AMPK 2002 – 2nd International Meeting on AMP-activated Protein Kinase

Independent Meeting held at the West Park Centre, University of Dundee, Scotland, on 12–14 September 2002. Edited by D. G. Hardie (Dundee) and D. Carling (Imperial College London). Sponsored by The Wellcome Trust, The Biochemical Society, BioDundee, Novo-Nordisk, Merck Sante, Aventis Pharma, Eli Lilly, Mercury Therapeutics, Merck & Co Inc., Pfizer Inc., Abbott, GlaxoSmithKline, Boehringer, AstraZeneca, Upstate and Roche.

AMP-activated protein kinase, super metabolic regulator

B. E. Kemp*[1], D. Stapleton*, D. J. Campbell*, Z.-P. Chen*, S. Murthy*, M. Walter*, A. Gupta*, J. J. Adams*, F. Katsis*, B. van Denderen*, I. G. Jennings*, T. Iseli*, B. J. Michell* and L. A. Witters†

*St. Vincent's Institute of Medical Research, and Department of Medicine, University of Melbourne, 41 Victoria Parade, Fitzroy, Vic. 3065, Australia, and †Departments of Medicine and Biochemistry, Dartmouth Medical School, Hanover, NH, U.S.A.

Abstract

The AMP-activated protein kinase (AMPK) is a metabolic-stress-sensing protein kinase that regulates metabolism in response to energy demand and supply by directly phosphorylating rate-limiting enzymes in metabolic pathways as well as controlling gene expression.

Introduction

AMP-activated protein kinase (AMPK) was first detected as an inhibitory activity in preparations of acetyl-CoA carboxylase [1] and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) [2] and was later found to be stimulated by AMP [3,4]. When it was found that the acetyl-CoA carboxylase- and the HMGR-associated kinases were one and the same, the name AMPK was adopted [5]. The link between AMPK and energy metabolism was recognized by Yeh et al. [3], who referred to the activation of AMPK by AMP as regulation by adenylate energy charge, a concept where the ratio of [ATP]/[ADP][AMP] modulates enzyme activity. The 'adenylate charge hypothesis' for the metabolic coupling of anabolic and catabolic pathways proposed by Atkinson [6] held that the ratio of adenine nucleotides modulated metabolic enzyme activities through allosteric control. Thus a decrease in the energy charge (decrease in ATP and increase in AMP) would switch off anabolic pathways such as fatty acid, triglyceride and cholesterol synthesis as well as protein synthesis and transcription and switch on catabolic pathways such as glycolysis and fatty acid oxidation (Figure 1).

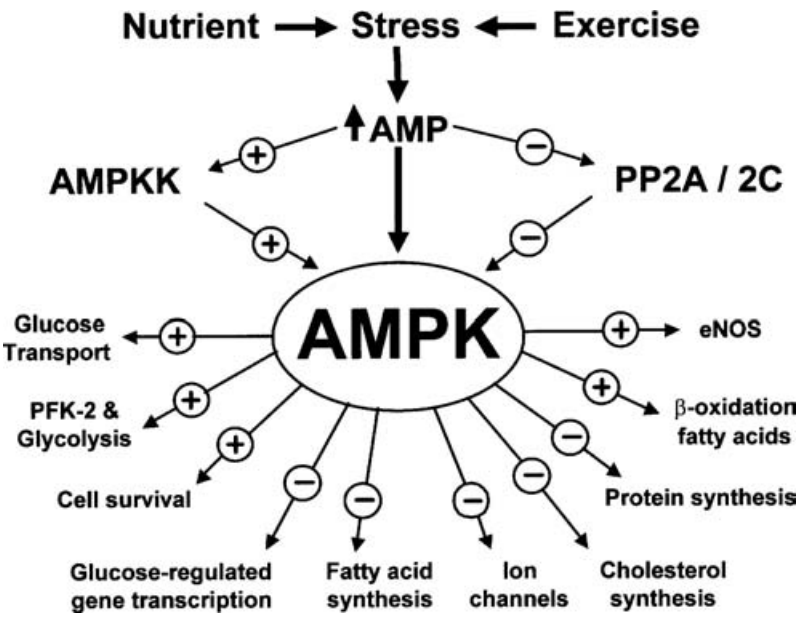
The purpose of this brief review is to highlight some recent advances in our understanding of AMPK's physiological and pathophysiological functions (for further details see earlier reviews [7–10]). When AMPK was purified from rat liver it was found to be an $\alpha\beta\gamma$ heterotrimer comprising an α catalytic subunit and $\beta\gamma$ non-catalytic subunits [11,12], with corresponding homologues in yeast belonging to the Snf1p kinase complex [11,13]. This discovery proved a revelation because Snf1p kinase was already known to control glucose-repressible gene expression in response to glucose deprivation [14] and mutations in the sucrose non-fermenting 1 (SNF1) gene blocked growth on alternative fermentable carbon sources such as sucrose. Snf1p and AMPK are functionally related and Snf1p phosphorylates and inhibits acetyl-CoA carboxylase [11] in response to glucose deprivation [15]. AMPK also inhibits glucose-activated gene expression [16,17] (see below). AMPK/Snf1p kinase homologues are found in all eukaryotes and represent an ancient metabolic-stress-sensing protein kinase subfamily.

