Minireview

Management of cellular energy by the AMP-activated protein kinase system

D. Grahame Hardie*, John W. Scott, David A. Pan, Emma R. Hudson

Division of Molecular Physiology, Wellcome Trust Biocentre, University of Dundee, Dow Street, Dundee DD1 5EH, UK

Received 20 March 2002; revised 8 May 2003; accepted 8 May 2003

First published online 26 May 2003

Edited by Richard Marais

Abstract The AMP-activated protein kinase is a sensor of cellular energy status that is found in all eukaryotic cells. It is activated by rising AMP and falling ATP by a complex mechanism that results in an ultrasensitive response. The functions of the different domains on the three subunits of the αβγ heterotrimer are slowly being unravelled, and a recent development has been the identification of a glycogen-binding domain on the β subunit. Along with findings that high cellular glycogen represses kinase activation, this suggests that the system may be a sensor of glycogen content as well as of AMP and ATP. New insights have been obtained into the sequence and structural features by which the kinase recognises its downstream target proteins, and these are discussed. Once activated by depletion of cellular energy reserves, the kinase switches on ATP-producing catabolic pathways and switches off ATP-consuming processes, both via direct phosphorylation of regulatory proteins and via indirect effects on gene expression. A survey of the range of downstream targets for this important signalling pathway is presented.

Key words: AMP-activated protein kinase; Cellular energy

1. Introduction

One of the most fundamental parameters that all living cells must maintain is a high ratio of ATP to ADP. The equilibrium ratio for ATP:ADP under intracellular conditions is around 10^{-7}:1, but living cells typically maintain this ratio at something like 10:1, i.e. eight orders of magnitude away from equilibrium. This non-equilibrium state of ATP and ADP is analogous to that of the chemicals in a fully charged electrical cell or battery, and just like the latter can be used to drive energy-requiring processes. In animal cells ADP and phosphate are converted to ATP (charging the battery) by catabolic reactions, i.e. glycolysis and oxidative phosphorylation. Almost every other cellular process requires energy and converts ATP to ADP and phosphate (or, in a few cases, to AMP and pyrophosphate), thus discharging the battery. The fact that the cellular ATP:ADP ratio is usually maintained within very narrow limits indicates that the rates of these energy-requiring processes in the cell are balanced almost perfectly by the rate of catabolism. However, if one stops to think about it, there is no a priori reason why this should be so. The explanation has to be that there are sophisticated regulatory systems in cells that maintain this balance, and the theme of this article is that the AMP-activated protein kinase (AMPK) is the key player in this process.

At first sight, what is needed is a system that can monitor the cellular ATP:ADP ratio. However, because adenylate kinase maintains the reaction 2ADP↔ATP+AMP close to equilibrium in all eukaryotic cells, the AMP:ATP ratio tends to vary as the square of the ADP:ATP ratio [1], so that the former is a more sensitive indicator of cellular energy status than the latter. By the 1960s a small number of metabolic enzymes had been found to be regulated directly by AMP and ATP and, extrapolating from these, Atkinson et al. [2] proposed that AMP, or the AMP to ATP ratio, would regulate enzymes involved at many branch points determining metabolic choices between anabolism and catabolism. This hypothesis now appears to be correct, except that what Atkinson had not anticipated was that in most cases this regulation would be performed by a protein kinase cascade rather than by direct allosteric regulation of the target enzymes. In this article we will concentrate on recent studies of the system, and readers should refer to previous reviews [1,3] for the earlier literature.

2. Structure and regulation of AMPK

AMPKs are heterotrimeric complexes comprising a catalytic α subunit and regulatory β and γ subunits [1,3]. Homologues of these three subunits are found in all eukaryotic species where genome sequences have been completed (Fig. 1). They are even found in the very primitive eukaryote *Giardia lamblia*, which does not possess mitochondria, nucleoli, or peroxisomes, and which according to molecular phylogenies is more than twice as remote from humans as fungi [4]. This suggests that the possession of AMPK complexes is a universal feature of eukaryotes. The number of genes encoding each

*Corresponding author. Fax: (44)-1382-345783.
E-mail address: d.g.hardie@dundee.ac.uk (D.G. Hardie).

Abbreviations: ACC1, acetyl-CoA carboxylase (1/α isoform); ACC2, acetyl-CoA carboxylase (2/β isoform); AICA, 5-aminoimidazole-4-carboxamide; AMPK, AMP-activated protein kinase; CFTR, cystic fibrosis transmembrane conductance regulator; eNOS, endothelial nitric oxide synthase; KIS, kinase interaction sequence; nNOS, neuronal nitric oxide synthase; PFK2, 6-phosphofructo-2-kinase; PI, phosphatidylinositol; PPAR-γ, peroxisome proliferator-activated receptor-γ

