

# The AMP-activated protein kinase pathway – new players upstream and downstream

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## Summary

The AMP-activated protein kinase (AMPK) cascade is a sensor of cellular energy status. Whenever the cellular ATP:ADP ratio falls, owing to a stress that inhibits ATP production or increases ATP consumption, this is amplified by adenylate kinase into a much larger increase in the AMP:ATP ratio. AMP activates the system by binding to two tandem domains on the  $\gamma$  subunits of AMPK, and this is antagonized by high concentrations of ATP. AMP binding causes activation by a sensitive mechanism involving phosphorylation of AMPK by the tumour suppressor LKB1. Once activated, AMPK switches on catabolic pathways that generate ATP while switching off ATP-consuming processes. As well as acting at the level of

the individual cell, the system also regulates food intake and energy expenditure at the whole body level, in particular by mediating the effects of hormones and cytokines such as leptin, adiponectin and ghrelin. A particularly interesting downstream target recently identified is TSC2 (tuberin). The LKB1→AMPK→TSC2 pathway negatively regulates the target of rapamycin (TOR), and this appears to be responsible for limiting protein synthesis and cell growth, and protecting against apoptosis, during cellular stresses such as glucose starvation.

Key words: AMP-activated protein kinase, LKB1, TSC2

## Introduction

A useful analogy can be drawn between the ubiquitous cellular nucleotides, ATP and ADP, and the chemicals in an electrical cell or battery. Living cells normally maintain a high ratio of ATP to ADP (typically around 10:1), which is many orders of magnitude away from the equilibrium ratio for ATP hydrolysis under cellular conditions. This represents a store of energy that can be used to drive energy-requiring processes. Catabolism (and photosynthesis when available) ‘charges up the battery’ by converting ADP to ATP, whereas most other cellular processes require ATP hydrolysis and tend to discharge the battery (Fig. 1). Most cells manage to maintain their ATP:ADP ratio within fairly narrow limits, which indicates that the rate of ATP synthesis exactly matches the rate of ATP consumption. How do they achieve this remarkable feat? In mammalian cells, it has recently become clear that the AMP-activated protein kinase (AMPK) system plays a crucial role.

One might expect that this system would respond to changes in the cellular ADP:ATP ratio. However, if the reaction catalysed by adenylate kinase ( $2\text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$ ) is maintained close to equilibrium (which appears to be the case in most eukaryotic cells), one can easily show that the AMP:ATP ratio varies as the square of the ADP:ATP ratio (Hardie and Hawley, 2001). The former ratio is therefore a much more sensitive indicator of cellular energy status than the latter, and is the parameter that is monitored by the AMPK system (Fig. 1).

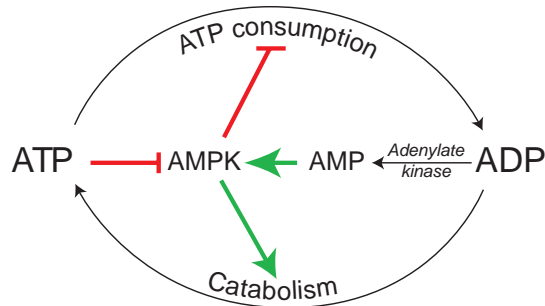
The AMPK field is developing very rapidly, and this Commentary cannot hope to be comprehensive. I therefore focus on recent developments, particularly the identification of new players acting upstream and downstream of AMPK.

Readers who are particularly interested in aspects related to medicine or physiology should consult other recent reviews (Hardie, 2003; Hardie et al., 2003; Carling, 2004; Hardie, 2004a; Hardie, 2004b).

## Structure of the AMPK complex

Mammalian AMPKs are heterotrimeric complexes consisting of a catalytic  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits (Hardie et al., 2003), each of which is encoded by two or three distinct genes ( $\alpha 1$ ,  $\alpha 2$ ;  $\beta 1$ ,  $\beta 2$ ;  $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 3$ ) (Fig. 2). All possible combinations of isoforms appear to be able to form complexes and, along with splice variants and the use of alternative promoters, this leads to a diverse array of different AMPK complexes. Although the mammalian kinase is the only example that is well characterized at the biochemical level, genes encoding orthologues of the  $\alpha$ ,  $\beta$  and  $\gamma$  subunits are found in all eukaryotic species whose genome sequences have been determined. This even includes the primitive parasite *Giardia lamblia* (Hardie et al., 2003), which lacks mitochondria and, according to molecular phylogenies, is more than twice as distant from humans as is *Saccharomyces cerevisiae* (Adam, 2000). Thus, the existence of AMPK orthologues appears to be a fundamental feature of all eukaryotic cells.

