S., Strynadka, K., Chohnan, S., Smith, W.W., Tamashiro, K.L., Ladenheim, E.E., Ronnett, G.V., Tu, Y., Birnbaum, M.J., Lopaschuk, G.D., Moran, T.H., 2007. Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. Proc. Natl. Acad. Sci. U.S.A. 104, 17358–17363.

Gleason, C.E., Lu, D., Witters, L.A., Newgard, C.B., Birnbaum, M.J., 2007. The role of AMPK and mTOR in nutrient sensing in pancreatic beta-cells. J. Biol. Chem. 282, 10341–10351.

Grant, S.F., Thorleifsson, G., Reynisdottir, I., Benediktsson, R., Manolescu, A., Sainz, J., Helgason, A., Stefansson, H., Emilsson, V., Helgadottir, A., Styrkarsdottir, U., Magnusson, K.P., Walters, G.B., Palsdottir, E., Jonsdottir, T., Gudmundsdottir, T., Gylfason, A., Saemundsdottir, J., Wilensky, R.L., Reilly, M.P., Rader, D.J., Bagger, Y., Christiansen, C., Gudnason, V., Sigurdsson, G., Thorsteinsdottir, U., Gulcher, J.R., Kong, A., Stefansson, K., 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat. Genet. 38, 320–323.

Gu, W., Li, X., Liu, C., Yang, J., Ye, L., Tang, J., Gu, Y., Yang, Y., Hong, J., Zhang, Y., Chen, M., Ning, G., 2006. Globular adiponectin augments insulin secretion from pancreatic islet beta cells at high glucose concentrations. Endocrine 30, 217–221.

Gu, Y.Z., Hogenesch, J.B., Bradfield, C.A., 2000. The PAS superfamily: sensors of environmental and developmental signals. Annu. Rev. Pharmacol. Toxicol. 40, 519–561, 519–561.

Hallows, K.R., Kobinger, G.P., Wilson, J.M., Witters, L.A., Foskett, J.K., 2003a. Physiological modulation of CFTR activity by AMP-activated protein kinase in polarized T84 cells. Am. J. Physiol. Cell. Physiol., 284.

Hallows, K.R., McCane, J.E., Kemp, B.E., Witters, L.A., Foskett, J.K., 2003b. Regulation of channel gating by AMP-activated protein kinase modulates cystic fibrosis

transmembrane conductance regulator activity in lung submucosal cells. J. Biol. Chem., 278.
Hallows, K.R., Raghuram, V., Kemp, B.E., Witters, L.A., Foskett, J.K., 2000. Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase. J. Clin. Invest. 105, 1711–1721.
Hansen, J.B., Arkhammar, P.O., Bodvarsdottir, T.B., Wahl, P., 2004. Inhibition of insulin secretion as a new drug target in the treatment of metabolic disorders. Curr. Med. Chem. 11, 1595–1615.
Hao, H.X., Cardon, C.M., Swiatek, W., Cooksey, R.C., Smith, T.L., Wilde, J., Boudina, S., Abel, E.D., McClain, D.A., Rutter, J., 2007. PAS kinase is required for normal cellular energy balance. Proc. Natl. Acad. Sci. U.S.A. 104, 15466–15471.
Hardie, D.G., 2007. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat. Rev. Mol. Cell. Biol. 8, 774–785.
Hardie, D.G., Carling, D., Carlson, M., 1998. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu. Rev. Biochem. 67, 821–855.
Hardie, D.G., 2003. Minireview: the AMP-activated protein kinase cascade—the key sensor of cellular energy status. Endocrinology 144, 5179–5183.
Hawley, S.A., Boudeau, J., Reid, J.L., Mustard, K.J., Udd, L., Makela, T.P., Alessi, D.R., Hardie, D.G., 2003. Complexes between the LKB1 tumor suppressor, STRADalpha/beta and MO25alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. J. Biol. 2, 28.
Hawley, S.A., Pan, D.A., Mustard, K.J., Ross, L., Bain, J., Edelman, A.M., Frenguelli, B.G., Hardie, D.G., 2005. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. Cell. Metab. 2, 9–19.
