COMMENTARY

The ubiquitin-associated domain of AMPK-related protein kinases allows LKB1-induced phosphorylation and activation

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The AMPK (AMP-activated protein kinase)-related protein kinase subfamily of the human kinome comprises 12 members closely related to the catalytic $\alpha 1/\alpha 2$ subunits of AMPK. The precise role of the AMPK-related kinases and their *in vivo* substrates is rather unclear at present, but some are involved in regulating cell polarity, whereas others appear to control cellular differentiation. Of the 12 human AMPK-related protein kinase family members, 11 can be activated following phosphorylation of their T-loop threonine residue by the LKB1 complex. Nine of these AMPK-related kinases activated by LKB1 contain an UBA (ubiquitin-associated) domain immediately C-terminal to the kinase catalytic domain. In this issue of the *Biochemical Journal*, Jaleel et al. show that the presence of an UBA domain in AMP-related kinases allows LKB1-induced phosphorylation and activation. The findings have implications for understanding the molecular mechanisms of activation of this fascinating family of protein kinases. Also, mutations in the UBA domains of the AMP-related kinase genes might be present in families with Peutz–Jehgers syndrome and in other cancer patients.

Key words: activation loop, cancer, cell differentiation, cell polarity, Peutz–Jeghers Syndrome, phosphorylation.

INTRODUCTION

AMPK (AMP-activated protein kinase) is highly conserved in eukaryotes, where it functions primarily as a sensor of cellular energy status [1]. The catalytic $\alpha 1/\alpha 2$ subunits of heterotrimeric AMPK have 12 close relatives in the human genome. These protein kinases are BRSKs (brain-specific kinases) 1 and 2, MARKs (microtubule-affinity-regulating kinases) 1, 2, 3 and 4, MELK (maternal embryonic leucine-zipper kinase), NUAK1 and NUAK2 [the sucrose-non-fermenting kinase-1 (SNF1)-like kinases], QIK (Qin-induced kinase), QSK and SIK (salt-induced kinase). BRSK1 (SAD1A) and BRSK2 (SAD1B) are related to SAD-1 kinase of *Caenorhabditis elegans*, a neuronal-cell polarity regulator. Indeed, SAD1A and SAD1B were shown to be required for neuronal polarization in mammalian cells [2]. The MARKs are homologues of PAR1 in *C. elegans* and *Drosophila melanogaster*. MARKs were originally identified after their role in regulating microtubule dynamics by phosphorylating microtubule-associated proteins, but were subsequently shown to be involved in the establishment of cell polarity [3]. MELK has been implicated in stem cell renewal, cell cycle progression and pre-mRNA splicing. NUAC1 is also known as ARK5 (AMP-related kinase 5), NUAC2 as SNARK (SNF1/AMPK-related kinase) and QIK as SIK2. NUAC1 might lie downstream of Akt/protein kinase B, suppresses apoptosis and is closely involved in tumour progression. NUAC2, like AMPK, is activated in response to various cellular stresses, notably glucose deprivation, ATP depletion and hyperosmotic stress [4]. QIK was expressed at a very early stage of adipogenesis, and interestingly phosphorylates the same residue on IRS1 (insulin-responsive substrate 1; Ser-794 in human IRS1) as that phosphorylated by AMPK and which correlated with an increase in insulin-stimulated, IRS1-associated phosphoinositide 3-kinase activity. SIK is involved in steroidogenesis and adipogenesis. To date, the role of QSK is unknown.

A common feature of the AMPK-related kinases is conservation of the activation loop (T-loop) threonine residue (Thr-172 in $\alpha 1/\alpha 2$-AMPK) located in a distinct motif between subdomains VII and VIII of the kinase domain. AMPK Thr-172 phosphorylation is essential for AMPK activation. After many years of intense research, the first upstream AMPK activating kinase was identified as the Peutz–Jehgers protein, LKB1 [5,6]. This followed the identification of three yeast kinases, namely Elm1, Pak1 and Tos3, which phosphorylate and activate SNF1, which is orthologous with mammalian AMPK. The most closely related protein kinases to Elm1, Pak1 and Tos3 in the human genome are members of the CAMKKs (calmodulin-dependent protein kinase kinases) and the tumour suppressor protein LKB1. Mutations in the LKB1 gene cause Peutz–Jeghers syndrome, a rare and hereditary form of cancer characterized by the presence of hamartomatous polyps in the intestine combined with pigmentation of mucous membranes. LKB1, like AMPK, is a heterotrimer associated with MO25 (mouse protein 25) and STRAD (STE20-related adaptor protein). STRAD is a pseudo-kinase, whereas MO25 acts as a scaffold and stabilizes the LKB1 complex. The *C. elegans* homologue of LKB1 is PAR4, which regulates cell polarity. Overexpression of LKB1 in various mammalian cells induced a G_1-phase cell cycle arrest, whereas activation of LKB1 by induced expression of STRAD led to complete polarization of intestinal epithelial cells in a cell-autonomous fashion [3]. Eleven of the AMPK-related kinases are activated by LKB1 complexes via phosphorylation of their T-loop threonine residue [7]. The exception is MELK, which autophosphorylates its T-loop residue. Recently, another protein kinase, SNRK (SNF1-related kinase), which lies on the same branch as $\alpha 1/\alpha 2$-AMPK in the human kinome tree, was also found to be phosphorylated at its T-loop residue and activated by LKB1 [8]. SNRK is highly expressed in testis, where it might be involved in regulating spermatogenesis and/or sperm motility.