What are the functions of the three subunits? The  $\alpha 1$  and  $\alpha 2$  subunits contain a conventional kinase domain at the N-terminus (Fig. 2), and a phylogenetic tree of the >500 such domains encoded in the human genome (Manning et al., 2002) reveals that these lie on a distinct branch containing eleven others (Fig. 3) that we now refer to as the AMPK-related kinase

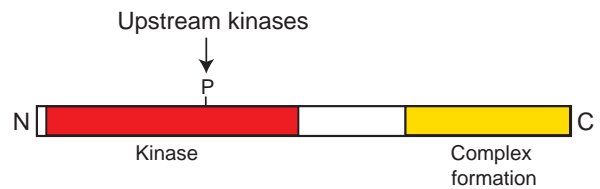


**Fig. 1.** Physiological role of AMPK in the cell. Catabolism ‘charges up the battery’ by converting ADP to ATP (bottom curved arrow) whereas ATP-consuming processes convert ATP to ADP (top curved arrow). If a cellular stress causes the rate of catabolism to fail to keep pace with the rate of ATP consumption, ADP levels will rise and ATP levels will fall. ADP is converted into AMP by adenylate kinase and this, combined with the fall in ATP, will activate AMPK. AMPK then promotes the restoration of energy balance by stimulating catabolism and inhibiting ATP-consuming processes.

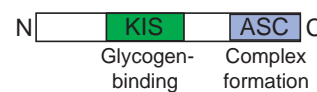
family (Lizcano et al., 2004). The C-terminal regions of the  $\alpha$  subunits are required to form a complex with the  $\beta$  and  $\gamma$  subunits (Crute et al., 1998). Comparison of  $\beta$  subunit sequences from different species revealed that they contained two conserved regions originally termed the KIS and ASC domains (Jiang and Carlson, 1997). Recent work has shown that, in mammals, the ASC domain is sufficient for the formation of a complex with the  $\alpha$  and  $\gamma$  subunits, whereas the KIS domain is not (as previously thought) involved in subunit interactions but is in fact a glycogen-binding domain (GBD) (Hudson et al., 2003) (Fig. 2). According to the protein families (Pfam) database, the GBD is a member of the isoamylase-N domain family (see entry PF02922 at <http://www.sanger.ac.uk/Software/Pfam/>). These non-catalytic domains are usually found in enzymes that metabolize the  $\alpha 1 \rightarrow 6$  branch points in  $\alpha 1 \rightarrow 4$ -linked glucans, such as glycogen and starch. Two lines of evidence confirmed that the GBD was indeed involved in glycogen binding. First, when the  $\alpha$ ,  $\beta$  and  $\gamma$  subunits are co-expressed in cultured human cells with a green fluorescent protein (GFP) tag on  $\alpha$ , fluorescence is associated with glycogen-containing granules in the cytoplasm, but this is abolished if the GBD is deleted from the  $\beta$  subunit (Hudson et al., 2003). Second, the GBD from rat  $\beta 1$  synthesized in bacteria binds to glycogen in cell-free assays (Polekhina et al., 2003).

The exact function(s) of the GBD remains unclear, although there are several experimental findings linking AMPK with glycogen; however, these observations are currently difficult to synthesize into a single, all-encompassing hypothesis. The GBD does cause a partial localization of AMPK to glycogen particles, where one of its known downstream targets – glycogen synthase – resides (Hudson et al., 2003). There is also indirect evidence that glycogen regulates AMPK activity: in both rat (Wojtaszewski et al., 2002) and human (Wojtaszewski et al., 2003) skeletal muscle, a high content of glycogen represses activation of AMPK. This makes physiological sense because if muscle glycogen content is high it tends to be used preferentially as fuel and, although AMPK activation stimulates the usage by muscle of alternative fuels such as blood glucose and fatty acids, it is not required for glycogen breakdown or glycolysis. As yet, repression of AMPK

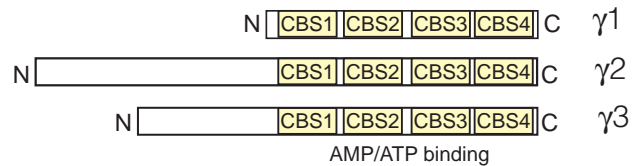
### A $\alpha$ subunits



### B $\beta$ subunits

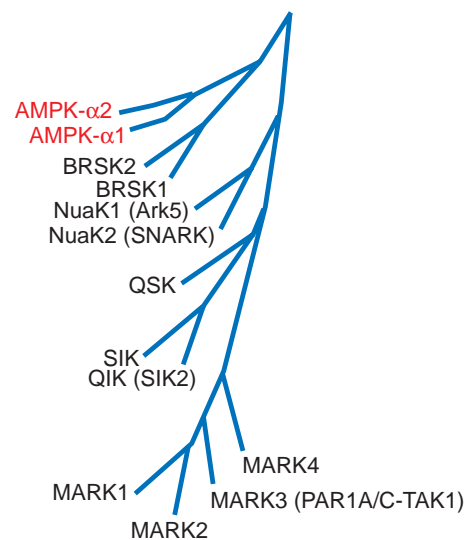


### C $\gamma$ subunits



**Fig. 2.** Conserved domains in AMPK subunits: (A)  $\alpha$  subunits, (B)  $\beta$  subunits and (C)  $\gamma$  subunits. The proposed function of each domain is indicated. The two isoforms of the  $\alpha$  ( $\alpha 1$ ,  $\alpha 2$ ) and  $\beta$  ( $\beta 1$ ,  $\beta 2$ ) subunits have very similar structures, but the three isoforms of the  $\gamma$  subunit ( $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 3$ ) contain variable N-terminal regions of unknown function, and are drawn separately. [Redrawn from Hardie et al. (Hardie et al., 2003).]