Key words: AMP-activated protein kinase metabolism, cholesterol, exercise, fatty acid, glucose.
Abbreviations used: AMPK, AMP-activated protein kinase; AMPKK, AMPK kinase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; GBD, glycogen-binding domain; AICAR, 5-amino-4-imidazolecarboxamide riboside; PFK-2, 6-phosphofructo-2-kinase; mTOR, mammalian target of rapamycin; eEF, eukaryotic initiation factor.

[1]To whom correspondence should be addressed (e-mail kemp@ariel.unimelb.edu.au).

Figure 1 | AMPK regulation and functions

Nutrient- or exercise-induced metabolic stress increases AMP levels and activates AMPK. Activation or inhibition of downstream pathways is indicated by arrows with plus or minus signs, respectively. AMPKK, AMPK kinase; PP, protein phosphatase; eNOS, endothelial nitric oxide synthase; PFK-2, 6-phosphofructo-2-kinase.



Subunit structural aspects

Mammals, insects, nematodes and plants have multiple genes encoding the α catalytic subunit of AMPK, whereas yeasts only have the one gene, SNF1. The two mammalian $\alpha 1$ and $\alpha 2$ subunits share 90% identity in their N-terminal catalytic domains (residues 1–270) but only 60% identity in their C-terminal domains [18]. Structure–function studies of both Snf1p and AMPK α subunits have shown that the C-terminal domain is responsible for binding the non-catalytic subunits $\beta\gamma$ (Figure 2). Thus truncating the α subunit from 1–548 to 1–392 results in loss of $\beta\gamma$ binding and catalytic activity. Further truncation to 1–312 results in a constitutively active (AMP-independent) kinase fragment indicating that the 312–392 sequence contains an autoregulatory sequence analogous to other protein kinases [19] (Figure 2).

There are two mammalian β subunits, $\beta 1$ and $\beta 2$, and three in yeast, Gal83p, sip1p and sip2p, that function as targeting scaffolds. In yeast they direct Snf1p to the nucleus, vacuole or cytoplasm, respectively [20]. The core of the β subunits contains a glycogen-binding domain (GBD; D. Stapleton et al., unpublished work) belonging to the isoamylase N domain subfamily (Figure 2) and more remotely related to domains present in glycogen-targeting phosphatase subunits and many starch-binding proteins. The β subunit GBD (residues 68–163) targets AMPK to glycogen and has recently been crystallized (D. Stapleton et al., unpublished work). Previously a kinase-interacting sequence was identified in the yeast β homologues Gal83p, sip1p and sip2p by genetic studies [21], but this corresponds to the GBD-targeting sequence. The C-terminus of the β subunit (186–270) is responsible for binding the α and γ subunits (Figure 2) and in yeast this sequence was termed the 'association with Snf1 complex' (ASC) region because it associates with the yeast homologue of the γ subunit, Snf4 [21].

In mammals there are three γ subunits ($\gamma 1$, $\gamma 2$ and $\gamma 3$) and like their yeast homologue Snf4p they contain four CBS domain motifs found in a number of enzymes and named after the corresponding domains in cystathionine β synthase (Figure 3). The γ isoforms differ in the length of their N-terminal sequence with $\gamma 2$ being the largest and $\gamma 1$ the smallest. The crystal structure of the two CBS domains present in bacterial inosine monophosphate dehydrogenase [22] provides clues to the γ structure (Figure 3). Several lines of evidence suggest that AMP regulation is mediated by the γ subunit since AMPK is labelled by the photoaffinity nucleotide analogue, 8-azido-[^{32}P]AMP [23]. Direct AMP-binding studies using glutathione S-tansferase fusion constructs of the γ subunit CBS domains have now shown that each pair of CBS domains provides an AMP-binding site (J. Scott and D.G. Hardie, personal communication). This parallels the situation in cystathionine β synthase where CBS domains mediate *S*-adenosylmethionine allosteric regulation and mutation in the CBS domain 1 (D444N) [24] causes homocystinuria, resulting in loss of adenosylmethionine stimulation. Similarly, the corresponding R70Q mutation in $\gamma 1$ causes a loss of AMP activation of AMPK [25]. Cystathionine β synthase is autoregulated by its C-terminal sequence interacting with the CBS domains [26] and AMPK may be autoregulated in a similar manner where a variety of mutations in the γ subunit may reverse the autoregulation.

Figure 2 | AMPK α and β subunit structures

(**A**) The domain structure of the AMPK α catalytic subunit is illustrated. The catalytic domain is modelled on the crystal structure of protein kinase A. The autoinhibitory sequence that has been shown to inhibit kinase activity [77] is followed by the $\beta\gamma$ binding domain (BD) [77]. (**B**) The domain structure for β1 and the post-translational modifications are illustrated. The hypothetical structure of the GBD is modelled on glycosyltrehalose trehalohydrolase [78] with side chains of potential glycogen-binding residues shown.

