Minireview

At the crossroads: AMP-activated kinase and the LKB1 tumor suppressor link cell proliferation to metabolic regulation

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The AMP-activated protein kinase (AMPK) is a metabolic master regulator that is activated in times of reduced energy availability (high cellular AMP:ATP ratios) and serves to inhibit anabolic processes [1-5]. In an AMP-dependent manner, AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC) [1,2], the rate-limiting enzyme in fatty-acid synthesis; ACC catalyzes the formation of malonyl-CoA, a potent inhibitor of fatty-acid oxidation. Accordingly, AMPK acts to elevate fat oxidation and reduce lipogenesis [1,2]. AMPK also catalyzes the AMP-dependent phosphorylation and inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, thus reducing cholesterol formation [1,2,5]. In addition, AMPK activation suppresses the expression of several lipogenic genes [2] and activates phosphofructokinase-1, thereby suppressing glucose oxidation and enhancing glycolysis (the Pasteur effect). AMPK is activated in exercise, where it triggers glucose uptake by skeletal muscle [2-4]. Thus, AMPK activators could alleviate glucose intolerance; in support of this idea, the biguanide drugs metformin and phenformin, as well as the thiazolidinedione rosiglitazone, all of which have at one time been used to treat type 2 diabetes (although phenformin is now banned due to hepatotoxicity), may exert their effects in part by activating AMPK [5-8]. In addition, mutations in the γ2 subunit of human AMPK have been linked to Wolff-Parkinson-White syndrome (WPW), a condition marked by cardiac hypertrophy and ventricular pre-excitation [9-11] associated with the accumulation in the myocardium of excess glycogen [10]. The WPW mutations in AMPK reduce the kinase’s sensitivity to AMP and, accordingly, the extent of its activation and overall activity in vivo and in vitro [11].

AMPK exists in the cell as a heterotrimer, the subunits of which are widely conserved in evolution. The α subunits (α1 and α2 in mammals) contain the protein kinase domain and are homologous to the Saccharomyces cerevisiae gene sucrose nonfermenting-1 (SNF1) [1]. The yeast Snf1p protein and its associated subunits (see below) function to enable cells to grow on sucrose or raffinose in the complete absence of glucose. The functions of the β and γ subunits
are still somewhat unclear, but all three subunits are necessary for assembly of an active enzyme [1]. The mammalian β subunits (β1 and β2 in mammals) are homologous to *S. cerevisiae* Sip1p, Sip2p and Gal83p and include amino-terminal N-isoamylase domains that enable AMPK to bind tightly to glycogen [12,13], a process that modestly inhibits AMPK but may also enable glycogen synthase phosphorylation [1-4]. The AMPK γ subunits (γ1-γ3 in mammals) are homologous to *S. cerevisiae* Snf4p and each contains four cystathionine-β-synthase (CBS) domains [1]. Inasmuch as the γ-subunit mutations of WPW reduce AMPK’s sensitivity to AMP [11], it is thought that the γ subunits contain the AMP-binding site. The reduced AMP sensitivity in WPW, by reducing AMPK-mediated inhibition of glycogen synthase, might account for the glycogen storage disorder associated with the disease.

Although AMP was originally identified as an allosteric activator of AMPK, the regulation of AMPK by AMP is complex. Thus, AMP also inhibits dephosphorylation and deactivation of the kinase, and AMP potentiates phosphorylation and activation of AMPK by an upstream kinase AMPK-kinase (AMPKK; Figure 1) [1]. The existence of an AMPKK was suggested by the observation that AMPK could be deactivated by protein phosphatases. An AMPKK was partially purified by several laboratories, and these preparations could phosphorylate the AMPK α subunit at Thr172 in the kinase activation loop [1,14,15]; this phosphorylation is required for optimal AMPK activity [14,15]. Interestingly, partially purified AMPKK appeared itself to rely on AMP for efficient activation of AMPK; it was proposed either that AMPKK, like AMPK itself, was allosterically regulated by AMP or that binding of AMP to AMPK made AMPK a better AMPKK substrate [14,15]. More recent work has shown that AMPKK activity can be resolved chromatographically into two peaks (AMPKK1 and AMPKK2) [14,16]. But despite heroic efforts, the mammalian AMPKK(s) have been resistant to traditional methods of protein purification and sequencing - until now [16].