activation by glycogen has not been reproduced in a cell-free system; so the molecular mechanism remains unclear. Given the ample evidence (discussed further below) that the AMPK system senses the immediate availability of cellular energy in the form of adenine nucleotides, an attractive hypothesis is that it can also sense longer-term reserves in the form of glycogen. Whatever its functions, the GBD is found universally in the  $\beta$  subunit orthologues of all eukaryotes, which suggests that its function(s) are ancient and important.



**Fig. 3.** Phylogenetic tree of the AMPK-related protein kinases. [Based on Manning et al. (Manning et al., 2002).]

Following their variable N-terminal regions, the  $\gamma$  subunits of AMPKs contain four tandem repeats of a sequence motif first identified by Bateman as a 'CBS domain' (Bateman, 1997) (Fig. 2). These motifs contain about 60 residues and are found in various proteins in archaea, bacteria and eukaryotes: the acronym derives from the enzyme cystathionine  $\beta$ -synthase, which has a pair of such motifs at the C-terminus. They invariably occur as tandem pairs, and the crystal structure of a bacterial inosine-5'-monophosphate (IMP) dehydrogenase (Zhang et al., 1999) shows that the repeats in a pair associate closely together through hydrophobic interactions. As it is now clear that the basic functional unit is a dimer, Kemp has suggested the term 'Bateman domain' to refer to the structure formed by two tandem repeats (Kemp, 2004). Initially, the function(s) of Bateman domains was not known, but recent studies have revealed that Bateman domains in the  $\gamma$  subunits represent the regulatory AMP- and ATP-binding sites of the AMPK complex (Scott et al., 2004). The N-terminal and C-terminal Bateman domains from  $\gamma 2$  can each bind one molecule of AMP or ATP in a mutually exclusive manner, whereas a construct containing both Bateman domains binds two molecules of AMP or ATP with strong positive cooperativity. The finding that there are two binding sites for AMP and ATP in a single  $\alpha\beta\gamma$  complex had not been anticipated. Strong evidence that the Bateman domains are indeed the regulatory AMP- and ATP-binding sites came from studies of mutations in  $\gamma 2$  that cause a human heart condition, Wolff-Parkinson-White syndrome. All five mutations caused both defective binding of AMP to the isolated Bateman domains (Scott et al., 2004) and defective activation of the intact  $\alpha\beta\gamma$  complex by AMP (Daniel and Carling, 2002; Scott et al., 2004), and the order of severity of the individual mutations was the same for both parameters.

Bateman domains from other proteins bind other adenosine derivatives (Scott et al., 2004). Those of IMP dehydrogenase and CLC chloride channels bind ATP, whereas those of cystathionine  $\beta$ -synthase bind *S*-adenosyl methionine, the common feature being the binding of an adenosine-containing ligand that activates protein function. Intriguingly, in all of these proteins, point mutations that interfere with ligand binding cause a variety of human hereditary diseases ranging from retinitis pigmentosa to epilepsy (Scott et al., 2004).

### Regulation of the AMPK complex

AMPK is activated by 5'-AMP in three distinct ways, all of which are antagonized by high concentrations of ATP.

First, AMP binding causes allosteric activation of mammalian AMPK. The magnitude of this effect varies between AMPK isoforms but is typically fivefold or less. A puzzling finding is that, although the  $\gamma$  subunits of the yeast and plant orthologues contain two Bateman domains that are very similar to those of the mammalian enzyme, allosteric activation of the yeast and plant kinases has not been demonstrated. This might simply be due to technical problems: because adenylate kinase converts ADP formed in the kinase assay to AMP, one cannot demonstrate AMP activation unless adenylate kinase is completely removed. AMP does activate the AMPK orthologue from *Drosophila* (Pan and Hardie, 2002), which shows that this regulatory mechanism is at least conserved between mammals and insects.

Second, AMP binding makes AMPK a better substrate for the upstream kinase. The upstream kinase, recently identified as the tumour suppressor LKB1 (see below), activates the kinase by phosphorylation of the  $\alpha$  subunit at a specific threonine residue (Thr172) (Hawley et al., 1996) in the so-called activation loop, within which phosphorylation causes activation of many other protein kinases. As phosphorylation of Thr172 causes at least 50- to 100-fold activation, it is quantitatively much more important than the allosteric activation. Like the allosteric effect, activation by the upstream kinase is stimulated by AMP and inhibited by high concentrations of ATP (Hawley et al., 1996). AMP stimulation is only observed if the intact  $\alpha\beta\gamma$  complex, and not the isolated kinase domain (which lacks the AMP-binding sites), is used as the substrate (Hawley et al., 2003). This shows that the effect is due to binding of AMP to the substrate, AMPK, and not to the upstream kinase. The critical threonine residue, and the sequence around it, is conserved in the  $\alpha$  subunit sequences of AMPK orthologues from all eukaryotes, and mutation of the equivalent threonine residue in the yeast  $\alpha$  subunit (Snf1p) causes a total loss of function in vivo (Estruch et al., 1992). Thus, although it is not yet clear whether the allosteric activation by AMP is conserved in all eukaryotes, the regulation by phosphorylation is certainly conserved.