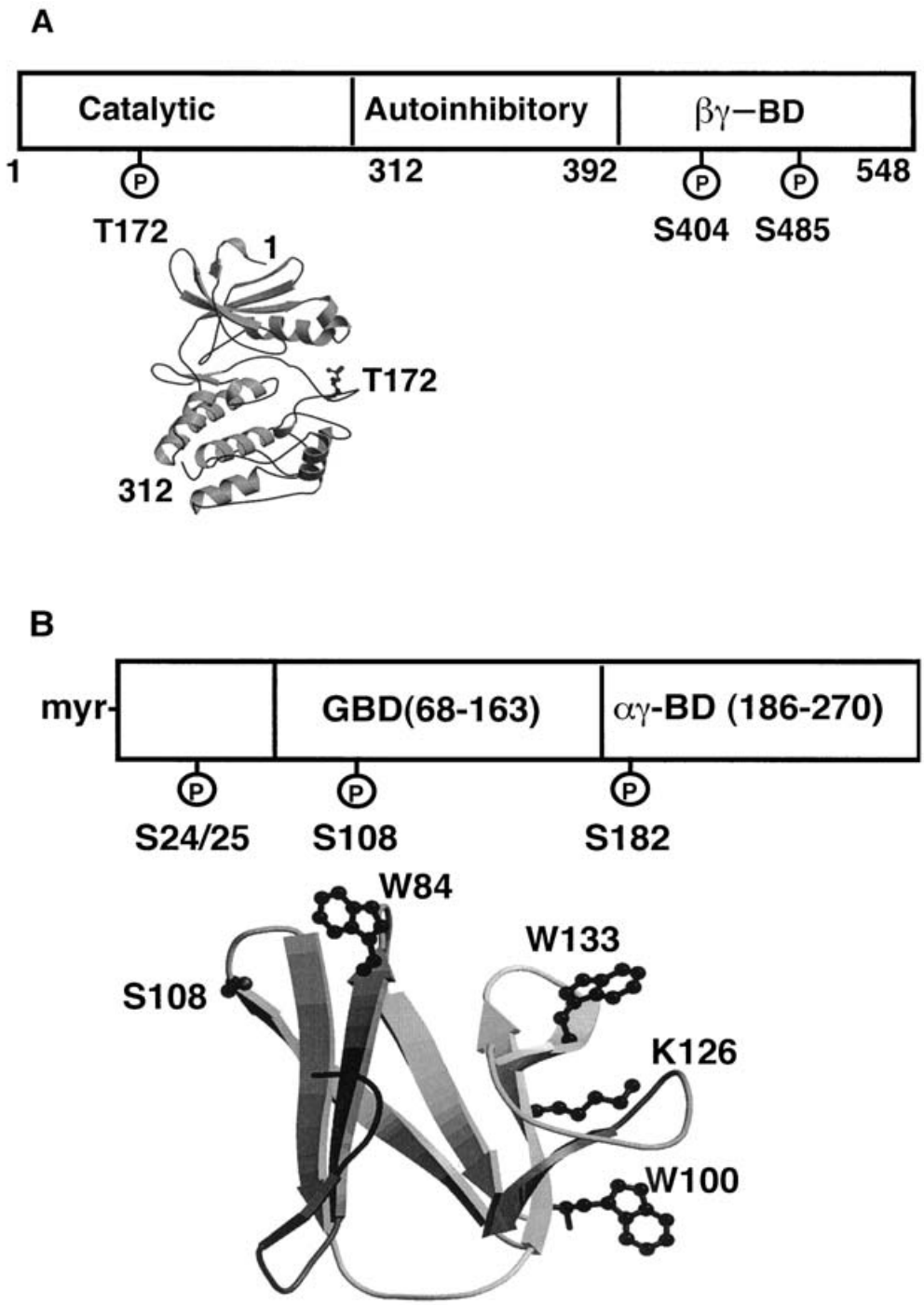
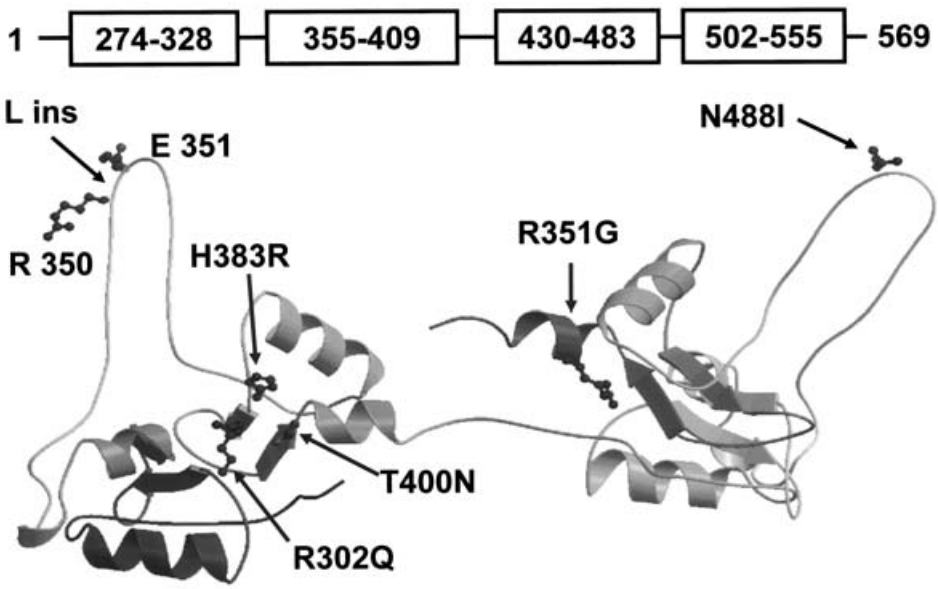