Hong, S.P., Leiper, F.C., Woods, A., Carling, D., Carlson, M., 2003. Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. Proc. Natl. Acad. Sci. U.S.A. 100, 8839–8843.
Hue, L., Beauloye, C., Bertrand, L., Horman, S., Krause, U., Marsin, A.S., Meisse, D., Vertommen, D., Rider, M.H., 2003. New targets of AMP-activated protein kinase. Biochem. Soc. Trans. 31, 213–215.
Huypens, P., Moens, K., Heimberg, H., Ling, Z., Pipeleers, D., Van de, C.M., 2005. Adiponectin-mediated stimulation of AMP-activated protein kinase (AMPK) in pancreatic beta cells. Life Sci. 77, 1273–1282.
Inoki, K., Zhu, T., Guan, K.L., 2003. TSC2 mediates cellular energy response to control cell growth and survival. Cell 115, 577–590.
Jenne, D.E., Reimann, H., Nezu, J., Friedel, W., Loff, S., Jeschke, R., Muller, O., Back, W., Zimmer, M., 1998. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat. Genet. 18, 38–43.
Jones, R.G., Plas, D.R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M.J., Thompson, C.B., 2005. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol. Cell 18, 283–293.
Kefas, B.A., Cai, Y., Kerckhofs, K., Ling, Z., Martens, G., Heimberg, H., Pipeleers, D., Van de Casteele, M., 2004. Metformin-induced stimulation of AMP-activated protein kinase in b-cells impairs their glucose-responsiveness and can lead to apoptosis. Biochem. Pharmacol. 68, 409–416.
Kefas, B.A., Cai, Y., Ling, Z., Heimberg, H., Hue, L., Pipeleers, D., Van de, C.M., 2003a. AMP-activated protein kinase can induce apoptosis of insulin-producing MIN6 cells through stimulation of c-Jun-N-terminal kinase. J. Mol. Endocrinol. 30, 151–161.
Kefas, B.A., Heimberg, H., Vaulont, S., Meisse, D., Hue, L., Pipeleers, D., Van de Casteele, M., 2003b. AICA-riboside induces apoptosis of pancreatic beta cells through stimulation of AMP-activated protein kinase. Diabetologia 46, 250–254.
Kim, J.W., Cho, J.H., Ko, S.H., Park, H.S., Ha, J., Song, K.H., Son, H.Y., Kim, S.S., Yoon, K.H., Suh-Kim, H., 2008. Transcriptional mechanism of suppression of insulin gene expression by AMP-activated protein kinase activator 5-amino-4-imidazolecarboxamide riboside (AICAR) in beta-cells. Biochem. Biophys. Res. Commun. 365, 614–620.
Kola, B., Boscaro, M., Rutter, G.A., Grossman, A.B., Korbonits, M., 2006. Expanding role of AMPK in endocrinology. Trends Endocrinol. Metab. 17, 205–215.
Kola, B., Farkas, I., Christ-Crain, M., Wittmann, G., Lolli, F., Amin, F., Harvey-White, J., Liposits, Z., Kunos, G., Grossman, A.B., Fekete, C., Korbonits, M., 2008. The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. PLoS ONE 3, e1797.
Koo, S.H., Flechner, L., Qi, L., Zhang, X., Screaton, R.A., Jeffries, S., Hedrick, S., Xu, W., Boussouar, F., Brindle, P., Takemori, H., Montminy, M., 2005. The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature 437, 1109–1111.
KurthKraczek, E.J., Hirshman, M.F., Goodyear, L.J., Winder, W.W., 1999. 5′-AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes 48, 1667–1671.
Leclerc, I., DaSilva Xavier, G., Rutter, G.A., 2004a. AMP-activated protein kinase: a new-cell glucose sensor? Regulation by amino acids and calcium ions. Diabetes 53, S67–S74.
Leclerc, I., Fernadez-Millan, E., Nyirenda, M., Rutter, G.A., 2007. Role of AMP-activated protein kinase in glucagon secretion. Diab. Med. 24, 1.