Third, AMP binding to AMPK also inhibits dephosphorylation of Thr172 by protein phosphatases (Davies et al., 1995). Unlike the allosteric activation, this mechanism of regulation by AMP has also been reported to operate in higher plants (Sugden et al., 1999).

Why should AMP activate the system by three mechanisms, all of which probably involve binding to the same sites on the  $\gamma$  subunit? Computer simulations have revealed that this causes the system to be ultrasensitive; thus, a small change in the initial input (AMP) can produce a much larger relative change in the final output (Hardie et al., 1999). This is an example of multistep sensitivity, which arises when a signal molecule affects more than one step in a cascade (Chock and Stadtman, 1977). Sensitivity in the AMPK system also arises because the upstream kinase has a very low  $K_m$  for AMPK (Hardie et al., 1999), a phenomenon referred to as zero-order ultrasensitivity (Goldbeter and Koshland, 1981). A factor that might increase the sensitivity of the AMPK system even further is the recent finding that the two Bateman domains on the  $\gamma$  subunits bind AMP cooperatively (see above). Overall, this exquisite sensitivity might be very important in the ability of AMPK to maintain the energy state of the cell within narrow limits.

### Mammalian AMPK is activated by stresses that cause ATP depletion

AMPK is therefore activated by any stress that depletes cellular ATP, such as metabolic poisoning, oxidative stress, hypoxia, or nutrient deprivation (Table 1). These can be regarded as pathological stresses that interfere with ATP production. A physiological stress that activates AMPK by increasing ATP consumption is exercise in skeletal muscle (Winder and Hardie, 1996). Indeed, numerous studies now suggest that AMPK is an important mediator of the varied metabolic responses to exercise. For full coverage of this topic, the reader should refer to other reviews (Hardie, 2003; Hardie, 2004a; Hardie, 2004b). It is becoming clear that AMPK may be

**Table 1. Stresses that activate AMPK**

Type of treatment	Treatment	Primary reference
Respiratory chain inhibitors	Antimycin A	Witters et al., 1991
	Azide	Witters et al., 1991
	Nitric oxide	Almeida et al., 2004
ATP synthase inhibitor	Oligomycin	Marsin et al., 2000
Mitochondrial uncouplers	Dinitrophenol	Witters et al., 1991
	Over-expression of UCP1	Matejkova et al., 2004
	Over-expression of UCP3	Schrauwen et al., 2004
TCA cycle inhibitor	Arsenite	Corton et al., 1994
Environmental stresses	Heat shock	Corton et al., 1994
	Oxidative stress	Choi et al., 2001
	Osmotic stress	Fryer et al., 2002
Metabolic stresses	Ischaemia	Kudo et al., 1995
	Hypoxia	Marsin et al., 2000
	Low glucose	Salt et al., 1998
	Exercise (muscle)	Winder and Hardie, 1996
	Contraction (muscle)	Hutber et al., 1997; Vavvas et al., 1997
	Metformin	Zhou et al., 2001
Biguanide drugs	Phenformin	Hawley et al., 2003
	Rosiglitazone	Fryer et al., 2002
Thiazolidinedione drugs	Pioglitazone	Saha et al., 2004

responsible for many of the health benefits of regular exercise, particularly with respect to prevention and treatment of obesity and Type 2 diabetes. Medical interest in the AMPK system was further stimulated by the finding that it is activated by two of the main classes of drugs currently used for treatment of Type 2 diabetes: biguanides and thiazolidinediones (Table 1). Both classes of drug inhibit complex I of the respiratory chain (Owen et al., 2000; Brunmair et al., 2004) and this might be how they activate AMPK, although in the case of metformin it has proved difficult to measure changes in cellular AMP:ATP ratios (Fryer et al., 2002; Hawley et al., 2002). With the possible exception of osmotic stress (Fryer et al., 2002), all of the stresses that activate AMPK are associated with changes in the cellular AMP:ATP ratio.