Figure 3 | γ Subunit domain structure

The γ subunits share four highly conserved CBS domains but vary in length at their N-termini. The amino acid positions of the four CBS domains (shown as boxes) of γ2, modelled on bacterial inosine monophosphate dehydrogenase [22], are shown. The positions of known mutations that cause cardiac disease are marked.



Regulation of AMPK by AMP and phosphorylation

AMPK is activated by one or more upstream AMPK kinases (AMPKKs) that are responsible for phosphorylating the AMPK α subunit at Thr-172 in the activation loop [27]. AMPK appears to be absolutely dependent on Thr-172 phosphorylation since bacterially expressed AMPK(1–312) is inactive unless phosphorylated on Thr-172 [28]. Although the identity of the AMPKK(s) or Snf1p kinase kinase(s) are not yet known, some of the regulatory features have been revealed using partially purified enzyme preparations. Direct activation of AMPKK by AMP has been reported [29] but we have not found AMP activation of partially purified AMPKK from heart, liver, kidney or skeletal muscle (S. Murthy, I.G. Jennings, L.A. Witters, D. Stapleton and B.E. Kemp, unpublished work). In addition, AMP may also act by binding to AMPK and suppressing dephosphorylation of Thr-172 by protein phosphatase 2C [30]. It has been argued that the multiple effects of AMP on regulating the phosphorylation of AMPK at Thr-172 serve to substantially amplify the system's responsiveness to AMP [31]. Observations using metabolically stressed hearts to manipulate AMP levels in the range 0.2–28 μM have shown that AMPK activity increases with an $A_{0.5}$ of approx. 2 μM AMP [32]. Recent studies, however, indicate additional complexity of the regulation of AMPK by phosphorylation. We have recently identified other sites of phosphorylation of the α subunit (Ser-485 and Ser-404; B.E. Kemp, D. Stapleton, S. Hamilton, J. Hurley and L.A. Witters, unpublished work), which appear to be important in the regulation of AMPK activity. The β subunit is likewise multi-site phosphorylated, with phospho-Ser-108 regulating AMPK activity and phosphorylation of Ser-24/Ser-25 and Ser-182 (a Ser-Pro site) regulating β subunit localization [33].

AMPK genes and mutations

Mammals possess seven genes encoding AMPK subunits. Several important mutations in the γ subunits have been reported recently, highlighting the physiological functions of AMPK. The RN^- autosomal dominant mutation in Hampshire pigs is due to an R200Q mutation in the γ3 subunit CBS 1 domain [34]. This amino acid substitution corresponds precisely with the D444N mutation in the CBS 1 domain of cystathionine β synthase that causes loss of *S*-adenosylmethionine regulation [24]. Pigs carrying the R200Q mutation have elevated skeletal muscle glycogen levels that are deleterious to meat processing. Based on the finding that the corresponding R70Q mutation in the γ1 subunit is strongly activating [25], we expect the R200Q mutation to be constitutively active, causing enhanced glucose uptake and glycogen accumulation. Analysis of pig γ3 subunit genes has revealed that the adjacent Ile-199 allele is associated with improved meat quality and lower glycogen levels [35], making it likely that AMPK genotyping is destined to alter pig breeding programmes.

Mutations in the γ2 subunit produce cardiac disease in humans. Gollob and his colleagues [36] found an R302Q mutation in the CBS domain 1 of γ2 in two families with

Wolf–Parkinson–White syndrome associated with cardiac hypertrophy due to glycogen accumulation [37]. These patients exhibit ventricular pre-excitation and early onset of atrial fibrillation and conduction disease, thought to be the consequence of an embryonic defect that results in altered electrical conduction between the atria and ventricles. Structurally the $\gamma 2$ R302Q mutation corresponds to the R200Q mutation seen in the pig $\gamma 3$ subunit CBS 1 domain. Thus far a total of five missense mutations plus an insert mutation have been detected in different families [36–40] (Figure 3). Several laboratories have now generated transgenic animals with γ mutations (M. Arad, I.P. Moskowitz, V.V. Patel, F. Ahmad, A.R. Perez-Atayde, D.B. Sawyer, M. Walter, G.H. Li, P.G. Burgon, C.T. Maguire, D. Stapleton, J.P. Schmitt, X.X. Guo, A. Pizard, S. Kuperschmidt, D.M. Roden, I. Charles, C.I. Berul, C.E. Seidman and J.G. Seidman, unpublished work; [40b,40c]), and these have the expected accumulation of glycogen in muscle.