Leclerc, I., Kahn, A., Doiron, B., 1998. The 5′-AMP-activated protein kinase inhibits the transcriptional stimulation by glucose in liver cells, acting through the glucose response complex. FEBS Lett. 431, 180–184.
Leclerc, I., Woltersdorf, W.W., daSilvaXavier, G., Rowe, R.L., Cross, S.E., Korbutt, G.S., Rajotte, R.V., Smith, R., Rutter, G.A., 2004b. Metformin, but not leptin, regulates AMP-activated protein kinase in pancreatic islets: impact on glucose-stimulated insulin secretion. Am. J. Physiol. Endocrinol. Metab. 286, E1023–E1031.
Leclerc, I., Rutter, G.A., 2004. AMP-activated protein kinase: a new beta-cell glucose sensor? Regulation by Amino Acids and Calcium Ions. Diabetes, 53.
Leclerc, I., Woltersdorf, W.W., da Silva Xavier, G., Rowe, R.L., Cross, S.E., Korbutt, G.S., Rajotte, R.V., Smith, R., Rutter, G.A., 2004c. Metformin, but not leptin, regulates AMP-activated protein kinase in pancreatic islets: impact on glucose-stimulated insulin secretion. Am. J. Physiol. Endocrinol. Metab., 286.
Lee, H., Cho, J.S., Lambacher, N., Lee, J., Lee, S.J., Lee, T.H., Gartner, A., Koo, H.S., 2008. The *C. elegans* AMP-activated protein kinase AAK-2 is phosphorylated by LKB1 and required for resistance to oxidative stress and for normal motility and foraging behavior. J. Biol. Chem..
Lee, J.H., Koh, H., Kim, M., Kim, Y., Lee, S.Y., Karess, R.E., Lee, S.H., Shong, M., Kim, J.M., Kim, J., Chung, J., 2007. Energy-dependent regulation of cell structure by AMP-activated protein kinase. Nature 447, 1017–1020.
Lefebvre, D.L., Bai, Y., Shahmolky, N., Sharma, M., Poon, R., Drucker, D.J., Rosen, C.F., 2001. Identification and characterization of a novel sucrose-non-fermenting protein kinase/AMP-activated protein kinase-related protein kinase, SNARK. Biochem. J. 355, 297–305.
Lefebvre, D.L., Rosen, C.F., 2005. Regulation of SNARK activity in response to cellular stresses. Biochim. Biophys. Acta 1724, 71–85.
Liang, Y., Bai, G., Doliba, N., Buettger, C., Wang, L., Berner, D.K., Matschinsky, F.M., 1996. Glucose metabolism and insulin release in mouse betaHC9 cells, as model for wild-type pancreatic beta-cells. Am. J. Physiol. Endocrinol. Metab. 270, E846–E857.
Lizcano, J.M., Goransson, O., Toth, R., Deak, M., Morrice, N.A., Boudeau, J., Hawley, S.A., Udd, L., Makela, T.P., Hardie, D.G., Alessi, D.R., 2004. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J. 23, 833–843.
Long, Y.C., Zierath, J.R., 2006. AMP-activated protein kinase signaling in metabolic regulation. J. Clin. Invest. 116, 1776–1783.
Luo, Z., Saha, A.K., Xiang, X., Ruderman, N.B., 2005. AMPK, the metabolic syndrome and cancer. Trends Pharmacol. Sci. 26, 69–76.
Lupi, R., Marchetti, P., Giannarelli, R., Coppelli, A., Tellini, C., Del Guerra, S., Lorenzetti, M., Carmellini, M., Mosca, F., Navalesi, R., 1997. Effects of glibenclamide and metformin (alone or in combination) on insulin release from isolated human pancreatic islets. Acta Diabetol. 34, 46–48.
Manning, G., Whyte, D.B., Martinez, R., Hunter, T., Sudarsanam, S., 2002. The protein kinase complement of the human genome. Science 298, 1912–1934.