### Physiological roles of AMPK orthologues in yeast and plants

The *SNF1* (for 'sucrose, non-fermenting') and *SNF4* genes respectively encode the  $\alpha$  and  $\gamma$  subunits of the yeast SNF1 complex, and were originally identified by genetic screens for mutations that prevent growth on sucrose and non-fermentable carbon sources (Hardie et al., 1998). Growth on sucrose requires the expression of the enzyme that breaks it down (invertase), whereas growth on non-fermentable carbon sources requires expression of mitochondrial genes needed for oxidative metabolism. Expression of all of these genes is repressed by glucose, and the *SNF1* and *SNF4* genes are required for their derepression. One mechanism by which this is mediated is the phosphorylation of the repressor protein Mig1p by the SNF1 complex (Smith et al., 1999). Phosphorylation causes Mig1p to bind to a nuclear export protein that promotes its removal from the nucleus (DeVit and Johnston, 1999).

Thus, the primary role of the AMPK orthologue in yeast is in the response to glucose starvation. In cultured cells from mammals (Salt et al., 1998) and *Drosophila* (Pan and Hardie, 2002), AMPK is also activated by glucose deprivation; so this seems to have been an ancient role for the system. In green plants, darkness is the equivalent of starvation: plants

synthesize carbohydrates by photosynthesis during light periods and then rely on breakdown of carbohydrate reserves (in the form of starch) during dark periods. The moss *Physcomitrella patens* has two genes encoding orthologues of the AMPK  $\alpha$  subunit. Disruption of both genes produces a complete failure to grow unless the plants are maintained under constant illumination (Thelander et al., 2004). Thus, the response to starvation also seems to be a fundamental role of AMPK orthologues in green plants.

### Regulation of whole body energy intake and expenditure

Although AMPK orthologues are present in single-celled eukaryotes, and AMPK initially appeared to regulate energy balance in a cell-autonomous manner, it has recently become clear that in multicellular organisms it also plays the same role at the whole body level. The first indications of this came with findings that AMPK mediates stimulation of fatty oxidation in skeletal muscle by leptin (Minokoshi et al., 2002) and adiponectin (Tomas et al., 2002; Yamauchi et al., 2002). These two hormones are so-called adipokines secreted by adipocytes, and are thought to represent signals that body lipid stores are adequate. AMPK activation in muscle also increases glucose uptake (Merrill et al., 1997) and mitochondrial biogenesis (Zong et al., 2002), and leptin and adiponectin would therefore increase energy expenditure by stimulating oxidation of glucose as well as fatty acids in muscle. As well as its effects on muscle, high leptin levels in vivo can cause adipocytes to switch from net synthesis to net oxidation of fatty acids, and this is associated with AMPK activation in the cells (Orci et al., 2004). Thus, AMPK stimulates 'burning' of fat when its storage is excessive (although why this fails in obese individuals remains unclear).

Remarkably, as well as increasing energy expenditure by stimulating fatty acid and glucose oxidation, AMPK also appears to regulate energy intake in mammals. In the regions of the hypothalamus involved in regulation of satiety and food intake, AMPK is inhibited in rodents by high levels of glucose, leptin and insulin, all of which repress food intake, whereas it

is activated by ghrelin, a gut hormone that stimulates food intake (Andersson et al., 2004; Minokoshi et al., 2004). These changes in AMPK activity seem to trigger the subsequent changes in food intake, because direct injection of the AMPK activator 5-aminoimidazole 4-carboxamide (AICA) riboside into the hypothalamus increases food intake (Andersson et al., 2004), whereas expression of activated and dominant-negative inhibitory mutants of AMPK in the hypothalamus stimulates and inhibits food intake, respectively (Minokoshi et al., 2004). One interesting aspect of these findings is that leptin inhibits AMPK in the hypothalamus yet activates it in skeletal muscle, raising interesting questions about the mechanisms involved. It is also intriguing that the AMPK system appears to be involved in increasing food intake in response to lowered blood glucose levels in mammals, just as its orthologue in yeast, the SNF1 complex, is involved in the response to lowered glucose levels in the extracellular medium.

### Identification of the upstream kinase, LKB1-STRAD-MO25

Although it had been clear for many years that the mechanism of AMPK activation by stresses involves an upstream kinase, which was partially purified from rat liver (Hawley et al., 1996), its identity remained a mystery. However, recently, we (Sutherland et al., 2003) and others (Hong et al., 2003a) identified three closely related protein kinases (Elm1p, Tak1p, Tos3p) capable of acting upstream of the yeast SNF1 complex *in vivo*. One of the closest sequence matches to these in humans was the protein kinase LKB1, and compelling evidence now indicates that this is the key upstream kinase in mammalian cells (Hawley et al., 2003; Woods et al., 2003; Shaw et al., 2004). LKB1 forms a complex with two accessory subunits: STRAD and MO25 (Baas et al., 2003; Boudeau et al., 2003). The upstream kinase purified from rat liver is a complex between LKB1, STRAD and MO25, and all three subunits are required for full activity (Hawley et al., 2003).

*LKB1* was first identified as the gene mutated in human Peutz-Jeghers syndrome (PJS) (Hemminki et al., 1998; Jenne et al., 1998). Subjects with PJS are heterozygous for a loss-of-function mutation in the *LKB1* gene. They have unusual skin pigmentation and also suffer from numerous gastrointestinal polyps that are classed as hamartomas, benign tumours caused by abnormal cell growth, in which the normal differentiated state of the cells is maintained. Subjects with PJS also have an increased risk of developing malignant tumours, which led to LKB1 being designated as a tumour suppressor.