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00560-X

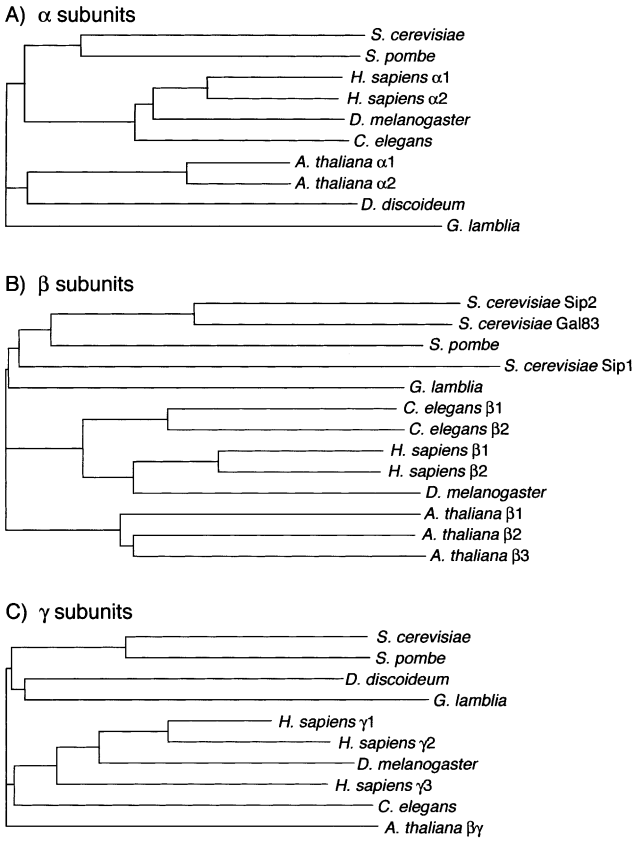

Fig. 1. Phylogenetic trees of homologues of the (A) α subunits, (B) β subunits and (C) γ subunits of AMPK from different species. Species analysed are human (*Homo sapiens*), fruit fly (*Drosophila melanogaster*), nematode worm (*Caenorhabditis elegans*), slime mould (*Dictyostelium discoideum*), thale cress (*Arabidopsis thaliana*), budding yeast (*Saccharomyces cerevisiae*), fission yeast (*Schizosaccharomyces pombe*), and *Giardia lamblia*. Names of isoforms are shown where relevant. Most sequences were found using BLAST searches of the respective genome web sites. Initial alignments were created using PILEUP and the trees were created using CLUSTALX by the neighbour-joining method.

subunit varies from species to species. In the fruit fly *Drosophila melanogaster*, for example, there are single genes encoding homologues of α, β and γ [5], while in humans each subunit is encoded by either two or three distinct genes (α1, α2, β1, β2, γ1, γ2, γ3), so that there are 12 possible αβγ combinations (even excluding splice variants).

As the name suggests, mammalian AMPK is allosterically activated by 5′-AMP. However, the complex is inactive unless it has been phosphorylated by upstream kinases (currently unidentified) at a threonine residue (Thr-172) in the 'activation segment' of the kinase domain, and our laboratory showed that AMP had three additional effects to activate the system, i.e. (i) activation of the upstream kinase, (ii) making AMPK a better substrate for the upstream kinase and (iii) making it a worse substrate for the protein phosphatase. The combination of these effects results in the system being 'ultrasensitive', i.e. the response curve is sigmoidal and (over the critical concentration range) a small change in AMP produces a large effect [6]. The simplest hypothesis is that the three effects of AMP that are due to binding to AMPK (as opposed to the upstream kinase) are due to binding at a single site, although this remains to be proven. All three effects are antagonised by high concentrations of ATP [7–9], suggesting that the allosteric site(s) bind AMP and ATP competitively, and also implying that the key signal that activates AMPK in vivo is rising AMP coupled with falling ATP.

What are the functions of the three subunits of AMPK? The α subunit sequences contain an N-terminal kinase domain and a C-terminal domain of approximately equal size (Fig. 2A). When expressed on its own in either bacterial [10] or mammalian [11] cells, the kinase domain is AMP-independent, although it still requires phosphorylation of Thr-172 to become active. Based on expression of C-terminal truncations, the extreme C-terminus of α1 (392–548) is required for formation of the complex with the β and γ subunits, while the region immediately C-terminal to the kinase domain (312–392) appears to be inhibitory [11].

The β subunits contain poorly conserved N-terminal regions followed by two regions, originally termed *KIS* and *ASC* domains [12], that are conserved across all eukaryotes (Fig. 2B). Based on two-hybrid analysis in the yeast system, it was originally thought that the *KIS* domain was required for interaction with the α subunit, while the *ASC* domain was required for the interaction with γ [12]. However, recent studies in which the mammalian β subunits were truncated from the N-terminus and co-expressed with α and γ have modified this view: only the *ASC* domain was required for the formation of a stable, active αβγ complex [13]. Recent bioinformatic analysis has also shown that the so-called *KIS* (kinase

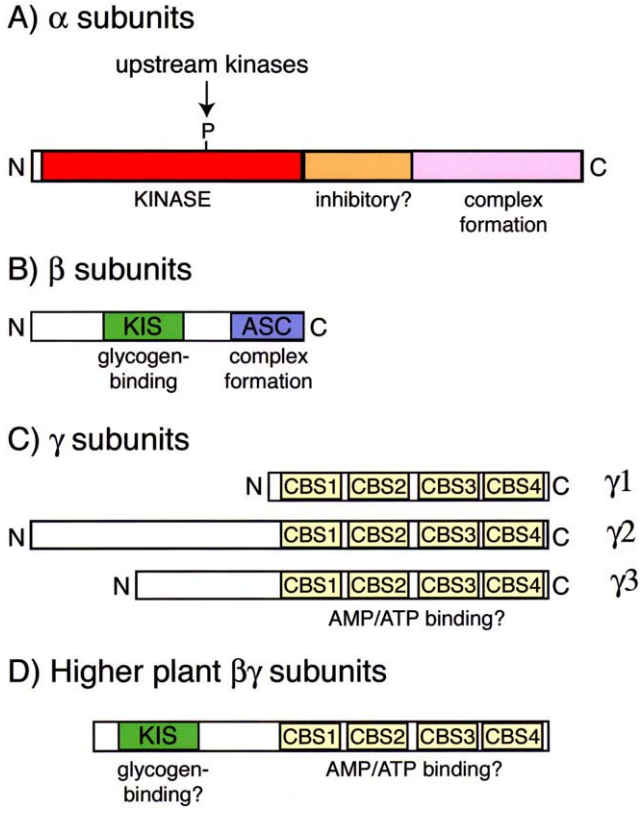

Fig. 2. Diagram showing the location of conserved domains in the (A) α subunits, (B) β subunits and (C) γ subunits of mammalian AMPK, and (D) the unusual βγ subunits found in higher plants. The proposed function of each domain is indicated underneath the subunit.

interaction sequence) domain may in fact have a function other than interaction with the kinase subunit. Its sequence is closely related to those of 'N-isoamylase domains' (see the *PFAM* database [14], entry PF02922), which are found in enzymes that metabolise the α1→6 branch points in α1→4-linked glucans such as starch and glycogen. In these enzymes the domains appear not to be catalytic but may instead act to position the enzymes on the substrate prior to catalysis. Our laboratory [13] and others [15] have recently obtained evidence that this domain is indeed involved in binding of glycogen. There is evidence that high cellular glycogen represses activation of AMPK in muscle in vivo [16,17], suggesting that AMPK can act as a 'glycogen sensor' as well as a sensor of AMP and ATP, and it is tempting to suggest that this involves the glycogen-binding domain. However, the regulation of purified AMPK by glycogen in vitro has not yet been convincingly demonstrated. Whatever the function of the glycogen-binding domain, it is conserved in the β subunits across all eukaryotes.