McCrimmon, R.J., Shaw, M., Fan, X., Cheng, H., Ding, Y., Vella, M.C., Zhou, L., McNay, E.C., Sherwin, R.S., 2008. Key role for AMP-activated protein kinase in the ventromedial hypothalamus in regulating counter regulatory hormone responses to acute hypoglycemia. Diabetes 57, 444–450.
Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y.B., Lee, A., Xue, B., Mu, J., Foufelle, F., Ferre, P., Birnbaum, M.J., Stuck, B.J., Kahn, B.B., 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428, 569–574.
Minokoshi, Y., Kahn, B.B., 2003. Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. Biochem. Soc. Trans. 31, 196–201.
Momcilovic, M., Hong, S.P., Carlson, M., 2006. Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. J. Biol. Chem. 281, 25336–25343.
Moule, S.K., Denton, R.M., 1998. The activation of p38 MAPK by the beta-adrenergic agonist isoproterenol in rat epididymal fat cells. FEBS Lett. 439, 287–290.
Mountjoy, P.D., Bailey, S.J., Rutter, G.A., 2007. Inhibition by glucose or leptin of neuropeptide Y-expressing hypothalamic neurons requires changes in AMP-activated protein kinase activity. Diabetologia 50, 168–177.
Mountjoy, P.D., Rutter, G.A., 2007. Glucose sensing by hypothalamic neurons and pancreatic islet cells: ample evidence for common mechanisms? Exp. Physiol. 92, 311–319.
Okamoto, M., Ohara-Imaizumi, M., Kubota, N., Hashimoto, S., Eto, K., Kanno, T., Kubota, T., Wakui, M., Nagai, R., Noda, M., Nagamatsu, S., Kadowaki, T., 2008. Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. Diabetologia 51, 827–835.
Otonkoski, T., Jiao, H., Kaminen-Ahola, N., Tapia-Piaz, I., Ullah, M.S., Parton, L.E., Schuit, F.C., Quintens, R., Sipila, I., Mayatepek, E., Meissner, T., Halestrap, A.P., Rutter, G.A., Kere, J., 2007. Physical exercise-induced hyperinsulinemic hypoglycemia caused by failure of monocarboxylate transporter 1 silencing in pancreatic beta cells. Am. J. Hum. Genet. 81, 467–474.
Owen, M.R., Doran, E., Halestrap, A.P., 2000. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem. J. 348 (Pt 3), 607–614, 607–614.
Pold, R., Jensen, L.S., Jessen, N., Buhl, E.S., Schmitz, O., Flyvbjerg, A., Fujii, N., Goodyear, L.J., Gotfredsen, C.F., Brand, C.L., Lund, S., 2005. Long-term AICAR administration and exercise prevents diabetes in ZDF rats. Diabetes 54, 928–934.
Ponting, C.P., Aravind, L., 1997. PAS: a multifunctional domain family comes to light. Curr. Biol. 7, R674–R677.
Ravnskjaer, K., Boergesen, M., Dalgaard, L.T., Mandrup, S., 2006. Glucose-induced repression of PPARalpha gene expression in pancreatic beta-cells involves PP2A activation and AMPK inactivation. J. Mol. Endocrinol. 36, 289–299.
Riboulet-Chavey, A., Diraison, F., Siew, L.K., Wong, F.S., Rutter, G.A., 2008. Inhibition of AMP-activated protein kinase protects pancreatic {beta}-cells from cytokine-mediated apoptosis and CD8+ T cell-induced cytotoxicity. Diabetes 57, 415–423.
Richards, S.K., Parton, L.E., Leclerc, I., Rutter, G.A., Smith, R.M., 2006. Over-expression of AMP-activated protein kinase impairs pancreatic beta-cell function in vivo. J. Endocrinol. 187, 225–235.

Rutter, G.A., daSilvaXavier, G., Leclerc, I., 2003. Roles of 5′-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. Biochem. J. 375, 1–16.
Rutter, G.A., Hill, E.V., 2006. Insulin vesicle release: walk, kiss, pause then run. Physiology 21, 189–196.