Studies of the regulation of yeast Snf1p paved the way to the identification of a mammalian AMPKK complex. Snf1p, the *S. cerevisiae* ortholog of the AMPK α subunit, is like its mammalian counterpart in requiring phosphorylation for activity; Thr210 is the site in the Snf1p activation loop analogous to Thr172 of mammalian AMPK α [1]. A small family of yeast protein kinases, known as polymerase alpha kinase-1 (Pak1p), not to be confused with mammalian p21-activated kinase-1, also abbreviated Pak1), Tos3p and Elm1p, were recently identified as Snf1p kinases [17-19]. Thus, mass-spectrometric analysis of proteins associated with the Snf1p complex identified Pak1p and Tos3p as Snf4p interactors [17,18]; Pak1p can also bind Snf1p [17]. This association of Pak1p with Snf1p is enhanced under the low glucose conditions in which Snf1p is activated [17]. Pak1p, Tos3p and Elm1p can all phosphorylate Snf1p at Thr210 [17-19] (indeed, Elm1p was selected in a proteomic screen for Snf1p Thr210 kinases [19]); but neither single nor double mutant strains carrying deletions of *pak*, *tos3*, or *elm1*, displays a *Snf* 

Interrogation of mammalian genomic databases indicates that the Pak1p/Tos3p/Elm1p family is most closely related
to mammalian calcium-calmodulin kinase-kinases (CaMKks) and to the tumor suppressor kinase LKB1. But although CaMKK can weakly phosphorylate the AMPK α subunit at Thr172, partially purified mammalian AMPKks - unlike CaMKks - are not dependent on Ca²⁺ and calmodulin [20], making it unlikely that CaMKks are physiologically relevant AMPKks. By contrast, Hong et al. [18] showed that, in vitro, LKB1 could phosphorylate the mammalian AMPK α subunit at Thr172; but it was unclear from this finding that LKB1 was, in fact, a physiologically relevant AMPKk. Hawley et al. [16] now present dramatic and convincing evidence that LKB1 is a major, physiologically relevant mammalian AMPKK. The regulatory relationship between LKB1 and AMPK provides a concrete link between the control of cell proliferation and nutrient regulation of cell metabolism.

The lkb1 gene encodes a serine/threonine kinase that is mutated in Peutz-Jeghers syndrome (PJS), an autosomal-dominant tumor-predisposition disorder that is characterized most notably by the development of hamartomatous polyps in the gastrointestinal tract [21]; PJS patients are especially at increased risk for the development of malignant tumors of the gastrointestinal tract. PJS arises from loss-of-function mutations (primarily in the kinase domain) in LKB1 and although PJS is dominantly inherited, it is not clear if tumor formation is due to haploinsufficiency or to loss of heterozygosity [21].

LKB1 is regulated by interactions with two adaptor proteins. Ste20-related adaptor (STRAD, with α and β isoforms in mammals) is a polypeptide of 45-48 kDa that is related to the Ste20 family of protein kinases. STRAD is a pseudokinase, however, as it lacks key residues (notably in the conserved phosphotransferase region) required for catalyzing protein phosphorylation. The binding of STRAD to LKB1 substantially activates LKB1’s autophosphorylating kinase activity and its ability to phosphorylate myelin basic protein. STRAD binding also targets LKB1 to the cytosol [22]. Mouse protein 25 (MO25, again in α and β forms) is a second adaptor protein of 40 kDa that regulates LKB1. MO25 binds STRAD and functions to stabilize the STRAD-LKB1 complex [23].