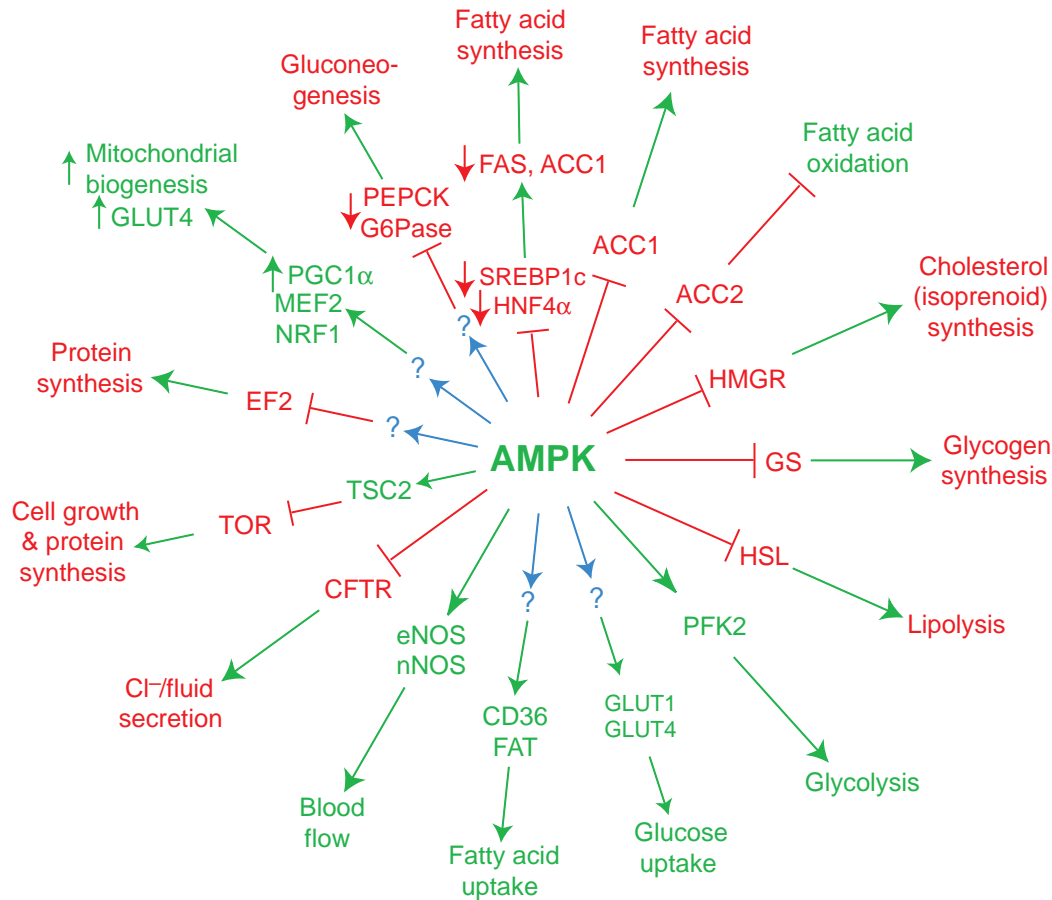
Heterozygous mice that lack one functional copy of the *LKB1* gene develop extensive gastrointestinal polyps, as in the human syndrome; however, there are conflicting reports as to whether these are caused by loss of heterozygosity, i.e. loss of the remaining functional copy of the gene (Bardeesy et al., 2002; Miyoshi et al., 2002; Rossi et al., 2002). One strain of these mice has increased incidence of liver cancers, which is associated with loss of heterozygosity (Nakau et al., 2002). Homozygous *LKB1*-knockout mice die during embryonic development, but immortalized mouse embryo fibroblasts (MEFs) can be derived from the embryos, and have provided an important tool that proved that LKB1 is necessary for AMPK activation (Hawley et al., 2003; Shaw et al., 2004).

Are the tumour suppressor effects of LKB1 due to its ability to activate AMPK? This is certainly possible, because AMPK activation in HepG2 cells causes a cell-cycle arrest in G1 phase through stabilization of p53 (Imamura et al., 2001), and expression of LKB1 in HeLa or G361 cells (tumour cell lines that do not normally express the kinase) also causes a p53-dependent G1 arrest (Tiainen et al., 1999; Tiainen et al., 2002). However, LKB1 lies upstream of all of the AMPK-related kinases shown in Fig. 3 (Lizcano et al., 2004). Although the four MARKs appear to have functions in regulating cell polarity (Baas et al., 2004), the functions and downstream targets of the other AMPK-related kinases are not well understood. Thus, some of the tumour suppressor effects of LKB1 might be mediated by one or more of the AMPK-related kinases rather than AMPK itself.