The γ subunits of AMPK contain N-terminal regions that in mammals are very variable in both size and sequence, followed by four tandem repeats of a motif known as a CBS domain (Fig. 2C). These domains of about 50–60 amino acids occur in a variety of different proteins and in all domains of life from archaea to eukaryotes ([18], see also the *PFAM* database [14], entry PF00571). CBS domains invariably occur as tandem pairs, although the γ subunits of AMPK are unique in having two pairs. The structure of a pair of domains from a bacterial IMP dehydrogenase [19] shows that they are intimately associated via hydrophobic interactions and suggests that a single domain would not be stable. Point mutations in CBS domains in other proteins cause a variety of human hereditary diseases [20–24], while six different point mutations occurring in the CBS domains of the AMPK γ2 subunit cause cardiac arrhythmias (ventricular pre-excitation, or Wolff–Parkinson–White syndrome) that can lead to sudden death at a young age from heart failure [25–28]). These observations indicate that CBS domains have important functions, yet they are not understood in any case. Using a photoaffinity analogue of AMP (8-azido-AMP) we obtained evidence that binding of the adenosine portion of AMP by the AMPK complex involves the γ subunit [29]. Since complexes containing any of the three γ subunit isoforms are activated by AMP, and the CBS domains are the only regions that are conserved between them, we believe that they represent the binding sites for the regulatory nucleotides, AMP and ATP. This hypothesis is currently being addressed in our laboratory.

In the higher plant *Arabidopsis thaliana* there is an intriguing variant on the distribution of the domains found in other species. In both maize and *A. thaliana*, genes have been found that encode proteins (denoted βγ in Fig. 1C) containing an N-terminal *KIS* domain and a C-terminal 'γ subunit' region with four CBS domains [30] (Fig. 2D). Intriguingly, genome sequencing also reveals another β subunit homologue in *A. thaliana* (protein ID At2g28060.1, shown as β3 in Fig. 1B) that appears to represent an *ASC* domain without an associated *KIS* domain. However, two genes in *A. thaliana* encoding more conventional β subunits with *KIS* and *ASC* domains (denoted β1 and β2 in Fig. 1B) have also been reported [31]. Direct biochemical analysis is required to determine how these various gene products assemble into complexes in plant cells.

3. Recognition of target proteins by AMPK

The original target proteins identified for AMPK were the lipid biosynthetic enzymes 3-hydroxy-3-methylglutaryl-CoA reductase [32,33] and the ACC1 (α) isoform of acetyl-CoA carboxylase [34,35]. Comparison of sites phosphorylated on these and other targets revealed conserved sequence motifs, especially hydrophobic residues at P−5 and P+4 (i.e. five residues N-terminal, or four residues C-terminal, to the phosphorylated amino acid) and a single basic residue at P−3 or P−4. The importance of these residues was confirmed [36,37] using a series of variants of two related peptides, one of which was the *SAMS* peptide, a 15 residue peptide based on the sequence around Ser-79 on rat ACC1 that has become the 'gold standard' substrate for AMPK assays [38]. The core recognition motif for AMPK defined by these studies was Hyd-(Basic, X)-X-X-Ser/Thr-X-X-X-Hyd, where Hyd is a hydrophobic residue (Leu, Met, Ile, Phe or Val), Basic is Arg, Lys or His (which can be at P−3 or P−4) and 'X' is any amino acid.

Molecular modelling of a peptide substrate bound to the kinase domain of AMPK (based on crystal structures for other kinase domains with bound inhibitory peptides) suggested that the interaction might in fact occur over a wider region of the substrate than this nine residue motif [10]. In particular, the model suggested that binding of substrate residues N-terminal to the P−5 position occurred via an amphipathic helix that fits into a hydrophobic groove on the large lobe of the kinase. Near the Ser-79 site on ACC1 there are hydrophobic residues not only at P−5 but also at P−9, P−13 and P−16 (Fig. 3A), and these would all lie on the same face of an α-helix. This model was tested using a recombinant protein in which residues 60–94 from the rat ACC1 sequence were expressed in bacteria as a fusion with glutathione *S*-transferase [10]. The protein containing the wild type sequence was an excellent substrate, while point mutations of the hydrophobic residues at P−5, P−9, P−13 and P−16 caused large decreases in k_{cat}/K_m. The predicted hydrophobic interaction between the methionine at P−5 on the substrate and a leucine (Leu-212) in the hydrophobic groove on the kinase was confirmed by making complementary mutations that converted it into an electrostatic interaction. Further support for our model came with the publication of the crystal structure of human HMG-CoA reductase [39], because the residues from Gly-860 (P−12) to Arg-871 (P−1) form three turns of α-helix, with the side chains of Val-863 (P−9) and Met-867 (P−5) pointing outwards into the solvent and therefore in a position accessible to interact with the hydrophobic groove on the kinase.

Our model [10] also suggested that the substrate arginine at P−4 bound to an acidic patch on the kinase domain formed by Glu-100 and Asp-103, while the histidine in the P−6 position (the N-terminal residue in the *SAMS* peptide, and previously only recognised as a positive determinant for the higher plant homologue [40]) bound to another acidic patch formed by Asp-215 and Asp-217. The importance of these interactions was confirmed by making mutations on both the substrate and the kinase. The only part of the original recognition motif whose interaction with the kinase domain could not be modelled precisely was the P+4 hydrophobic residue, although we did confirm by mutation that glutamine is accepted at this position, as found in some target proteins,