Hawley et al. [16] show that all three components of the LKB1 complex - LKB1, STRADα and MO25α - coelute precisely on anion exchange columns with both rat liver AMPKK peaks. The LKB1 immunoreactivity in the AMPKK1 peak runs faster on SDS polyacrylamide gels than that of AMPKK2; and this is not due to reduced phosphorylation, because phosphatase treatment fails to enhance the mobility of the LKB1 immunoreactivity in the AMPKK2 peak [16]. It is not known if this difference in gel mobility accounts for the resolution of AMPKK1 and AMPKK2 as separate peaks upon ion-exchange chromatography. Immunoprecipitation of LKB1 can almost completely deplete either AMPKK peak of AMPK-activating activity; and recombinant LKB1-STRADs-MO25α purified from transfected cells also phosphorylates the AMPK α subunit on Thr172. LKB1 expressed alone is incapable of phosphorylating the AMPK α subunit, however, indicating a requirement for the STRAD and MO25 subunits [16]. By comparison with a STRADα and MO25α complex, LKB1 in a complex with STRADβ and MO25β is a poor AMPKK [16]. Of note, the LKB1-STRAD-MO25 complex can phosphorylate both isolated, bacterially expressed AMPK α subunit, and the α subunit as part of an intact AMPK heterotrimer [16].

As noted above, AMP has been shown to enhance phosphorylation of AMPK by AMPKK (Figure 1) [14,15,20]. Addition of AMP enhances phosphorylation of intact AMPK heterotrimers by LKB1-STRAD-MO25 heterotrimers, but fails to enhance phosphorylation of the isolated, bacterially expressed AMPK α subunit by LKB1-STRAD-MO25 [16]. This result suggests that AMP does not directly activate the LKB1 complex, but that binding of AMP to the AMPK complex renders AMPK a better LKB1-STRAD-MO25 subunit [16]. It will be important to confirm this finding with intact AMPK heterotrimers harboring mutations in the AMP-binding site - once this site has been precisely mapped. The regulation by AMP of the phosphorylation of AMPK by LKB1-STRAD-MO25 is somewhat similar to the indirect regulation by inositol lipids of the activation of protein kinase-B (PKB)/Akt by 3’-phosphoinositide-dependent kinase-1 (PDK1) [24].

Hawley et al. [16] provide genetic evidence that attests to the physiologic relevance of LKB1 to AMPK regulation. Thus, HeLa cells do not naturally express LKB1; in these cells, the drugs AICAR and phenformin fail to activate AMPK, and transient transfection of LKB1 restores this activation [16]. Disruption of murine lkb1 produces embryonic lethality; but lkb1+/- mouse embryonic fibroblasts (MEFs) have been generated. In contrast to lkb1+/- MEFs, in which AICAR and phenformin readily activate AMPK, the AMPK in lkb1+/- MEFs is not activated by either treatment [16]. Thus, LKB1 is both necessary and sufficient for activation of AMPK.

These mammalian cell, biochemical and genetic data present an interesting contrast with the situation in yeast, in which three Snf1p kinases have been identified [17-19]. While it is certainly possible that additional AMPKKs will be identified in cell types other than HeLa or MEFs, the paucity of LKB-like kinases in the human genome argues against this idea. This difference between yeast and mammalian cells may reflect the more extreme metabolic demands faced by unicellular eukaryotes, as compared with mammalian cells,
which have numerous methods for storing and distributing metabolites. Alternatively, LKB1 may interact with regulatory proteins other than STRAD and MO25, allowing for a measure of heterogeneous regulation and/or functional redundancy. With this in mind, the prominence of endocrine versus metabolic control of AMPK and the function of LKB1 in these processes are important areas of investigation. For example, leptin and adiponectin, hormones produced by adipocytes, stimulate fatty acid oxidation and glucose utilization via activation of AMPK [25,26]. It will be important to determine whether LKB1 - in complex with STRAD and MO25 isoform(s) - mediates the actions of leptin and adiponectin.

The link between LKB1 and PJS, and the identification of LKB1 as a tumor suppressor and now as the long-sought AMPKK, provide a molecular basis for the interaction between metabolism and cell proliferation. It is possible that AMPK-activating drugs could prove promising in the treatment of LKB1-deficient cancers. Furthermore LKB1 now joins AMPK as an attractive target for activating drugs that would be useful in the treatment of obesity and type 2 diabetes.

