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

Guy A. Rutter^{1†} and Isabelle Leclerc²

Sections of ¹Cell Biology and ²Endocrinology and Metabolic Medicine, Division of Medicine, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, Exhibition Road, South Kensington, London SW7 2AZ

[†]To whom correspondence should be addressed:

Guy A. Rutter PhD

Professor and Head of Department of Cell Biology, Division of Medicine, Sir Alexander Fleming Building, Imperial College, London, Exhibition Road, London SW7 2AZ, UK

E-mail: g.rutter@imperial.ac.uk

Website: <http://www1.imperial.ac.uk/medicine/about/divisions/medicine/cellbiology/>

Phone: +44 (0)20.759.43340

Abstract

AMP-activated protein kinase (AMPK) is a widely conserved Ser/Thr specific protein kinase, homologous to *S. 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, *et al* *Biochem J.* 375 (Pt 1):1-16, 2003). In the subsequent five 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.

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 *S. cerevisiae* (Celenza and Carlson, 1984), to the plant SNF-1 related kinase, SnRK (Alderson *et al.*, 1991) and to *C. 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 twenty years (Stapleton *et al.*, 1996; 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.

AMPK structure

As reviewed in detail elsewhere (Hardie, 2007), mammalian AMPK exists as a heterotrimeric complex of a catalytic, α -subunit ($\alpha 1$ or $\alpha 2$), a β - (scaffold) subunit ($\beta 1$, $\beta 2$ or $\beta 3$) and a “regulatory” γ -subunit ($\gamma 1$, $\gamma 2$ or $\gamma 3$). 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.

Upstream regulators

One of the most important breakthroughs in the past five 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. cerevisiae* 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, CaMKK α and CaMKK β (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-1 β -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).

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 *et al.*, 2006). Whilst there is substantial evidence implicating AMPK activation by these agents, as 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 phosphoenolpyruvate 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 as 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.

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),(daSilvaXavier *et al.*, 2003) and by depolarisation-stimulated increases in free Ca^{2+} concentration (Leclerc *et al.*, 2004a) implies that both LKB1 and CaMKK α/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 PPAR α 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;Kefas *et al.*, 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;Kefas *et al.*, 2003b;Kefas *et al.*, 2004), acting in part via enhanced mitochondrial production of reactive oxygen species (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.

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 colleagues (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 (ROS) 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 supraphysiological 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.* (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 selectivity/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 AMPK $\alpha 2$ 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 AMPK $\alpha 1$ displayed no obvious defects in glucose homeostasis (Viollet *et al.*, 2003a). Mice lacking both catalytic isoforms die *in utero*).

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 - *versus* other cell types. Interestingly, AMPK $\alpha 1$ 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 $\alpha 2$ 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, $\alpha 1$ immunoreactivity was restricted to the cytosol, whereas $\alpha 2$

immunoreactivity was present in both the cytosol and nucleus, and blockade of $\alpha 2$ activity (but not $\alpha 1$) 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 $\alpha 1$ isoform in controlling electrical activity or Ca^{2+} homeostasis in β - 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 $\alpha 2$ 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 (ie the equilibrium between globular and fibrous forms) may well be involved. Whether the predominance of AMPK $\alpha 1$ versus $\alpha 2$ 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 C. *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 AMPK $\alpha 1$ 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.

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 colleagues (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 haemopoietic cells or testis, the main sites of PASK expression (GAR, G. da Silva Xavier, unpublished)

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 α TC1-9-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).

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, the search for important targets has largely involved candidate gene approaches. Phosphorylation and inhibition of acetyl-CoA carboxylase-1 (ACC1), and the consequent decrease 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 *D. melanogaster* 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* AMPK α 2 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 Ca²⁺ signals, by as yet undefined mechanisms; note that this represents a very different role for AMPK in the β - versus other (eg 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; Hallows *et al.*, 2003a; Hallows *et al.*, 2003b), the two pore calcium channel TASK (Wyatt *et al.*, 2007) or large conductance Ca²⁺-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.