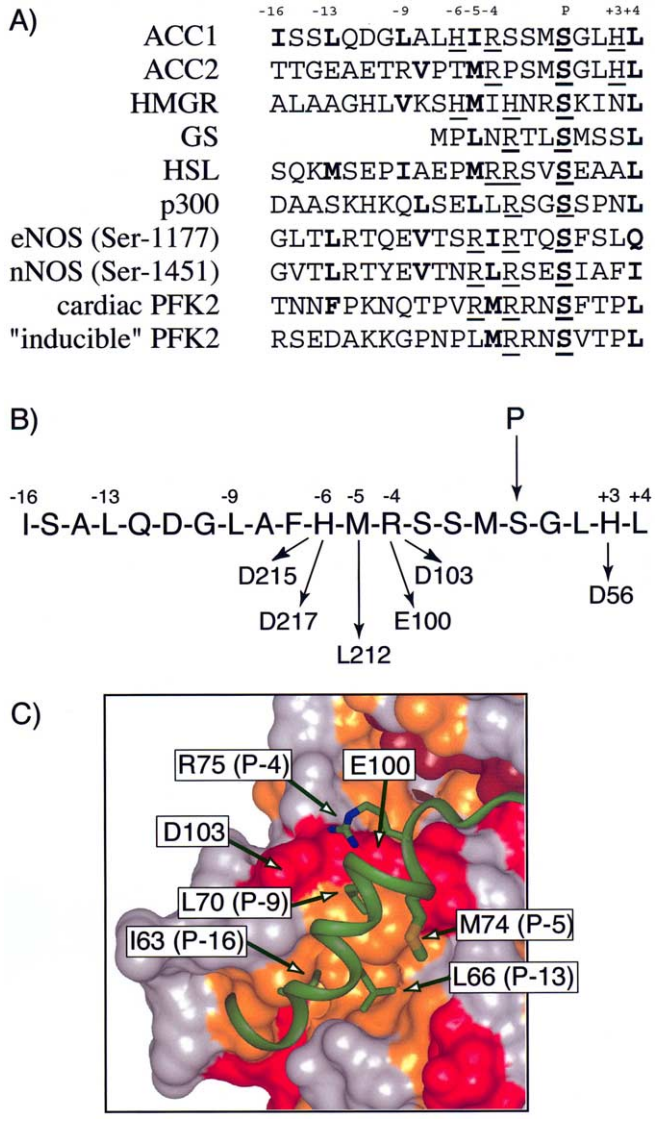

Fig. 3. A: Alignment of sequences (from humans) around target sites for AMPK on the ACC1 and ACC2 isoforms of acetyl-CoA carboxylase, HMG-CoA reductase (HMGR), glycogen synthase, hormone-sensitive lipase (HSL), the co-activator p300, the endothelial and neuronal isoforms of NO synthase (eNOS and nNOS) and the cardiac and 'inducible' isoforms of 6-phosphofructo-2-kinase (PFK2). The phosphorylated serine is shown in bold and underlined, while basic and hydrophobic residues believed to be involved in recognition are highlighted by underlining and bold type respectively. B: Sequence (single letter code) around Ser-79 on rat ACC1, showing the residues involved in recognition by AMPK, with arrows pointing to the residues (numbered) on the kinase domain with which they are proposed to interact. The phosphorylated serine is marked with 'P' and an arrow. C: View of model of a protein substrate bound to the kinase domain of AMPK [10]. The substrate is shown as a green backbone 'ribbon' with selected side chains shown in 'wireframe', while the kinase domain is shown in 'spacefill' mode with acidic residues in red and hydrophobic residues in orange. This view highlights how the arginine side chain at P−4 on the substrate (R75) interacts with an acidic patch formed by Glu-100 (E100) and Asp-103 (D103), while residues N-terminal to it form an amphipathic helix with hydrophobic side chains, i.e. Met-74 (M74), Leu-70 (L70), Leu-66 (L66) and Ile-63 (I63) on one face. The helix fits into a hydrophobic groove on the large lobe of the kinase domain.

e.g. endothelial nitric oxide synthase (eNOS) (Fig. 3B). The model also suggested that His-82 (at P+3, not previously recognised as a positive determinant and only found in ACC1 and ACC2) formed an electrostatic interaction with Asp-56 on the small lobe of the kinase domain. This was confirmed by performing 'charge swap' mutations. The extended substrate recognition determinants derived from this study (together with information about the residues on the kinase domain with which they interact) are shown in Fig. 3A, and an alignment of the sequences around a few physiological target sites in Fig. 3B. The latter reveals that only ACC1 seems to have all of these determinants, but that most sites have at least a sub-set of them. It is also interesting that in the NOS and 6-phosphofructo-2-kinase (PFK2) isoforms the basic–hydrophobic–basic motif usually found in the P−6 to P−4 position is displaced towards the phosphorylated serine by one residue, suggesting that some flexibility in the manner of substrate binding is allowed.

4. Regulation of AMPK in vivo and in intact cells

Because of its reciprocal regulation by AMP and ATP, mammalian AMPK is activated in intact cells by any stress that either increases ATP consumption or inhibits ATP pro-

duction. These include heat shock, metabolic inhibitors such as arsenite or oligomycin, hypoxia or ischaemia, and glucose deprivation (reviewed in [1], see also [41–43]). These can all be regarded as pathological stresses, but more recently a number of more physiological stimuli have been found to activate AMPK, including exercise and contraction in skeletal muscle [1] and the peptide hormones, leptin and adiponectin [44,45]. These findings that the system is activated by exercise, and by 'adipocytokines' involved in control of appetite, body weight and insulin sensitivity, point to its potential importance in the treatment of the current world-wide 'epidemic' of obesity and type 2 diabetes. Indeed, AMPK is activated in intact cells by two of the main classes of drug currently used to treat type 2 diabetes, i.e. the biguanides (e.g. metformin) and the thiazolidinediones (e.g. rosiglitazone) [41,46,47].

Identification of targets for the system in vivo required the development of methods to manipulate the activity of the system other than cellular stresses that deplete ATP, which might have many secondary consequences unrelated to AMPK activation. The first method available was the incubation of cells with the nucleoside analogue 5-aminoimidazole-4-carboxamide (AICA) riboside, which is taken up by cells and converted to the respective monophosphate, ZMP, an analogue of AMP. While still very widely used, this method is not completely specific in that ZMP is an effector of other enzymes that are regulated by AMP, such as phosphorylase and fructose-1,6-bisphosphatase. Incubation of cells with the anti-diabetic drug metformin represents a second method to activate the kinase in intact cells. Metformin does not deplete ATP and the mechanism by which it activates AMPK remains unknown [41,47]. However, since it clearly works by a different mechanism from AICA riboside it is a useful adjunct to the latter. As yet there are no readily available, specific pharmacological inhibitors of AMPK, although expression of a kinase-inactive mutant α subunit acts as a dominant negative inhibitor. This approach works because the α subunit is unstable in the absence of β and γ, so that if an inactive α mutant is over-expressed it replaces the endogenous, active α subunit because the maximal level of α subunit expression is limited by the availability of β and γ [48].