Is the LKB1 complex itself regulated? Although this possibility cannot be excluded, recent results suggest that it is constitutively active. First, during stimulation of cultured muscle cells by several stress treatments that activate AMPK, LKB1 activity was constant (Woods et al., 2003). Second, during activation of AMPK in MEFs by the anti-diabetic drug phenformin, there was no change in LKB1 activity (Lizcano et al., 2004), despite the fact that activation requires the presence of LKB1 (Hawley et al., 2003). Third, during treatment of rat skeletal muscle with different AMPK-activating stimuli, the activities of LKB1, and several of the AMPK-related kinases downstream of LKB1, did not change (Sakamoto et al., 2004).

### Downstream targets of AMPK

A full discussion of the downstream targets and processes regulated by AMPK can be found elsewhere (Hardie, 2003; Hardie et al., 2003; Hardie, 2004a; Hardie, 2004b). Fig. 4 summarizes some of the well-established downstream targets, and a few of the more recently identified targets are discussed below. In general, activation of AMPK downregulates biosynthetic pathways such as fatty acid and cholesterol biosynthesis, yet switches on catabolic pathways that generate ATP, such as fatty acid oxidation, glucose uptake and glycolysis (Fig. 1). It achieves this not only through direct phosphorylation of metabolic enzymes, but also through effects on gene expression, such as upregulation of the glucose transporter GLUT4 and mitochondrial genes in muscle, and downregulation of genes encoding enzymes of fatty acid synthesis and gluconeogenesis in liver. Although in no single case do we completely understand the detailed mechanisms by which AMPK activation regulates the expression of a particular gene, it has many effects on individual transcription factors and coactivators. For example, it upregulates the expression of the co-activator PGC1 $\alpha$  (Terada et al., 2002), which may be involved in the increased expression of mitochondrial genes in muscle (Zong et al., 2002), yet it downregulates the transcription factors SREBP-1c (Zhou et al., 2001) and HNF4 $\alpha$  (Leclerc et al., 2001; Hong et al., 2003b), which may be involved in decreased expression of genes involved in lipogenesis, glucose uptake and glycolysis in liver. In muscle, it causes increased DNA binding by the transcription factors NRF1 (Bergeron et al., 2001) and MEF2 (Zheng et al., 2001), which may be involved in regulation of mitochondrial genes and GLUT4, respectively. Finally, it phosphorylates the ubiquitous co-activator p300 at a specific site (Ser89), reducing



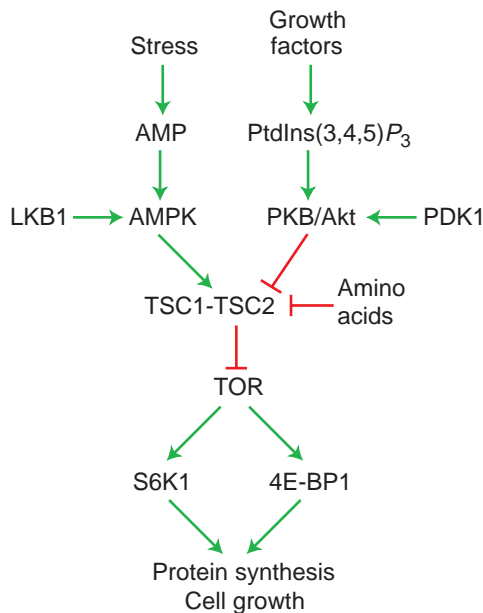
**Fig. 4.** Targets for AMPK. Target proteins and processes activated by AMPK activation are shown in green, and those inhibited by AMPK activation are shown in red. Where the effect is caused by a change in gene expression, an upward-pointing green arrow next to the protein indicates an increase, whereas a downward-pointing red arrow indicates a decrease in expression. Abbreviations: ACC1/ACC2, 1 ( $\alpha$ ) and 2 ( $\beta$ ) isoforms of acetyl-CoA carboxylase; CD36/FAT, CD36/fatty acid translocase; CFTR, cystic fibrosis transmembrane regulator; EF2, elongation factor-2; eNOS/nNOS, endothelial/neuronal isoforms of nitric oxide synthase; FAS, fatty acid synthase; G6Pase, glucose-6-phosphatase; GLUT1/4, glucose transporters; GS, glycogen synthase; HMGR, 3-hydroxy-3-methyl-CoA reductase; HSL, hormone-sensitive lipase; MEF2, myocyte-specific enhancer factor-2; NRF1, nuclear respiratory factor-1; PEPCK, phosphoenolpyruvate carboxykinase; PGC1 $\alpha$ , peroxisome proliferator-activated receptor- $\gamma$  co-activator-1 $\alpha$ ; TOR, mammalian target of rapamycin.

its ability to bind to nuclear hormone receptors and thus activate their target genes (Yang et al., 2001).