References
1. Hardie DG, Carling D, Carlson M: The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu Rev Biochem 1998, 67:821-855.
2. Winder WW, Hardie DG: The AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol 1999, 277:E1-E10.
3. Goodyear LJ: AMP-activated protein kinase: a critical signaling intermediary for exercise-stimulated glucose uptake? Exerc Sport Sci Rev 2000, 28:113-116.
4. Mu J, Brozinick JT, Vallardes O, Bucan M, Birnbaum MA: A role for AMP-activated protein kinase in contraction and hypoxia-regulated glucose transport in skeletal muscle. Mol Cell 2001, 7:1085-1094.
5. Moller DE: New drug targets for type 2 diabetes and the metabolic syndrome. Nature 2001, 414:821-827.
6. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyl-Melody J, Wu M, Ventre J, Doeber T, Fuji N, et al.: Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001, 108:1167-1179.
7. Fryer LG, Parbu-Patel A, Carling D: The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 2002, 277:25226-25232.
8. Hawley SA, Gadalla AE, Olsen GS, Hardie DG: The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. Diabetes 2002, 51:2420-2425.
9. Blair E, Redwood C, Ashrafhan H, Oliveira M, Broxholme J, Kerr B, Salmon A, Osmann-Smith I, Watkins H: Mutations in the β(2) subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: evidence for the central role of energy compromise in disease pathogenesis. Hum Mol Genet 2001, 10:2125-1220.
10. Arad M, Benson DW, Perez-Atayade AR, McKenna WJ, Sparks EA, Kanter RJ, McGarry K, Seidman JG, Seidman CE: Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. J Clin Invest 2002, 109:357-362.
11. Daniel T, Carling D: Functional analysis of mutations in the γ 2 subunit of AMP-activated protein kinase associated with cardiac hypertrophy and Wolff-Parkinson-White syndrome. J Biol Chem 2002, 277:51017-51024.
12. Hudson ER, Pan DA, James J, Lucoco JM, Hawley SA, Green KA, Baba O, Terashima T, Hardie DG: A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias. Curr Biol 2003, 13:861-866.
13. Polekhina G, Gupta A, Michelli Bj, van Denderen B, Murthy S, Feil SC, Jennings IG, Campbell DJ, Winters LA, Parker MW, et al.: AMPK β subunit targets metabolic stress sensing to glycogen. Curr Biol 2003, 13:867-871.
14. Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, Hardie DG: Characterization of the AMP-activated protein kinase kinase from rat liver, and identification of threonine-172 as the major site at which it phosphorylates and activates AMP-activated protein kinase. J Biol Chem 1996, 271:27879-27887.
15. Stein SC, Woods A, Jones NA, Davison MD, Carling D: The regulation of AMP-activated protein kinase by phosphorylation. Biochem J 2000, 345:437-443.
16. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP, Alessi DR, Hardie DG: Complexes between the LKB1 tumour suppressor, STRADαβ and MO25αβ are upstream kinases in the AMP-activated protein kinase cascade. J Biol Chem 2003, 2:28.
17. Nath N, McCartney RR, Schmidt MC: Yeast Pkl1 kinase associates with and activates Snf1. Mol Cell Biol 2003, 23:3909-3917.
18. Hong S-P, Leiper FC, Woods A, Carling D, Carlson M: Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. Proc Natl Acad Sci USA 2003, 100:8839-8843.
19. Sutherland CM, Hawley SA, McCartney RR, Leech A, Stark MJ, Schmidt MC, Hardie DG: Elk1p is one of three upstream kinases for the Saccharomyces cerevisiae Snf1 complex. Curr Biol 2003, 13:1299-1305.
20. Hawley SA, Selbert MA, Goldstein EG, Edelman AM, Carling D, Hardie DG: 5′-AMP activates the AMP-activated protein kinase cascade and Ca²⁺/calmodulin the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. J Biol Chem 1995, 270:2186-2191.
21. Boudeau J, Sakopila G, Alessi DR: LKB1, a protein kinase regulating cell proliferation and polarity. FEBS Lett 2003, 546:159-165.
22. Baas AF, Boudeau J, Sakopila GP, Smic L, Medema R, Morricle NA, Alessi DR, Clevers HC: Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. EMBO J 2003, 22:3062-3072.
23. Boudeau J, Baas AF, Deak M, Morricle NA, Kieloch A, Schoutowski M, Prescott AR, Clevers HC, Alessi DR: MO25αβ interact with STRADαβ enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. EMBO J 2003, 22:5102-5114.
24. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PRJ, Reese CB, Cohen P: Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Ba. Curr Biol 1997, 7:261-269.
25. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002, 415:339-343.
26. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, et al.: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002, 8:1288-1295.