Another approach to studying physiological functions in vivo is the use of transgenic mice. As yet there are no reports of α1 knockout mice or of mice lacking both catalytic subunits, but results with mice having a germ-line disruption of the α2 gene have recently been presented [49]. Intriguingly, they have impaired glucose tolerance (i.e. a larger increase in blood glucose following oral intake) and impaired insulin sensitivity (i.e. a reduced whole body glucose turnover during a clamp procedure when insulin is raised but blood glucose kept constant). These are both diagnostic features of diabetes, although in other respects the mice do not display typical features of the disease. In particular, their fasting blood glucose, insulin release by isolated pancreatic islets and insulin-stimulated glucose uptake by isolated muscles were all normal. Moreover, the glucose intolerance of the knockout mice could be rescued by injection of the α-adrenergic blocker, phentolamine, and catecholamines were increased in urine. The authors therefore suggested that the effects of the α2 knockout on glucose tolerance and insulin sensitivity were secondary to an increase in catecholamine release via an unknown mechanism [49].

A complicating factor with the α2 knockout mice was that the expression of α1 was up-regulated, at least in muscle [49], and this might be partly compensating for the lack of α2. Birnbaum and co-workers have created mice that express, from a muscle-specific promoter, a kinase-inactive α1 subunit that acts as a dominant negative mutant (see above) [50,51]. In these 'quasi-knockout' mice the AMPK activity in muscle was undetectable, and results obtained with them are discussed further below.

5. Downstream targets and physiological role of the AMPK system in vivo

Some of the well-established downstream target proteins and pathways for AMPK that have been identified by the methods described in Section 4 are summarised in Fig. 4. As space is limited we will concentrate here on more recent findings, and previous reviews [1,3] should be consulted for earlier literature. In line with its proposed role in the management of cellular energy status, AMPK activation switches off anabolic pathways and other processes that consume ATP, while switching on catabolic pathways that generate ATP. Examples of the former include acute inhibition of lipid biosynthesis by phosphorylation and inactivation of key metabolic enzymes such as ACC1 (fatty acid synthesis), glycerol phosphate acyl transferase (triacylglycerol synthesis), and HMG-CoA reductase (cholesterol/isoprenoid biosynthesis). AMPK also inactivates the muscle isoform of glycogen synthase in cell-free assays [52], and perfusion of rat hind-limb muscle with AICA riboside also produces an inactivation of the enzyme that appears to be due to phosphorylation, although the sites modified have not yet been determined [17]. If AMPK does phosphorylate glycogen synthase in vivo one might expect that the enzyme would become inactivated during exercise, but confirming this was complicated by the fact that exercise causes glycogen breakdown, which relieves feedback inhibition of glycogen synthase by glycogen. However, subjects with McArdle's disease cannot break down glycogen rapidly due to a hereditary lack of phosphorylase, so these

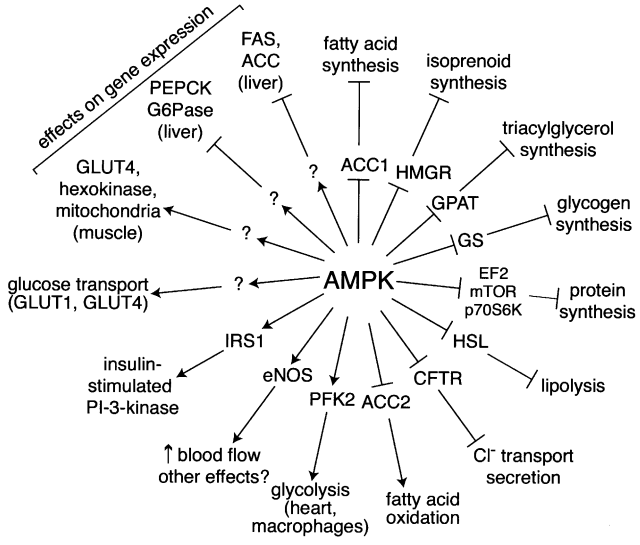

Fig. 4. Known physiological target proteins and pathways regulated by the AMPK system. The list is not exhaustive: other targets have been proposed but in some cases the evidence is not yet conclusive.

confounding effects are eliminated. In such subjects, exercise causes a marked activation of AMPK and concomitant inactivation of glycogen synthase, as expected [53].

Thus, AMPK activation inhibits both lipid and carbohydrate biosynthesis, and recently it has also been found to inhibit protein synthesis in isolated hepatocytes via phosphorylation of elongation factor-2 brought about by an activation of EF-2 kinase [54]. Although these authors found no effect on phosphorylation of 4E-BP1 (a protein that regulates *initiation* of translation) another group did report that in muscle of rats injected with AICA riboside, inhibition of protein synthesis correlated with reduced phosphorylation of 4E-BP1 at a site whose phosphorylation activates initiation of translation [55]. There was also reduced phosphorylation of other protein kinases, including mTOR and p70 S6 kinase (at Thr-389). Phosphorylation of p70 S6 kinase by mTOR at Thr-389 is thought to be involved in activation of the kinase, leading to phosphorylation of ribosomal protein S6, thus stimulating translation of '5′-TOP' mRNAs encoding ribosomal proteins and elongation factors. A problem with the interpretation of this paper [55] is that it is difficult to know whether the effects of AICA riboside were direct effects of muscle or indirect effects on the levels of other circulating factors. However, Hue's group have reported that the amino acid-induced activation of p70 S6 kinase (involving increased phosphorylation of Thr-389) in isolated hepatocytes was blocked by AICA riboside [56].