Another mechanism by which AMPK activation affects the expression of proteins is through effects on mRNA stability. Through an undefined mechanism, AMPK activation reduces the cytoplasmic level of the RNA-binding protein HuR, which stabilizes specific mRNAs in the cytoplasm by binding to their 3'-untranslated regions (Wang et al., 2002). Target mRNAs for HuR include proteins that regulate the cell cycle, such as cyclin A, cyclin B1 and p21. The same group showed that activation of AMPK by an elevated AMP:ATP ratio in senescent fibroblasts in culture can explain many of the features of the senescent phenotype (Wang et al., 2003). Indeed, LKB1<sup>-/-</sup> MEF cells, in which AMPK cannot be activated by agents that elevate cellular AMP levels (phenformin) or that mimic the effects of AMP (AICA riboside) (Hawley et al., 2003), are resistant to passage-induced senescence (Bardeesy et al., 2002).

Another key biosynthetic pathway that is downregulated by AMPK is translation, which can account for around 20% of energy turnover in growing cells and is particularly sensitive

to decreases in ATP synthesis (Buttgereit and Brand, 1995). Inhibition occurs by at least two mechanisms: (1) phosphorylation and activation of elongation factor-2 kinase (Horman et al., 2002; Browne et al., 2004), which switches off the elongation step in translation; and (2) inhibition of the target of rapamycin (TOR) pathway (Bolster et al., 2002; Krause et al., 2002; Kimura et al., 2003), which is a major positive stimulus for protein synthesis, cell growth and cell size. The TOR pathway is activated by growth factors and amino acids, and is thought to stimulate translation, and hence cell growth, by two mechanisms: (1) activation of ribosomal protein S6 kinase (S6K1); and (2) increased phosphorylation of elongation factor-4E binding protein 1 (4E-BP1), which stimulates the initiation step of translation (Carrera, 2004). Recent studies suggest that inhibition of the TOR pathway by AMPK might occur through phosphorylation of TSC2 (Inoki et al., 2003) (Fig. 5). TSC1 and TSC2 (also known as hamartin and tuberlin) are partners that form a stable complex in the cell, and mutations in either leads to the human disease tuberous sclerosis complex. This is similar to PJS in that it is an



**Fig. 5.** Regulation of protein synthesis and cell growth by AMPK and PKB/Akt by the mTOR pathway. Cellular stresses activate AMPK because the increase in AMP promotes its phosphorylation by LKB1; whereas growth factors activate PKB/Akt because the increase in phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5) $P_3$ ] promotes its phosphorylation by PDK1. AMPK and PKB/Akt phosphorylate TSC2 at different sites, and this stimulates or inhibits, respectively, the ability of the TSC1-TSC2 complex to inhibit TOR. Amino acids also stimulate TOR through the TSC complex. TOR in turn stimulates protein synthesis, and hence cell growth, through ribosomal protein S6 kinase 1 (S6K1) and elongation factor-4E binding protein 1 (4E-BP1). The molecular events immediately upstream and downstream of TOR in this pathway are not shown in detail and remain incompletely understood.

autosomal dominant condition resulting in hamartomas, although they are not restricted to the intestine. Studies in *Drosophila* suggest that the TSC1-TSC2 complex negatively regulates cell growth, and acts upstream of TOR to cause its inhibition (Gao et al., 2002). AMPK phosphorylates TSC2 at two sites; by mutating these site, Guan and colleagues provided evidence that these phosphorylation events increase the ability of the TSC complex to inhibit the TOR pathway, and that phosphorylation of TSC2 by AMPK is involved in the mechanism by which the TSC complex inhibits cell growth and protects against apoptosis during glucose starvation (Inoki et al., 2003). Interestingly, Shaw et al. reported that LKB1<sup>-/-</sup> MEF cells are more susceptible to apoptosis in response to AMPK-activating stresses (Shaw et al., 2004). Given that the LKB1→AMPK→TSC2 pathway (Fig. 5) seems to limit cell growth and cell size by inhibiting TOR, the idea that the hamartomas caused by heterozygosity for mutations in LKB1 (which lies upstream of AMPK) are related to those caused by heterozygosity for mutations in TSC2 (which lies downstream of AMPK) is attractive, although why the lesions are restricted to the intestine in the case of LKB1 remains unclear. However, it is also possible that the lesions in PJS are caused in part by dysregulation of downstream kinases other than AMPK, such as the MARKs.

## Conclusions and perspectives

The identification of LKB1 as the upstream kinase ended one era in research on AMPK but initiated another. In particular, the work on TSC2 raises the intriguing possibility, as discussed previously (Shaw et al., 2004), that the AMPK system acts as an 'energy checkpoint' that determines whether cellular energy status is sufficient before the cell commits to a programme of growth triggered by the TOR pathway. There are also indications that AMPK activation in response to low energy status inhibits cell division through effects on DNA replication and mitosis, although the detailed mechanisms remain to be elucidated. Finally, the AMPK system seems to have evolved in single-celled eukaryotes, where it might have had a primary role in orchestrating the response to glucose starvation. However, the recent findings that AMPK is regulated by cytokines such as leptin and adiponectin in mammals show that, during the evolution of multicellular organisms, it became involved in the regulation of energy intake and expenditure at the whole body level.

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