Regulation of lipid metabolism

AMPK regulates multiple aspects of lipid metabolism both acutely as well as through transcriptional control (see below). Five important substrate targets have been reported. These are HMGR, hormone-sensitive lipase (HSL), acetyl-CoA carboxylase, glycerophosphate acyltransferase (GPAT) and malonyl-CoA decarboxylase (MCD) [41], but due to space restriction they will not be discussed further in this review. AMPK's role in lipid metabolism has also been highlighted by recent studies on leptin signalling that indicate that AMPK mediates the effects of leptin on fatty acid metabolism in muscle and possibly other tissues [42].

Regulation of glucose transport

AMPK contributes to the regulation of glucose uptake by skeletal muscle, cardiac muscle and adipocytes. Physical exercise or experimentally induced contraction of skeletal muscle activates AMPK, in association with an acute, insulin-independent increase in glucose transport into muscle [43–47]. Furthermore, studies of 5-amino-4-imidazole-carboxamide riboside (AICAR)-treated muscle provide evidence that AMPK increases glucose uptake by promoting GLUT4 translocation to the cell surface [48–50] and GLUT4 gene transcription [51].

The role of AMPK in glucose transport has been investigated using a transgenic mouse model overexpressing a dominant-negative kinase-dead subunit (AMPK $\alpha 2$DN) in skeletal muscle and heart [52]. There was no change in steady-state levels of muscle GLUT4 or glucose uptake [52], suggesting that AMPK does not play a role in basal glucose uptake. Hypoxia-stimulated glucose uptake and GLUT4 translocation were completely blocked in AMPK $\alpha 2$DN mice. In contrast, there was only an approx. 30% decrease in deoxyglucose uptake and GLUT4 translocation to the cell surface in response to electrical stimulation and no alteration of insulin-stimulated glucose uptake. While AMPK appears to be an obligate intermediate in hypoxia-driven glucose uptake and GLUT4 translocation, it would appear that AMPK transmits only a portion of the signal by which muscle contraction increases glucose uptake, and that other AMPK-independent pathways also contribute to the response [52]. AMPK phosphorylates insulin receptor substrate-1 (IRS-1) on Ser-789 [53] and may mediate the increased insulin sensitivity accompanying exercise. AMPK may also regulate glucose transport through GLUT1, involving either AMPK $\alpha 1$ or $\alpha 2$ depending on the stimulus [54,55].

Regulation of glycolysis and tumour cell growth

Glycolysis is an important source of metabolic energy for many tissues, including the heart, under hypoxic conditions. Recently it was found that AMPK phosphorylates and activates 6-phosphofructo-2-kinase (PFK-2) in cardiac muscle under ischaemic conditions [56]. There are multiple isoforms of PFK-2; neither the liver nor the skeletal muscle form contains AMPK phosphorylation sites but both the inducible and placental forms of PFK-2 do. Since the inducible PFK-2 form is common in tumour cells and contributes to survival [57], it seems likely that AMPK will play an important role in protecting tumours from hypoxic stress. Indeed, this idea has received support from RNA antisense experiments showing that depletion of AMPK in a number of tumour cell lines sensitizes them to nutritional stress [58].

Regulation of gene transcription

Since the discovery of Snf1p regulation of the Mig1p transcription repressor in yeast [59] there has been rapid progress in identifying genes whose transcription is controlled by AMPK. Two transcription factors, HNF-4α [60] and the transcriptional co-activator p300 [61], have been identified as substrates. Phosphorylation of p300 by AMPK occurs on Ser-89 and inhibits interaction with the nuclear receptors peroxisome proliferator-activated receptor, thyroid receptor, retinoic acid receptor and retinoid X receptor. Phosphorylation of Ser-568 in the newly discovered carbohydrate-response-element-binding protein (ChREBP) by AMPK blocks DNA binding and mediates the fatty acid-induced inhibition of glucose-induced gene transcription, including liver pyruvate kinase, fatty acid synthase and acetyl-CoA carboxylase [62]. Transcriptional control by AMPK may be important in treating type 2 diabetes because AMPK activation by metformin or AICAR suppresses expression of sterol-regulated-element-binding protein-1, a key lipogenic transcription factor [63]. Glucose starvation and AICAR treatment strongly repress glucose-6-phosphatase expression due to the disappearance of the transcription factor FKHR ('forkhead in rhabdosarcoma'), indicating that AMPK may control transcription by regulating protein degradation in some cases [64].

Obesity and diabetes

Impaired fuel metabolism is an early pathogenic factor in obesity and type 2 diabetes. Skeletal muscle is a principal site of glucose and fatty acid use, and is one of the primary tissues responsible for insulin resistance in obesity and type 2 diabetes. Activation of skeletal muscle AMPK now offers a new approach to combating insulin resistance by promoting glucose uptake and reducing lipid synthesis. Recent studies indicate that the actions of the anti-diabetic agents metformin and rosiglitazone are mediated by AMPK [63,65, 66], indicating that AMPK activators may have a role as anti-diabetic agents [67–69]. Moreover, chronic activation of AMPK by administration of AICAR or the creatine analogue β-guanadinopropionic acid (β-GPA) produces changes in skeletal muscle biochemistry similar to some of the biochemical adaptations to endurance exercise training [69,70]. Using the insulin-resistant Zucker rat model it was found that long-term administration of AICAR improves glucose tolerance, lipid profiles and abdominal fat, and reduces systolic blood pressure [71]. The obesity-related insulin resistance in the Zucker rat is associated with an isoform-specific impairment in AMPK α1 activation in response to contraction, but the impairment does not appear to affect contraction-stimulated glucose transport [72].