Another recently identified target of AMPK is the cystic fibrosis transmembrane conductance regulator (CFTR), the product of the gene that is mutated in cystic fibrosis. CFTR is a Cl^- channel involved in transepithelial ion transport and fluid secretion, whose open probability is increased by phosphorylation by cyclic AMP-dependent protein kinase. Since opening and closing of the channel requires turnover of ATP, and the transepithelial ion transport that it brings about also requires ATP hydrolysis by the plasma membrane Na^+/K^+ pump, inhibition of CFTR would conserve ATP. Hallows' group [57] reported that CFTR interacted with the α subunits of AMPK and was phosphorylated by AMPK in cell-free assays. When the two proteins were co-expressed in *Xenopus laevis* oocytes the cyclic AMP-stimulated Cl^- conductance was also reduced. More recently, the same group reported that activation of AMPK inhibited cyclic AMP-stimulated Cl^- currents in cells derived from human lung and colon, and that in several types of secretory epithelia, AMPK was partly co-localised with CFTR at the apical membrane [58,59].

Examples of the manner in which AMPK activation can generate ATP by stimulating catabolism include its stimulation of fatty acid oxidation via phosphorylation of ACC2, and its stimulation of glucose uptake via activation of both glucose transporter (GLUT) 1 and GLUT4 (reviewed in [1], see also [60]). Although the target protein(s) whose phosphorylation regulates glucose uptake are not known, AICA riboside-stimulated glucose uptake by muscle is completely abolished in the 'quasi-knockout' mice lacking functional AMPK [51], confirming that AMPK is involved. Another recently found mechanism by which ATP generation is increased by AMPK is the phosphorylation of PFK2, which stimulates the production of fructose-2,6-bisphosphate, a physiological activator of the key enzyme of glycolysis, 6-phosphofructo-1-kinase. There are four isoforms of PFK2, but only the cardiac isoform and the so-called inducible isoform are targets for AMPK. Phosphorylation of the cardiac isoform represents a mechanism to stimulate glycolytic production of ATP during ischaemia or hypoxia in the heart, a new mechanism to explain the classical 'Pasteur effect' [42]. Expression of the 'inducible' isoform is switched on in monocytes by bacterial lipopolysaccharide. Phosphorylation and activation of this isoform by AMPK [43] may be a mechanism that allows monocytes to produce more ATP by glycolysis, and thus operate in the often hypoxic environment of infected or diseased tissues. Intriguingly, the 'inducible' isoform is also expressed by many tumour cells [61], suggesting that activation of PFK2 by AMPK could be partly responsible for their high glycolytic rate (the 'Warburg effect').

Another physiological target of AMPK is NOS, of which both the endothelial and neuronal isoforms (eNOS and nNOS) appear to be phosphorylated, although only the former has been shown to be activated [62]. Phosphorylation of eNOS has been shown to occur in heart muscle during ischaemia [62], and of nNOS in skeletal muscle during exercise [63]. There is evidence that NOS activity is required for the effects of AMPK on glucose uptake in muscle [64] and, since the classical effect of nitric oxide is to increase blood flow by relaxing vascular smooth muscle, another effect of eNOS phosphorylation might be to increase the local blood flow to hypoxic tissues.

The protein IRS-1, which is phosphorylated by the activated insulin receptor and then acts as binding site for SH2 domain-containing proteins such as phosphatidylinositol (PI) 3-kinase, is phosphorylated at Ser-789 in cell-free assays by AMPK, and in a muscle cell line in response to AICA riboside [65]. This phosphorylation appears to increase the binding of PI 3-kinase to the protein, thus potentially accentuating effects of insulin. However, further work is required to confirm whether the insulin signalling pathways downstream of PI 3-kinase are regulated by this mechanism.

Finally, the expression of numerous genes is regulated by AMPK. In a recent microarray study of the 'quasi-knockout' mice expressing dominant negative AMPK in muscle, a total of 234 genes were up-regulated and 130 down-regulated by more than two-fold compared with control mice [66]. In muscle, AMPK activation had already been shown to up-regulate expression of GLUT4, hexokinase, and mitochondrial enzymes, and in the case of GLUT4, experiments with mice expressing a reporter gene from the GLUT4 promoter show that this is an effect on transcription [67]. Similar changes in expression of these genes are seen in response to endurance training, or in response to chronic ATP depletion in muscle by feeding rodents the creatine analogue β-guanidinopropionic acid. Recent experiments with the 'quasi-knockout' mice expressing dominant negative AMPK suggest that the effects of the creatine analogue on mitochondrial biogenesis may be entirely mediated by AMPK [50] (these mice do not tolerate exercise well [51], so the effects of endurance training could not be studied). By up-regulating GLUT4, hexokinase and mitochondrial biogenesis via the AMPK pathway, regular aerobic exercise would increase the capacity of muscle to take up glucose and oxidise glucose and fatty acids, thus making the same bout of exercise less of a metabolic stress on subsequent occasions. Consistent with this, the activation of AMPK in response to the same level of exercise in both rats [68] and humans [69] was reduced after a regime of endurance training.

In liver cells, activation of AMPK decreases the expression of enzymes of gluconeogenesis (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase), both in cell culture [70] and in vivo [45]. Since elevated gluconeogenesis is a major cause of the high blood glucose in type 2 diabetes, this effect is probably a major factor in the success of AMPK-activating drugs in treating this condition. Another class of genes that are switched off in liver are those encoding enzymes of fatty acid biosynthesis (fatty acid synthase and ACC1) [48]. This brings us round 'full circle' in Fig. 4, and emphasises that fatty acid synthesis is switched off both acutely (by phosphorylation of ACC1) and chronically (by reduced expression of fatty acid synthase and ACC1). Fatty acid synthesis can be regarded as a 'luxury' pathway that is used as a means to store excess calories as fat rather than carbohydrate during 'times of plenty', and it is not surprising that it is one of the pathways that is most sensitive to inhibition by AMPK.

At present, the target proteins for AMPK that are responsible for the effects on gene expression are not known, although the co-activator p300 is phosphorylated at Ser-89 (shown in Fig. 3) and this reduces its interaction with nuclear hormone receptors such as peroxisome proliferator-activated receptor-γ (PPAR-γ) [71]. Moreover, AMPK activation has also been shown (by unknown mechanisms) to reduce the *expression* of several important transcription factors, including sterol response element binding protein-1C [46], hepatocyte nuclear factor-4α [72], C/EBPα and PPAR-γ [71].