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, Kahn and colleagues (Minokoshi *et al.*, 2004) and from Anderson et

al (Andersson *et al.*, 2004) each of whom used stereotaxic injection 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 AMPK α 1 or α 2 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 and others 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).

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 α 1-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.

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Figure legends.

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

Figure 2. **Subunit structure and upstream regulators of AMPK.**

Figure 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).

Figure 4. **Modified dendrogram 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)

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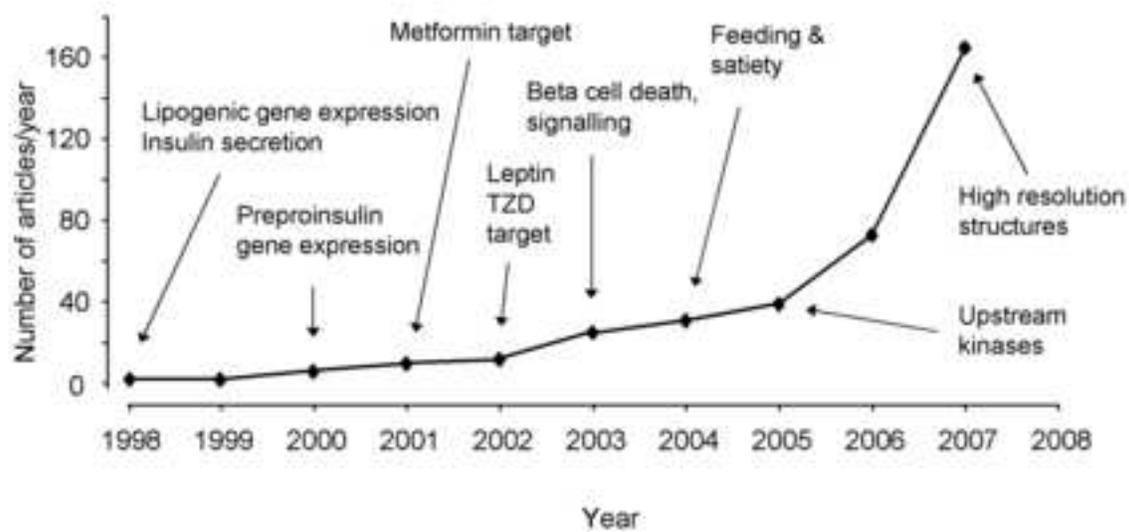