Protein synthesis

Since protein synthesis is a major energy-consuming process it is therefore expected that AMPK would link it to energy metabolism. A number of laboratories have now shown that activation of AMPK is associated with inactivation of p70 S6 kinase [73–75]. However, AMPK does not directly phosphorylate p70 S6 kinase. Instead, it appears to act upstream of mammalian target of rapamycin (mTOR) since the rapamycin-resistant p70 S6 kinase is insensitive to ATP depletion [75a]. Hypoxia inhibits protein synthesis in hepatocytes and this is mediated by AMPK-dependent activation of enkaryotic elongation factor 2 (eEF2) kinase and phosphorylation of eEF2 [76]. This regulatory pathway allows protein translation to pause during elongation until ATP levels are restored. The underlying mechanisms involved in AMPK inhibition of protein synthesis remain tantalizing since neither mTOR nor eEF2 kinase is a direct substrate.

Conclusions

Since the purification and identification of AMPK in 1994 interest in this important regulatory enzyme has grown dramatically. Given that all physiological processes depend on energy, there are potentially many links with AMPK and we can expect that it will soon rival protein kinase A in numbers of substrates. Rapid progress in the field has also created great expectations that AMPK will be an important therapeutic target for the treatment of diabetes, obesity, cancer and cardiovascular disease. Irrespective of these outcomes we can be assured that AMPK research will provide many new insights into the integration of metabolism and physiological functions.

References

1 Carlson, C.A. and Kim, K.H. (1973) J. Biol. Chem. **248**, 378–380
2 Beg, Z.H., Stonik, J.A. and Brewer, Jr, H.B. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 3678–3682
3 Yeh, L.A., Lee, K.H. and Kim, K.H. (1980) J. Biol. Chem. **255**, 2308–2314
4 Ferrer, A., Caelles, C., Massot, N. and Hegardt, F.G. (1985) Biochem. Biophys. Res. Commun. **132**, 497–504
5 Carling, D., Zammit, V.A. and Hardie, D.G. (1987) FEBS Lett. **223**, 217–222
6 Atkinson, D.E. (1970) Adv. Enzyme Regul. **9**, 207–219
7 Hardie, D.G., Carling, D. and Carlson, M. (1998) Annu. Rev. Biochem. **67**, 821–855
8 Kemp, B.E., Mitchelhill, K.I., Stapleton, D., Michell, B.J., Chen, Z.P. and Witters, L.A. (1999) Trends Biochem. Sci. **24**, 22–25
9 Winder, W.W. (2001) J. Appl. Physiol. **91**, 1017–1028
10 Hardie, D.G. and Hawley, S.A. (2001) Bioessays **23**, 1112–1119
11 Mitchelhill, K.I., Stapleton, D., Gao, G., House, C., Michell, B., Katsis, F., Witters, L.A. and Kemp, B.E. (1994) J. Biol. Chem. **269**, 2361–2364
12 Davies, S.P., Hawley, S.A., Woods, A., Carling, D., Haystead, T.A. and Hardie, D.G. (1994) Eur. J. Biochem. **223**, 351–357
13 Carling, D., Aguan, K., Woods, A., Verhoeven, A.J., Beri, R.K., Brennan, C.H., Sidebottom, C., Davison, M.D. and Scott, J. (1994) J. Biol. Chem. **269**, 11442–11448
14 Celenza, J.L. and Carlson, M. (1986) Science **233**, 1175–1180
15 Woods, A., Munday, M.R., Scott, J., Yang, X., Carlson, M. and Carling, D. (1994) J. Biol. Chem. **269**, 19509–19515
16 Foretz, M., Carling, D., Guichard, C., Ferre, P. and Foufelle, F. (1998) J. Biol. Chem. **273**, 14767–14771
17 Leclerc, I., Kahn, A. and Doiron, B. (1998) FEBS Lett. **431**, 180–184
18 Stapleton, D., Mitchelhill, K.I., Gao, G., Widmer, J., Michell, B.J., Teh, T., House, C.M., Fernandez, C.S., Cox, T., Witters, L.A. and Kemp, B.E. (1996) J. Biol. Chem. **271**, 611–614
19 Kobe, B. and Kemp, B.E. (1999) Nature (London) **402**, 373–376
20 Vincent, O., Townley, R., Kuchin, S. and Carlson, M. (2001) Genes Dev. **15**, 1104–1114
21 Jiang, R. and Carlson, M. (1997) Mol. Cell. Biol. **17**, 2099–2106
22 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
23 Cheung, P.C., Salt, I.P., Davies, S.P., Hardie, D.G. and Carling, D. (2000) Biochem. J. **346**, 659–669
24 Kluijtmans, L.A., Boers, G.H., Stevens, E.M., Renier, W.O., Kraus, J.P., Trijbels, F.J., van den Heuvel, L.P. and Blom, H.J. (1996) J. Clin. Invest. **98**, 285–289
25 Hamilton, S.R., Stapleton, D., O'Donnell, Jr, J.B., Kung, J.T., Dalal, S.R., Kemp, B.E. and Witters, L.A. (2001) FEBS Lett. **500**, 163–168
26 Janosik, M., Kery, V., Gaustadnes, M., Maclean, K.N. and Kraus, J.P. (2001) Biochemistry **40**, 10625–10633
27 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
28 Hamilton, S.R., O'Donnell, Jr, J.B., Hammet, A., Stapleton, D., Habinowski, S.A., Means, A.R., Kemp, B.E. and Witters, L.A. (2002) Biochem. Biophys. Res. Commun. **293**, 892–898
29 Hawley, S.A., Selbert, M.A., Goldstein, E.G., Edelman, A.M., Carling, D. and Hardie, D.G. (1995) J. Biol. Chem. **270**, 27186–27191
30 Davies, S.P., Helps, N.R., Cohen, P.T. and Hardie, D.G. (1995) FEBS Lett. **377**, 421–425
31 Hardie, D.G., Salt, I.P., Hawley, S.A. and Davies, S.P. (1999) Biochem. J. **338**, 717–722
32 Frederich, M. and Balschi, J.A. (2002) J. Biol. Chem. **277**, 1928–1932
33 Warden, S.M., Richardson, C., O'Donnell, Jr, J., Stapleton, D., Kemp, B.E. and Witters, L.A. (2001) Biochem. J. **354**, 275–283
34 Milan, D., Jeon, J.T., Looft, C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L. et al. (2000) Science **288**, 1248–1251
35 Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G. and Rothschild, M. (2001) Genetics **159**, 1151–1162
36 Gollob, M.H., Green, M.S., Tang, A.S., Gollob, T., Karibe, A., Ali Hassan, A.S., Ahmad, F., Lozado, R., Shah, G., Fananapazir, L. et al. (2001) N. Engl. J. Med. **344**, 1823–1831