6. Conclusions

Recent work on the AMPK system fully confirms the prediction made by Atkinson et al. in 1964 [2] that AMP, or the AMP:ATP ratio, regulates metabolic choices between anabolism and catabolism. These nucleotides achieve this, however, not just by direct allosteric regulation of metabolic enzymes as Atkinson had proposed, but also by activation of a complex signalling cascade that regulates gene expression as well as the activity of pre-existing proteins. Since genes encoding α, β and γ subunits related to those of AMPK occur in all eukaryotic genomes, this is likely to be an ancient mechanism, and this also implies that 5′-AMP is an intracellular signal molecule of importance at least equal to that of its more famous cousin, cyclic AMP. A caveat here is that attempts to demonstrate the regulation of non-mammalian homologues of AMPK by AMP, especially those from yeast and higher plants, have as yet not been successful. However, we recently reported conditions in which the *D. melanogaster* homologue is activated by AMP in cell-free assays and, like the mammalian kinase, it was found to be activated by hypoxia, oligomycin and glucose deprivation in intact cells [5]. All eukaryotic γ subunit homologues contain the four CBS domains that we now believe to represent the AMP-binding sites, and we therefore think that the question whether non-mammalian homologues of AMPK are regulated by AMP should be re-examined. Finally, although there are differences in detail, in many respects the functions of the mammalian AMPK complex and the yeast SNF1 complex are similar. The latter is required for the switch from the fermentative metabolism of glucose (using glycolysis to generate ATP) to the use of alternative carbon sources and to oxidative metabolism. Similarly, in mammalian cells the AMPK complex is not required for anaerobic metabolism of endogenous fuel (i.e. glycogen), but it is involved in up-regulating the uptake of glucose and the oxidative metabolism of both glucose and fatty acids.

Acknowledgements: Research in this laboratory was supported by a programme grant from the Wellcome Trust, an RTD Contract (QLG1-CT-2001-01488) from the European Commission, and project grants from the Medical Research Council and Diabetes UK.

References

[1] Hardie, D.G. and Hawley, S.A. (2001) BioEssays 23, 1112–1119.
[2] Ramaiah, A., Hathaway, J.A. and Atkinson, D.E. (1964) J. Biol. Chem. 239, 3619–3622.
[3] Hardie, D.G., Carling, D. and Carlson, M. (1998) Annu. Rev. Biochem. 67, 821–855.
[4] Adam, R.D. (2000) Int. J. Parasitol. 30, 475–484.
[5] Pan, D.A. and Hardie, D.G. (2002) Biochem. J. 367, 179–186.
[6] Hardie, D.G., Salt, I.P., Hawley, S.A. and Davies, S.P. (1999) Biochem. J. 338, 717–722.
[7] Corton, J.M., Gillespie, J.G., Hawley, S.A. and Hardie, D.G. (1995) Eur. J. Biochem. 229, 558–565.
[8] Hawley, S.A., Davison, M., Woods, A., Davies, S.P., Beri, R.K., Carling, D. and Hardie, D.G. (1996) J. Biol. Chem. 271, 27879–27887.
[9] Davies, S.P., Helps, N.R., Cohen, P.T.W. and Hardie, D.G. (1995) FEBS Lett. 377, 421–425.
[10] Scott, J.W., Norman, D.G., Hawley, S.A., Kontogiannis, L. and Hardie, D.G. (2002) J. Mol. Biol. 317, 309–323.
[11] Crute, B.E., Seefeld, K., Gamble, J., Kemp, B.E. and Witters, L.A. (1998) J. Biol. Chem. 273, 35347–35354.
[12] Jiang, R. and Carlson, M. (1997) Mol. Cell. Biol. 17, 2099–2106.
[13] Hudson, E.R. et al. (2003) Curr. Biol. (in press).
[14] Bateman, A. et al. (2002) Nucleic Acids Res. 30, 276–280.
[15] Polekhina, G. et al. (2003) Curr. Biol. (submitted).
[16] Wojtaszewski, J.F., MacDonald, C., Nielsen, J.N., Hellsten, Y., Hardie, D.G., Kemp, B.E., Kiens, B. and Richter, E.A. (2002) Am. J. Physiol. Endocrinol. Metab. (10.1152/ajpendo.00436.2002).
[17] Wojtaszewski, J.F.P., Jørgensen, S.B., Hellsten, Y., Hardie, D.G. and Richter, E.A. (2002) Diabetes 51, 284–292.
[18] Bateman, A. (1997) Trends Biochem. Sci. 22, 12–13.
[19] Zhang, R., Evans, G., Rotella, F.J., Westbrook, E.M., Beno, D., Huberman, E., Joachimiak, A. and Collart, F.R. (1999) Biochemistry 38, 4691–4700.
[20] Bowne, S.J. et al. (2002) Hum. Mol. Genet. 11, 559–568.
[21] Kennan, A. et al. (2002) Hum. Mol. Genet. 11, 547–557.
[22] Pusch, M. (2002) Hum. Mutat. 19, 423–434.
[23] Lloyd, S.E. et al. (1997) Hum. Mol. Genet. 6, 1233–1239.
[24] Konrad, M. et al. (2000) J. Am. Soc. Nephrol. 11, 1449–1459.
[25] Gollob, M.H., Seger, J.J., Gollob, T.N., Tapscott, T., Gonzales, O., Bachinski, L. and Roberts, R. (2001) Circulation 104, 3030–3033.
[26] Gollob, M.H. et al. (2001) New Engl. J. Med. 344, 1823–1831.
[27] Blair, E. et al. (2001) Hum. Mol. Genet. 10, 1215–1220.
[28] Arad, M. et al. (2002) J. Clin. Invest. 109, 357–362.
[29] Cheung, P.C.F., Salt, I.P., Davies, S.P., Hardie, D.G. and Carling, D. (2000) Biochem. J. 346, 659–669.
[30] Lumbreras, V., Alba, M.M., Kleinow, T., Koncz, C. and Pages, M. (2001) EMBO Rep. 2, 55–60.
[31] Bouly, J.P., Gissot, L., Lessard, P., Kreis, M. and Thomas, M. (1999) Plant J. 18, 541–550.
[32] Beg, Z.H., Allmann, D.W. and Gibson, D.M. (1973) Biochem. Biophys. Res. Comm. 54, 1362–1369.
[33] Clarke, P.R. and Hardie, D.G. (1990) EMBO J. 9, 2439–2446.
[34] Carlson, C.A. and Kim, K.H. (1973) J. Biol. Chem. 248, 378–380.
[35] Sim, A.T.R. and Hardie, D.G. (1988) FEBS Lett. 233, 294–298.
[36] Weekes, J., Ball, K.L., Caudwell, F.B. and Hardie, D.G. (1993) FEBS Lett. 334, 335–339.
[37] Dale, S., Wilson, W.A., Edelman, A.M. and Hardie, D.G. (1995) FEBS Lett. 361, 191–195.
[38] Davies, S.P., Carling, D. and Hardie, D.G. (1989) Eur. J. Biochem. 186, 123–128.