Fig. 1

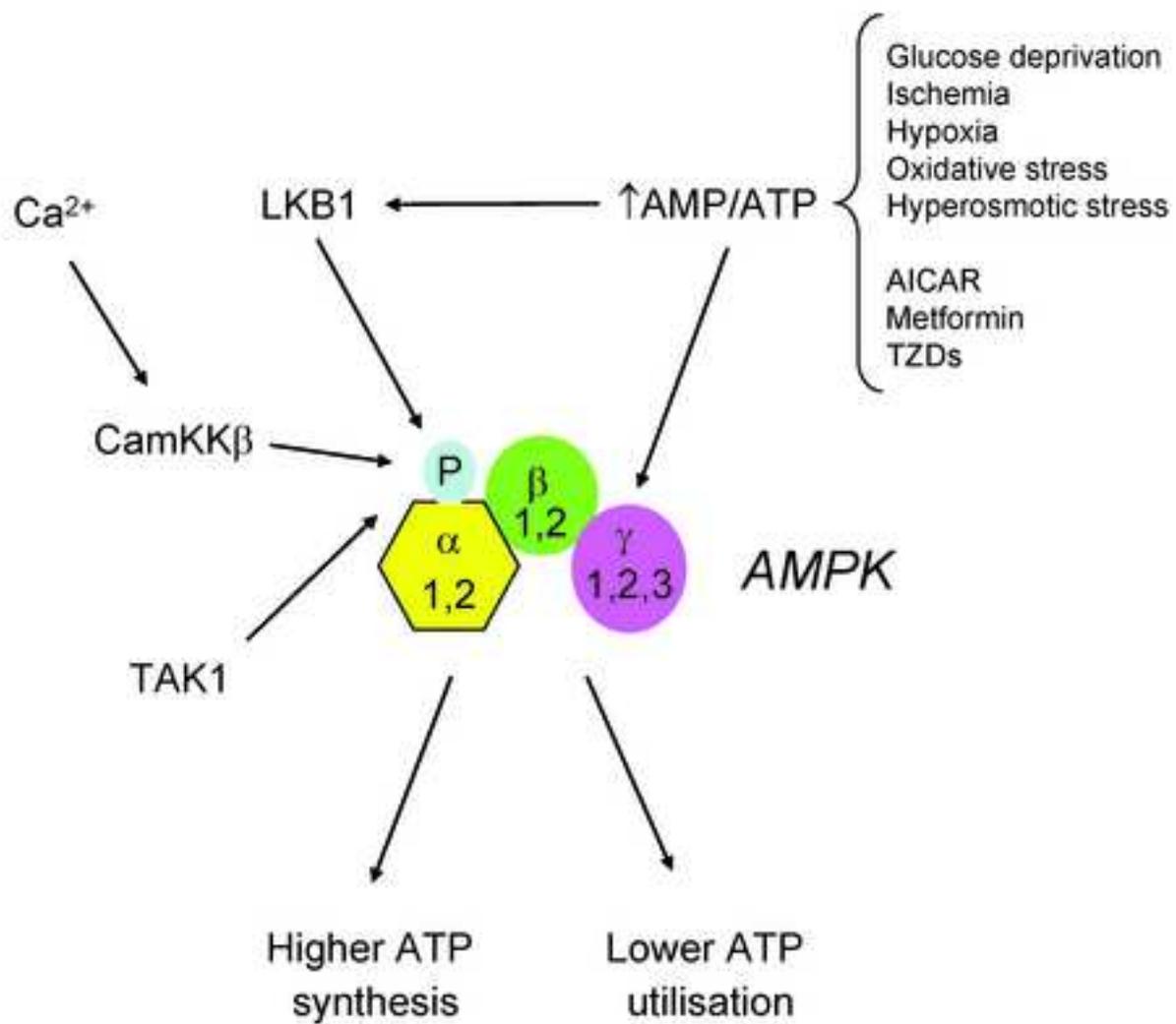


Fig. 2