37 Arad, M., Benson, D.W., Perez-Atayde, A.R., McKenna, W.J., Sparks, E.A., Kanter, R.J., McGarry, K., Seidman, J.G. and Seidman, C.E. (2002) J. Clin. Invest. **109**, 357–362
38 Gollob, M.H., Green, M.S., Tang, A.S. and Roberts, R. (2002) Curr. Opin. Cardiol. **17**, 229–234
39 Blair, E., Redwood, C., Ashrafian, H., Oliveira, M., Broxholme, J., Kerr, B., Salmon, A., Ostman-Smith, I. and Watkins, H. (2001) Hum. Mol. Genet. **10**, 1215–1220
40 Gollob, M.H., Seger, J.J., Gollob, T.N., Tapscott, T., Gonzales, O., Bachinski, L. and Roberts, R. (2001) Circulation **104**, 3030–3033
40a Reference deleted
40b Andersson, L. (2003) Biochem. Soc. Trans. **31**, 232–235
40c Musi, N., Yu, H. and Goodyear, J.J. (2003) Biochem. Soc. Trans. **31**, 191–195
41 Park, H., Kaushik, V.K., Constant, S., Prentki, M., Przybytkowski, E., Ruderman, N.B. and Saha, A.K. (2002) J. Biol. Chem. **277**, 32571–32577
42 Minokoshi, Y., Kim, Y.B., Peroni, O.D., Fryer, L.G., Muller, C., Carling, D. and Kahn, B.B. (2002) Nature (London) **415**, 339–343
43 Goodyear, L.J. and Kahn, B.B. (1998) Annu. Rev. Med. **49**, 235–261
44 Wasserman, D.H. and Halseth, A.E. (1998) Adv. Exp. Med. Biol. **441**, 1–16
45 Hutber, C.A., Hardie, D.G. and Winder, W.W. (1997) Am. J. Physiol. **272**, E262–E266
46 Vavvas, D., Apazidis, A., Saha, A.K., Gamble, J., Patel, A., Kemp, B.E., Witters, L.A. and Ruderman, N.B. (1997) J. Biol. Chem. **272**, 13255–13261
47 Merrill, G.F., Kurth, E.J., Hardie, D.G. and Winder, W.W. (1997) Am. J. Physiol. **273**, E1107–E1112
48 Kurth-Kraczek, E.J., Hirshman, M.F., Goodyear, L.J. and Winder, W.W. (1999) Diabetes **48**, 1667–1671
49 Russell, III, R.R., Bergeron, R., Shulman, G.I. and Young, L.H. (1999) Am. J. Physiol. **277**, H643–H649
50 Ojuka, E.O., Nolte, L.A. and Holloszy, J.O. (2000) J. Appl. Physiol. **88**, 1072–1075
51 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
52 Mu, J., Brozinick, Jr, J.T., Valladares, O., Bucan, M. and Birnbaum, M.J. (2001) Mol. Cell **7**, 1085–1094
53 Jakobsen, S.N., Hardie, D.G., Morrice, N. and H.Tornqvist, E. (2001) J. Biol. Chem. **276**, 46912–46916
54 Abbud, W., Habinowski, S., Zhang, J.Z., Kendrew, J., Elkairi, F.S., Kemp, B.E., Witters, L.A. and Ismail-Beigi, F., (2000) Arch. Biochem. Biophys. **380**, 347–352
55 Barnes, K., Ingram, J.C., Porras, O.H., Barros, L.F., Hudson, E.R., Fryer, L.G., Foufelle, F., Carling, D., Hardie, D.G. and Baldwin, S.A. (2002) J. Cell. Sci. **115**, 2433–2442
56 Marsin, A.S., Bertrand, L., Rider, M.H., Deprez, J., Beauloye, C., Vincent, M.F., Van den Berghe, G., Carling, D. and Hue, L. (2000) Curr. Biol. **10**, 1247–1255
57 Chesney, J., Mitchell, R., Benigni, F., Bacher, M., Spiegel, L., Al-Abed, Y., Han, J.H., Metz, C. and Bucala, R. (1999) Proc. Natl. Acad. Sci. U.S.A. **96**, 3047–3052