[39] Istvan, E.S., Palnitkar, M., Buchanan, S.K. and Deisenhofer, J. (2000) EMBO J. 19, 819–830.
[40] Toroser, D. and Huber, S.C. (1998) Arch. Biochem. Biophys. 355, 291–300.
[41] Hawley, S.A., Gadalla, A.E., Olsen, G.S. and Hardie, D.G. (2002) Diabetes 51, 2420–2425.
[42] Marsin, A.S. et al. (2000) Curr. Biol. 10, 1247–1255.
[43] Marsin, A.S., Bouzin, C., Bertrand, L. and Hue, L. (2002) J. Biol. Chem. 277, 30778–30783.
[44] Minokoshi, Y., Kim, Y.B., Peroni, O.D., Fryer, L.G., Muller, C., Carling, D. and Kahn, B.B. (2002) Nature 415, 339–343.
[45] Yamauchi, T. et al. (2002) Nat. Med. 6, 1288–1295.
[46] Zhou, G. et al. (2001) J. Clin. Invest. 108, 1167–1174.
[47] Fryer, L.G., Parbu-Patel, A. and Carling, D. (2002) J. Biol. Chem. 277, 25226–25232.
[48] Woods, A., Azzout-Marniche, D., Foretz, M., Stein, S.C., Lemarchand, P., Ferre, P., Foufelle, F. and Carling, D. (2000) Mol. Cell. Biol. 20, 6704–6711.
[49] Viollet, B. et al. (2003) Biochem. Soc. Trans. 31, 216–219.
[50] Zong, H., Ren, J.M., Young, L.H., Pypaert, M., Mu, J., Birnbaum, M.J. and Shulman, G.I. (2002) Proc. Natl. Acad. Sci. USA 99, 15983–15987.
[51] Mu, J., Brozinick, J.T., Valladares, O., Bucan, M. and Birnbaum, M.J. (2001) Mol. Cell 7, 1085–1094.
[52] Carling, D. and Hardie, D.G. (1989) Biochim. Biophys. Acta 1012, 81–86.
[53] Nielsen, J.N. et al. (2003) Biochem. Soc. Trans. 31, 186–190.
[54] Horman, S. et al. (2002) Curr. Biol. 12, 1419–1423.
[55] Bolster, D.R., Crozier, S.J., Kimball, S.R. and Jefferson, L.S. (2002) J. Biol. Chem. 277, 23977–23980.
[56] Krause, U., Bertrand, L. and Hue, L. (2002) Eur. J. Biochem. 269, 3751–3759.
[57] Hallows, K.R., Raghuram, V., Kemp, B.E., Witters, L.A. and Foskett, J.K. (2000) J. Clin. Invest. 105, 1711–1721.
[58] Hallows, K.R., Kobinger, G.P., Wilson, J.M., Witters, L.A. and Foskett, J.K. (2003) Am. J. Physiol. Cell Physiol. (in press).
[59] Hallows, K.R., McCane, J.E., Kemp, B.E., Witters, L.A. and Foskett, J.K. (2003) J. Biol. Chem. 278, 998–1004.
[60] Fryer, L.G., Foufelle, F., Barnes, K., Baldwin, S.A., Woods, A. and Carling, D. (2002) Biochem. J. 363, 167–174.
[61] Chesney, J. et al. (1999) Proc. Natl. Acad. Sci. USA 96, 3047–3052.
[62] Chen, Z.P. et al. (1999) FEBS Lett. 443, 285–289.
[63] Chen, Z.P., McConell, G.K., Michell, B.J., Snow, R.J., Canny, B.J. and Kemp, B.E. (2000) Am. J. Physiol. 279, E1202–E1206.
[64] Fryer, L.G.D., Hajduch, E., Rencurel, F., Salt, I.P., Hundal, H.S., Hardie, D.G. and Carling, D. (2000) Diabetes 49, 1978–1985.
[65] Jakobsen, S.N., Hardie, D.G., Morrice, N. and Tornqvist, H.E. (2001) J. Biol. Chem. 276, 46912–46916.
[66] Mu, J., Barton, E.R. and Birnbaum, M.J. (2003) Biochem. Soc. Trans. 31, 236–241.
[67] Zheng, D., MacLean, P.S., Pohnert, S.C., Knight, J.B., Olson, A.L., Winder, W.W. and Dohm, G.L. (2001) J. Appl. Physiol. 91, 1073–1083.
[68] Durante, P.E., Mustard, K.J., Park, S.H., Winder, W.W. and Hardie, D.G. (2002) Am. J. Physiol. 283, E178–E186.
[69] Nielsen, J.N. et al. (2003) J. Appl. Physiol. 94, 631–641.
[70] Lochhead, P.A., Salt, I.P., Walker, K.S., Hardie, D.G. and Sutherland, C. (2000) Diabetes 49, 896–903.
[71] Habinowski, S.A. and Witters, L.A. (2001) Biochem. Biophys. Res. Commun. 286, 852–856.
[72] Leclerc, I., Lenzner, C., Gourdon, L., Vaulont, S., Kahn, A. and Viollet, B. (2001) Diabetes 50, 1515–1521.