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Minireview

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

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Abstract

The tumor suppressor kinase LKB1 has been identified as a physiologic activator of the key metabolic regulator 5′-AMP-activated protein kinase, establishing a possible molecular link between the regulation of metabolism and cell proliferation.

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 in an insulin-independent manner, and phosphorylates and inhibits glycogen synthase [1-4].

In vivo, pharmacologic activation of AMPK with 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) mimics exercise and triggers insulin-independent 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

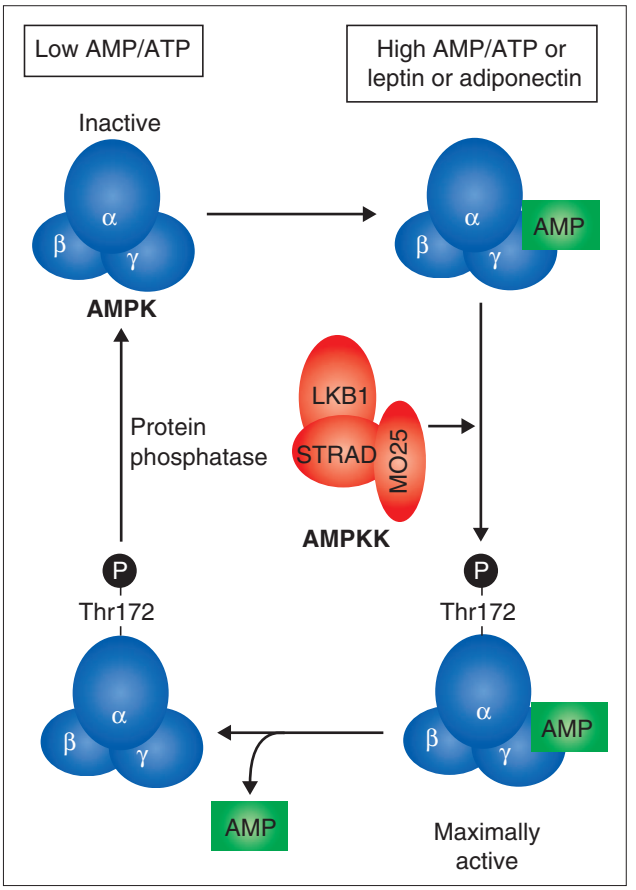

Figure 1
Regulation of AMPK. AMPK (blue) becomes activated under conditions of high AMP/ATP (metabolic depletion), or in response to the hormones leptin and adiponectin [1,25,26]. Under these circumstances, AMP binds to AMPK, facilitating phosphorylation at Thr172 and activation, in a reaction catalyzed by the LKB1-STRAD-MO25 complex (AMPKK; red). AMP also prevents dephosphorylation and deactivation of AMPK and serves as an allosteric activator of AMPK. See text for further details.

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*$^-$ phenotype (inability to grow on sucrose in the absence of glucose) [17-19]. Only a triple *pak1-tos3-elm1* deletion mutant showed a *Snf*$^-$ phenotype [18,19], suggesting a high degree of functional redundancy among the yeast Snf1p kinases.

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 Ca^{2+} 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-STRADα-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 substrate [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 metabolite control of AMPK and the function of LKB 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.

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