58 Kato, K., Ogura, T., Kishimoto, A., Minegishi, Y., Nakajima, N., Miyazaki, M. and Esumi, H. (2002) Oncogene **21**, 6082–6090
59 Smith, F.C., Davies, S.P., Wilson, W.A., Carling, D. and Hardie, D.G. (1999) FEBS Lett. **453**, 219–223
60 Leclerc, I., Lenzner, C., Gourdon, L., Vaulont, S., Kahn, A. and Viollet, B. (2001) Diabetes **50**, 1515–1521
61 Yang, W., Hong, Y.H., Shen, X.Q., Frankowski, C., Camp, H.S. and Leff, T. (2001) J. Biol. Chem. **276**, 38341–38344
62 Kawaguchi, T., Osatomi, K., Yamashita, H., Kabashima, T. and Uyeda, K. (2002) J. Biol. Chem. **277**, 3829–3835
63 Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N. et al. (2001) J. Clin. Invest. **108**, 1167–1174
64 Barthel, A., Schmoll, D., Kruger, K.D., Roth, R.A. and Joost, H.G. (2002) Endocrinology **143**, 3183–3186
65 Fryer, L.G., Parbu-Patel, A. and Carling, D. (2002) J. Biol. Chem. **277**, 25226–25232
66 Hawley, S.A., Gadalla, A.E., Olsen, G.S. and Hardie, D.G. (2002) Diabetes **51**, 2420–2425
67 Winder, W.W. and Hardie, D.G. (1999) Am. J. Physiol. **277**, E1–E10
68 Musi, N., Fujii, N., Hirshman, M.F., Ekberg, I., Froberg, S., Ljungqvist, O., Thorell, A. and Goodyear, L.J. (2001) Diabetes **50**, 921–927
69 Bergeron, R., Ren, J.M., Cadman, K.S., Moore, I.K., Perret, P., Pypaert, M., Young, L.H., Semenkovich, C.F. and Shulman, G.I. (2001) Am. J. Physiol. Endocrinol. Metab. **281**, E1340–E1346
70 Winder, W.W., Holmes, B.F., Rubink, D.S., Jensen, E.B., Chen, M. and Holloszy, J.O. (2000) J. Appl. Physiol. **88**, 2219–2226
71 Buhl, E.S., Jessen, N., Pold, R., Ledet, T., Flyvbjerg, A., Pedersen, S.B., Pedersen, O., Schmitz, O. and Lund, S. (2002) Diabetes **51**, 2199–2206
72 Barnes, B.R., Ryder, J.W., Steiler, T.L., Fryer, L.G., Carling, D. and Zierath, J.R. (2002) Diabetes **51**, 2703–2708
73 Krause, U., Bertrand, L. and Hue, L. (2002) Eur. J. Biochem. **269**, 3751–3759
74 Dubbelhuis, P.F. and Meijer, A.J. (2002) FEBS Lett. **521**, 39–42
75 Bolster, D.R., Crozier, S.J., Kimball, S.R. and Jefferson, L.S. (2002) J. Biol. Chem. **277**, 23977–23980
75a Kimura, N., Tokunaga, C., Dalal, S., Richardson, C., Yoshino, K., Hara, K., Kemp, B.E., Witters, L.A., Mimura, O. and Yonezawa, K. (2003) Genes Cells **7**, in the press
76 Horman, S., Browne, G., Krause, U., Patel, J., Vertommen, D., Bertrand, L., Lavoinne, A., Hue, L., Proud, C. and Rider, M. (2002) Curr. Biol. **12**, 1419–1423
77 Crute, B.E., Seefeld, K., Gamble, J., Kemp, B.E. and Witters, L.A. (1998) J. Biol. Chem. **273**, 35347–35354
78 Feese, M.D., Kato, Y., Tamada, T., Kato, M., Komeda, T., Miura, Y., Hirose, M., Hondo, K., Kobayashi, K. and Kuroki, R. (2000) J. Mol. Biol. **301**, 451–464

Received 10 September 2002