Rutter, J., Michnoff, C.H., Harper, S.M., Gardner, K.H., McKnight, S.L., 2001. PAS kinase: an evolutionarily conserved PAS domain-regulated serine/threonine kinase. Proc. Natl. Acad. Sci. U.S.A. 98, 8991–8996.
Salt, I.P., Connell, J.M.C., Gould, G.W., 2000. 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR) inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes. Diabetes 49, 1649–1656.
Salt, I.P., Johnson, G., Ashcroft, S.J., Hardie, D.G., 1998. AMP-activated protein kinase is activated by low glucose in cell lines derived from pancreatic beta cells, and may regulate insulin release. Biochem. J. 335, 533–539.
Saxena, R., Voight, B.F., Lyssenko, V., Burtt, N.P., de Bakker, P.I., Chen, H., Roix, J.J., Kathiresan, S., Hirschhorn, J.N., Daly, M.J., Hughes, T.E., Groop, L., Altshuler, D., Almgren, P., Florez, J.C., Meyer, J., Ardlie, K., Bengtsson, K., Isomaa, B., Lettre, G., Lindblad, U., Lyon, H.N., Melander, O., Newton-Cheh, C., Nilsson, P., Orho-Melander, M., Rastam, L., Speliotes, E.K., Taskinen, M.R., Tuomi, T., Guiducci, C., Berglund, A., Carlson, J., Gianniny, L., Hackett, R., Hall, L., Holmkvist, J., Laurila, E., Sjogren, M., Sterner, M., Surti, A., Svensson, M., Svensson, M., Tewhey, R., Blumenstiel, B., Parkin, M., Defelice, M., Barry, R., Brodeur, W., Camarata, J., Chia, N., Fava, M., Gibbons, J., Handsaker, B., Healy, C., Nguyen, K., Gates, C., Sougnez, C., Gage, D., Nizzari, M., Gabriel, S.B., Chirn, G.W., Ma, Q., Parikh, H., Richardson, D., Ricke, D., Purcell, S., 2007. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316, 1331–1336.
Schuit, F., DeVos, A., Farfari, S., Moens, K., Pipeleers, D., Brun, T., Prentki, M., 1997. Metabolic fate of glucose in purified islet cells—glucose-regulated anaplerosis in beta cells. J. Biol. Chem. 272, 18572–18579.
Scott, L.J., Mohlke, K.L., Bonnycastle, L.L., Willer, C.J., Li, Y., Duren, W.L., Erdos, M.R., Stringham, H.M., Chines, P.S., Jackson, A.U., Prokunina-Olsson, L., Ding, C.J., Swift, A.J., Narisu, N., Hu, T., Pruim, R., Xiao, R., Li, X.Y., Conneely, K.N., Riebow, N.L., Sprau, A.G., Tong, M., White, P.P., Hetrick, K.N., Barnhart, M.W., Bark, C.W., Goldstein, J.L., Watkins, L., Xiang, F., Saramies, J., Buchanan, T.A., Watanabe, R.M., Valle, T.T., Kinnunen, L., Abecasis, G.R., Pugh, E.W., Doheny, K.F., Bergman, R.N., Tuomilehto, J., Collins, F.S., Boehnke, M., 2007. A genome-wide association study of type 2 diabetes in finns detects multiple susceptibility variants. Science 316, 1341–1345.
Sekine, N., Cirulli, V., Regazzi, R., Brown, L.J., Gine, E., Tamarit-Rodriguez, J., Girotti, M., Marie, S., MacDonald, M.J., Wollheim, C.B., Rutter, G.A., 1994. Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic β-cell: potential role in nutrient sensing. J. Biol. Chem. 269, 4895–4902.
Shaw, R.J., Kosmatka, M., Bardeesy, N., Hurley, R.L., Witters, L.A., DePinho, R.A., Cantley, L.C., 2004. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proc. Natl. Acad. Sci. U.S.A. 101, 3329–3335.
Shaw, R.J., Lamia, K.A., Vasquez, D., Koo, S.-H., Bardeesy, N., DePinho, R.A., Montminy, M., Cantley, L.C., 2005. The kinase LKB1 Mediates glucose homeostasis in liver and therapeutic effects of metformin. Science, 310.