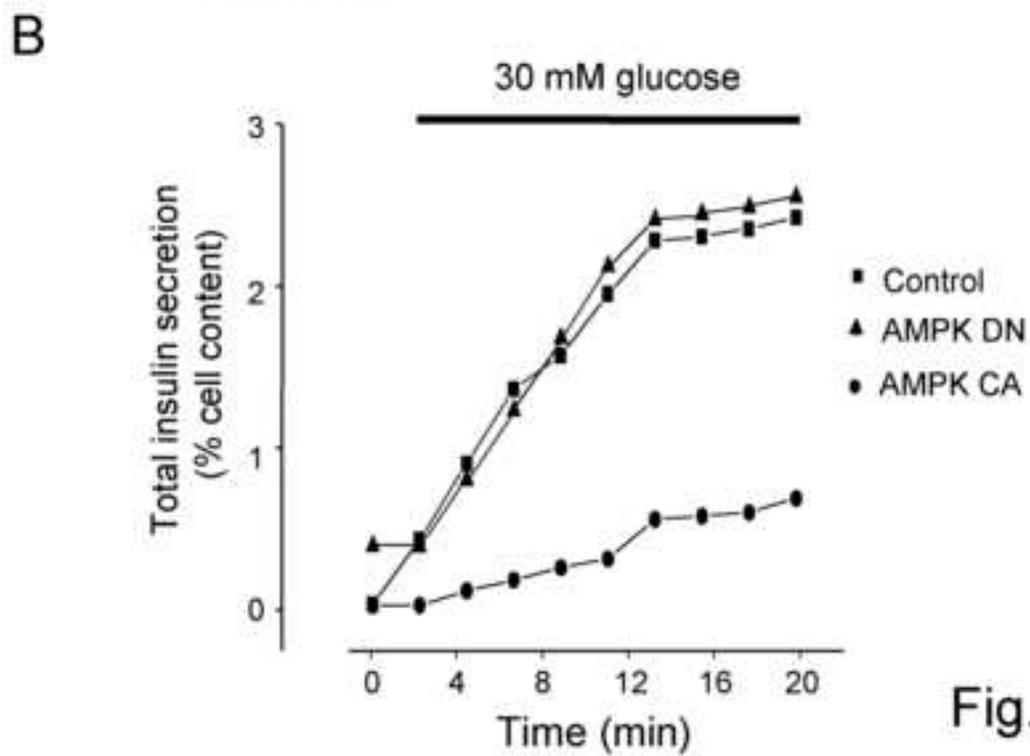
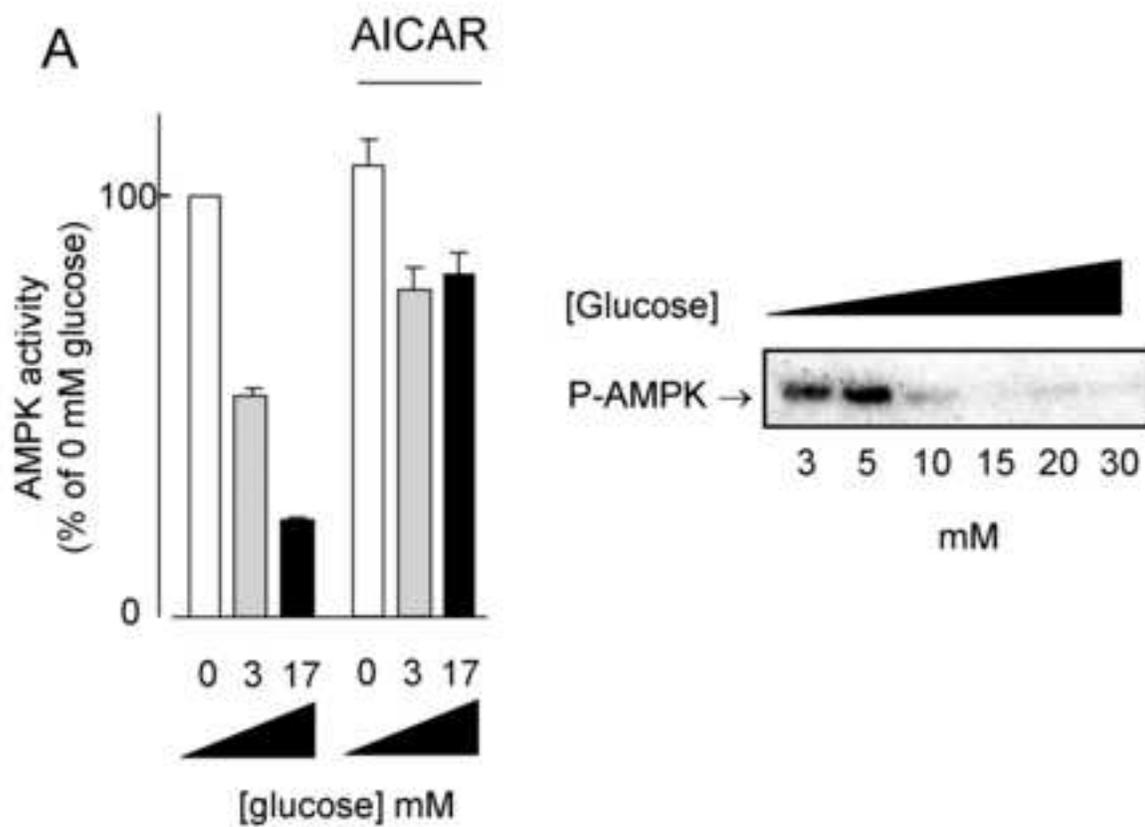