In this issue of the *Biochemical Journal*, Jaleel et al. [9] provide exciting new insights into the molecular mechanism by which

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AMPK-related kinases are activated by the LKB1 complex. Nine of the human AMPK-related kinases that are activated by LKB1, plus SNRK, possess an UBA (ubiquitin-associated) domain immediately C-terminal to the kinase catalytic domain. The authors show that the UBA domain plays a unique role in allowing AMPK-related kinases to be phosphorylated and activated by LKB1. UBA domains were originally identified by sequence analyses as a domain present in a number of proteins thought to be involved in the ubiquitin–proteasome pathway. The domain is widespread throughout eukaryotes, and in the relatively small genome of the fission yeast *Schizosaccharomyces pombe* there are at least 15 UBA-domain-containing proteins. UBA domains are approx. 45 amino acids long and fold into a bundle of three tightly packed helices, exposing a hydrophobic patch that interacts with ubiquitin or polyubiquitin chains [10]. The residues conserved in this exposed region are conserved in the UBA domains of AMPK-related kinases, including a glycine residue found in most UBA domains.

Jaleel et al. [9] first found that MARK2 and MARK3, expressed as active forms in HEK-293 cells, contained a protease-resistant fragment encompassing the catalytic and UBA domains (residues 6–350 of MARK2). Using small-angle X-ray scattering, they looked at whether there were differences in overall conformation between inactive MARK2-(6–350), expressed in *Escherichia coli*, and the active LKB1-phosphorylated fragment. The X-ray scattering analysis suggested that the LKB1-activated MARK-2 was more compact than inactive MARK2. Moreover, from the scattering data they were able to create a three-dimensional model of the active and inactive forms of MARK2-(6–350). This indicated that the structure of inactive MARK2 was elongated, with the UBA domain protruding from the larger catalytic domain. By contrast, in the active MARK2 structure, the UBA domain was closely associated with the large lobe of the catalytic domain. They then went on to test each of the ten protein kinases that contain the UBA domain to see if there was any interaction between the full-length proteins, or their isolated UBA domains, and different polyubiquitin species. However, they were unable to detect any significant interaction; nor were they able to detect binding to monoubiquitin or the ubiquitin-like SUMO (small ubiquitin-related modifier) proteins. They next looked at the role of the UBA domain in controlling the activity of AMPK-related kinases. Wild-type MARK4 and SIK, when transfected in HEK-293 cells endogenously expressing LKB1, were active and T-loop-phosphorylated, as expected. Interestingly, mutation of most of the conserved residues in the UBA domain, including the surface glycine residue, markedly reduced kinase activity and T-loop phosphorylation. Likewise, mutation of the conserved glycine residue to alanine in BRSK2 and QIK drastically decreased catalytic activity and T-loop phosphorylation. Fragments of MARK3, MARK4 and SIK that contained the kinase core but lacked the UBA domain were almost devoid of activity and T-loop phosphorylation. They then went on to investigate how mutations in the UBA domains of SIK and MARK2, expressed as non-phosphorylated forms in *E. coli*, affected phosphorylation and activation by the LKB1 complex *in vitro*. When the UBA-domain-conserved glycine residue of these kinases was changed to alanine, the mutants were barely phosphorylated or activated on treatment with LKB1 and MgATP. Likewise, fragments of SIK and MARK2 lacking the UBA domain were not significantly activated or phosphorylated by LKB1. Interestingly, in a mutant of SIK in which the T-loop threonine had been mutated to glutamate to mimic phosphorylation and activation, additional mutation of the conserved UBA glycine residue to alanine markedly reduced activity, suggesting that a functional UBA domain is required for full activation of T-loop-phosphorylated SIK. The UBA domain does not appear to function as a docking site for the LKB1 complex, since overexpression of the UBA domains of SIK, MARK3 and BRSK2 in HEK-293 cells did not affect the activity of endogenously expressed SIK, MARK2, MARK3 or QSK. Also, the *in vitro* phosphorylation and activation of SIK by LKB1 was unaffected by the inclusion of an excess of SIK UBA domain. No significant binding partners of isolated SIK UBA domains were identified in HEK-293 cells by tandem affinity purification. Lastly, the authors provide evidence that the UBA domain of SIK is required for the punctate nuclear localization of this particular AMPK-related kinase.

The important information arising from this work is that the UBA domains of AMPK-related kinases, rather than binding polyubiquitin, are necessary for their efficient phosphorylation and activation by LKB1. It is likely that the UBA domain interacts directly with the catalytic domain, inducing a conformational change to allow phosphorylation by LKB1. Three-dimensional structures of AMP-related kinases are eagerly awaited to elucidate the detailed molecular mechanisms involved. The mammalian $\alpha 1/\alpha 2$-AMPK subunits do not possess an obvious UBA domain, whereas the domain is present in the kinase subunit of SNF1 from *Saccharomyces cerevisiae* and from *S. pombe*. Interestingly, SNF1 is not stimulated by AMP, whereas AMP binding to the γ-subunits of mammalian AMPK is somehow thought to transmit a conformational change to the catalytic α-subunits which allows phosphorylation and activation by LKB1. Therefore during evolution, control of AMPK activation by upstream kinases seems to have moved from the catalytic subunits to the regulatory γ-subunit. Although some of the AMPK-related kinases interact with other proteins, none appear to require regulatory subunits, and they probably function as monomers. With the recent discovery that CAMKKs can also activate AMPK via T-loop phosphorylation [11–13], the question that immediately springs to mind is whether CAMKKs could also be upstream activating kinases for AMPK-related kinases, and, if so, whether the UBA domain would be necessary for phosphorylation and activation. Since a significant number of Peutz–Jehgers syndrome patients do not possess mutations in the LKB1 gene, it would be interesting to test families for mutations in the AMPK-related kinase genes and particularly in the region encoding the UBA domain.

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Received 31 January 2006; accepted 2 February 2006
Published on the Internet 24 February 2006, doi:10.1042/BJ20060184