Sladek, R., Rocheleau, G., Rung, J., Dina, C., Shen, L., Serre, D., Boutin, P., Vincent, D., Delisle, A., Hadjadj, S., Balkau, B., Heude, B., Charpentier, G., Hundson, T.J., Montpetit, A., Pshezhetsky, A.V., Prentki, M., Posner, B.I., Balding, D.J., Meyre, D., Polychronakos, C., Froguel, P., 2007. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445, 881–885.
Stapleton, D., Mitchelhill, K.I., Gao, G., Widmer, J., Michell, B.J., Teh, T., House, C.M., Fernandez, C.S., Cox, T., Witters, L.A., Kemp, B.E., 1996. Mammalian AMP-activated protein kinase subfamily. J. Biol. Chem. 271, 611–614.
Sun, Y., Ren, M., Gao, G.Q., Gong, B., Xin, W., Guo, H., Zhang, X.J., Gao, L., Zhao, J.J., 2008. Chronic palmitate exposure inhibits AMPKalpha and decreases glucose-stimulated insulin secretion from beta-cells: modulation by fenofibrate. Acta Pharmacol. Sin. 29, 443–450.
Targonsky, E.D., Dai, F., Koshkin, V., Karaman, G.T., Gyulkhandanyan, A.V., Zhang, Y., Chan, C.B., Wheeler, M.B., 2006. Alpha-lipoic acid regulates AMP-activated protein kinase and inhibits insulin secretion from beta cells. Diabetologia 49, 1587–1598.
Townley, R., Shapiro, L., 2007. Crystal structures of the adenylate sensor from fission yeast AMP-activated protein kinase. Science 315, 1726–1729.
Tsuboi, T., daSilvaXavier, G., Leclerc, I., Rutter, G.A., 2003. 5′-AMP-activated protein kinase controls insulin-containing secretory vesicle dynamics. J. Biol. Chem. 278, 52042–52051.
Viollet, B., Andreelli, F., Jorgensen, S.B., Perrin, C., Flamez, D., Mu, J., Wojtaszewski, J.F., Schuit, F.C., Birnbaum, M., Richter, E., Burcelin, R., Vaulont, S., 2003a. Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. Biochem. Soc. Trans. 31, 216–219.
Viollet, B., Andreelli, F., Jorgensen, S.B., Perrin, C., Geloen, A., Flamez, D., Mu, J., Lenzner, C., Baud, O., Bennoun, M., Gomas, E., Nicolas, G., Wojtaszewski, J.F., Kahn, A., Carling, D., Schuit, F.C., Birnbaum, M.J., Richter, E.A., Burcelin, R., Vaulont, S., 2003b. The AMP-activated protein kinase alpha2 catalytic subunit controls whole-body insulin sensitivity. J. Clin. Invest. 111, 91–98.
Wang, C.-Z., Wang, Y., Di, A., Magnuson, M.A., Ye, H., Roe, M.W., Nelson, D.J., Bell, G.I., Philipson, L.H., 2005. 5-Amino-imidazole carboxamide riboside acutely potentiates glucose-stimulated insulin secretion from mouse pancreatic islets by KATP channel-dependent and -independent pathways. Biochem. Biophys. Res. Commun., 330.
Wang, X., Zhou, L., Shao, L., Qian, L., Fu, X., Li, G., Luo, T., Gu, Y., Li, F., Li, J., Zheng, S., Luo, M., 2007. Troglitazone acutely activates AMP-activated protein kinase and inhibits insulin secretion from beta cells. Life Sci. 81, 160–165.
Wilcock, C., Bailey, C.J., 1990. Sites of metformin-stimulated glucose metabolism. Biochem. Pharmacol. 39, 1831–1834.
Witters, L.A., Kemp, B.E., Means, A.R., 2006. Chutes and Ladders: the search for protein kinases that act on AMPK. Trends Biochem. Sci. 31, 13–16.