Fig. 3

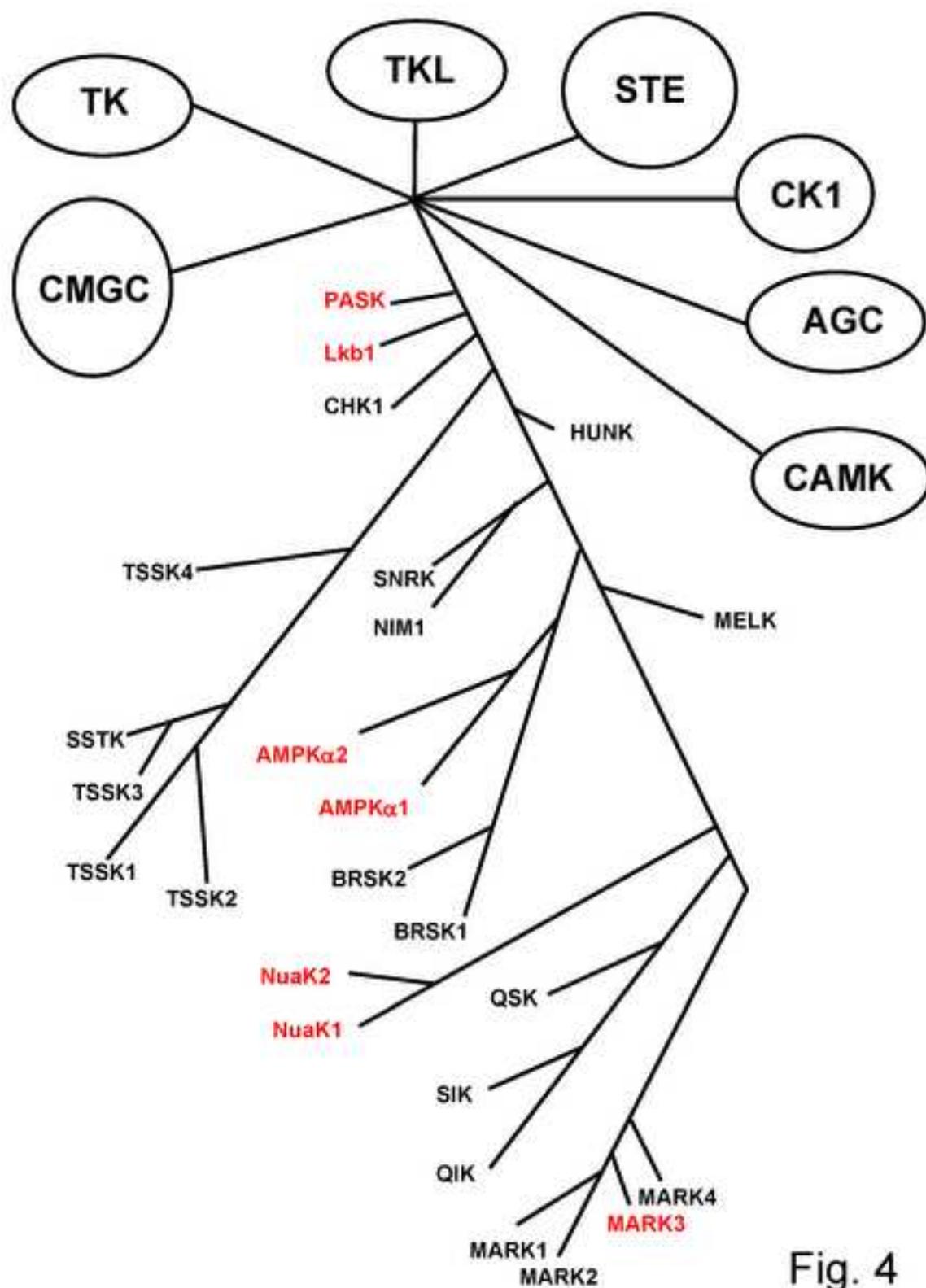


Fig. 4