Woods, A., Dickerson, K., Heath, R., Hong, S.P., Momcilovic, M., Johnstone, S.R., Carlson, M., Carling, D., 2005. Ca^{2+}/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell. Metab. 2, 21–33.
Woods, A., Johnstone, S.R., Dickerson, K., Leiper, F.C., Fryer, L.G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., Carling, D., 2003. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Curr. Biol. 13, 2004–2008.
Woods, A., Azzout-Marniche, D., Foretz, M., Stein, S.C., Lemarchand, P., Ferre, P., Foufelle, F., Carling, D., 2000. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. Mol. Cell. Biol., 20.
Wyatt, C.N., Mustard, K.J., Pearson, S.A., Dallas, M.L., Atkinson, L., Kumar, P., Peers, C., Hardie, D.G., Evans, A.M., 2007. AMP-activated protein kinase mediates carotid body excitation by hypoxia. J. Biol. Chem. 282, 8092–8098.
Xiao, B., Heath, R., Saiu, P., Leiper, F.C., Leone, P., Jing, C., Walker, P.A., Haire, L., Eccleston, J.F., Davis, C.T., Martin, S.R., Carling, D., Gamblin, S.J., 2007. Structural basis for AMP binding to mammalian AMP-activated protein kinase. Nature 449, 496–500.
Xie, M., Zhang, D., Dyck, J.R., Li, Y., Zhang, H., Morishima, M., Mann, D.L., Taffet, G.E., Baldini, A., Khoury, D.S., Schneider, M.D., 2006. A pivotal role for endogenous TGF-beta-activated kinase-1 in the LKB1/AMP-activated protein kinase energy-sensor pathway. Proc. Natl. Acad. Sci. U.S.A. 103, 17378–17383.
Yamaguchi, S., Katahira, H., Ozawa, S., Nakamichi, Y., Tanaka, T., Shimoyama, T., Takahashi, K., Yoshimoto, K., Imaizumi, M.O., Nagamatsu, S., Ishida, H., 2005. Activators of AMP-activated protein kinase enhance GLUT4 translocation and its glucose transport activity in 3T3-L1 adipocytes. Am. J. Physiol. Endocrinol. Metab. 289, E643–E649.
Yin, W., Mu, J., Birnbaum, M.J., 2003. Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis in 3T3-L1 adipocytes. J. Biol. Chem. 278, 43074–43080.
Yu, J.G., Kruszynska, Y.T., Mulford, M.I., Olefsky, J.M., 1999. A comparison of troglitazone and metformin on insulin requirements in euglycemic intensively insulin-treated type 2 diabetic patients. Diabetes 48, 2414–2421.
Zeggini, E., Weedon, M.N., Lindgren, C.M., Frayling, T.M., Elliott, K.S., Lango, H., Timpson, N.J., Perry, J.R., Rayner, N.W., Freathy, R.M., Barrett, J.C., Shields, B., Morris, A.P., Ellard, S., Groves, C.J., Harries, L.W., Marchini, J.L., Owen, K.R., Knight, B., Cardon, L.R., Walker, M., Hitman, G.A., Morris, A.D., Doney, A.S., McCarthy, M.I., Hattersley, A.T., 2007. Replication of genome-wide association signals in U.K. samples reveals risk loci for type 2 diabetes. Science 316, 1336–1341.
Zhao, C., Wilson, C.M., Schuit, F., Halestrap, A.P., Rutter, G.A., 2001. Expression and distribution of lactate/monocarboxylate transporter (MCT) isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50, 361–366.
Zhao, C., Rutter, G.A., 1998. Overexpression of lactate dehydrogenase A attenuates glucose-induced insulin secretion in stable MIN-6 beta-cell lines. FEBS Lett. 430, 213–216.
Zheng, B., Cantley, L.C., 2007. Regulation of epithelial tight junction assembly and disassembly by AMP-activated protein kinase. Proc. Natl. Acad. Sci. U.S.A. 104, 819–822.
Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M.F., Goodyear, L.J., Moller, D.E., 2001. Role of AMP-activated protein kinase in mechanism of metformin action. J. Clin. Invest. 108, 1167–1174.