AMPKbeta subunits, more than just a scaffold in the formation of AMPK complex

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Abbreviations: AMPK, AMP-activated protein kinase; $\alpha\gamma$-SBS, $\alpha\gamma$-subunit binding sequence; CIDEA, cell-death-inducing DFFA-like effector A; CBM, carbohydrate binding module; CBS, cystathionine-beta-synthase; DUB, deubiquitinating enzyme; PIASy, protein inhibitor of activated STAT; SENP, sentrin-specific protease (desumoylating enzyme); SUMO, small ubiquitin-like modifier.

Keywords: AMPK, CBM, phosphorylation, myristoylation, ubiquitination, sumoylation, activators.

ABSTRACT

AMP-activated protein kinase (AMPK) is a sensor of energy status composed of a catalytic subunit (AMPKα), a scaffolding subunit (AMPKβ) and a regulatory subunit involved in nucleotide binding (AMPKγ). Activation of AMPK results in enhancement of catabolic processes and downregulation of anabolic pathways with the aim to equilibrate the energy status of the cell. The study of the regulation of the activity of the AMPK complex has been traditionally focused on modifications of AMPKα and AMPKγ subunits by post-translational changes (i.e. phosphorylation of the catalytic subunit) and allosteric activation by AMP. In this review we summarize recent reports that indicate that AMPKβ subunits are also critical players in AMPK function, since they can regulate the phosphorylation status and activity of the AMPK complex. AMPKβ1- and AMPKβ2-containing complexes differ in their capacity to be activated by specific drugs (i.e., A769622, salicylate) and also by the ability to undergo post-translational modifications. This selective behavior opens the possibility to design specific drugs that activate AMPK complexes containing specific β-isoforms.

INTRODUCTION

AMP-activated protein kinase (AMPK) is a sensor of energy status. AMPK is a heterotrimer composed of one catalytic subunit (AMPKα, with two isoforms α1 and α2), one scaffolding subunit (AMPKβ, with two isoforms β1 and β2) and a regulatory subunit involved in nucleotide binding (AMPKγ, with three different isoforms γ1, γ2 and γ3). The AMPKγ subunit contains four tandem repeats of a structural module called a CBS motif, described initially in the enzyme cystathionine-β-synthase [1], which is involved in AMP, ADP and ATP binding [2]. Activation of AMPK is achieved by two complementary mechanisms. On one hand, AMPK is allosterically activated by the binding of AMP to the AMPKγ subunit, and on the other hand, the AMPKα catalytic subunit is phosphorylated in residue Thr172 in the kinase domain by specific upstream kinases (LKB1, CaMKKβ). It has recently been proposed that upon binding of AMP or ADP to the AMPKγ subunit, the AMPK complex undergoes a conformational change that makes it a poorer substrate for inactivating dephosphorylation ([3], [4]).

Therefore, an AMP/ADP-induced conformational switch and the ensuing phosphorylation of the catalytic subunit on Thr172 are the key factors that determine the activity of AMPK. The combined action of AMP binding and phosphorylation of Thr172 results in a 1,000 fold activation of the AMPK complex. Activation of AMPK results in an enhancement of catabolic processes and downregulation of anabolic pathways with the aim to equilibrate the energy status of the cell. Excellent reviews have been recently published which cover a comprehensive view of the importance of AMPK in cell physiology ([5], [6], [7], [8], [9]). The involvement of AMPKα and AMPKγ on the regulation of AMPK activity has also been recently reviewed ([5], [6], [7], [8], [9]). However, much less attention has been paid to the putative function of AMPKβ subunits on the regulation of AMPK function. In this review, we summarize recent reports that indicate that AMPKβ subunits are also critical players in AMPK function. Increasing evidence indicate that AMPKβ subunits regulate the phosphorylation status and activity of the AMPK complex and several reports indicate that these subunits can be viewed as new targets for therapeutic intervention in the development of drugs that activate AMPK complexes containing specific β-isoforms.

1.- Proteins, tissue functions and interacting domains.

Two isoforms of the AMPKβ subunit have been described, namely AMPKβ1 and AMPKβ2. Human AMPKβ1 is a protein of 270 aa (38 kDa) encoded by the PRKAB1 gene, and AMPKβ2 is a protein of 272 aa (34 kDa) encoded by the PRKAB2 gene. They are 71% identical and differ only at the N-terminus [10].

AMPKβ1 is mainly expressed in liver, pancreas, kidney, brown fat and brain, whereas AMPKβ2 is mainly expressed in cardiac and skeletal muscle ([10], [11], [12]). The generation of AMPKβ1 and AMPKβ2 null mice has revealed the distinct action of AMPK complexes containing each subunit in different tissues. For example, AMPKβ1 null mice show a dramatic effect in the liver, presenting reduced fasting gluconeogenesis and enhanced hepatic insulin sensitivity, what leads them to reduced food intake and body mass, and

preserves them from developing high-fat diet induced obesity [13]. AMPKβ1 null mice have also profound brain abnormalities due to reduced AMPK activity in this tissue [14]. In addition, the use of AMPKβ1 null mice has demonstrated a critical role of this subunit in controlling AMPK activity in the hypothalamus [13] and macrophages [15]. AMPKβ1-containing complexes play also a role in oxidative muscles as soleus, being the expression of AMPKβ1 dramatically up-regulated in the absence of endogenous AMPKβ2 subunit, suggesting that AMPKβ1 subunit can compensate for muscle function in some situations ([16], [17]). On the other hand, studies from AMPKβ2 null mice have demonstrated the critical role of AMPK complexes containing this subunit in skeletal muscle function, regulating glucose, glycogen and lipid metabolism during metabolic stress: AMPKβ2 null mice show hyperglycemia, glucose intolerance and insulin resistance when maintained on a high-fat diet and were unable to maintain muscle ATP levels during exercise ([16], [17]). Recently, specific skeletal muscle double AMPKβ1β2 null mice have been reported. They are physically inactive and have impaired contraction-stimulated glucose uptake, demonstrating a key role of AMPK in skeletal muscle function [18]. This unique tissue specific expression profile of AMPKβ subunits opens up the possibility of designing therapeutic strategies that target functions of AMPK in specific tissues.

AMPKβ subunits contain a carbohydrate binding module (CBM) in their N-terminal part, which extends from residues 68 to 163. In addition, AMPKβ subunits contain in their C-terminal part [residues 184 to 270 (β1)/272 (β2)] the αγ-SBS (αγ-Subunit Binding Sequence) domain, involved in binding to AMPKα and AMPKγ subunits (Fig. 1).

1.1.- Carbohydrate binding module (CBM).

The CBM found in AMPKβ subunits is remarkably similar to the major carbohydrate binding site of the starch binding domains in cyclodextrin glycosyltransferase, β-amylase and glucoamylase. Recently, all protein modules that bind starch, glycogen or related carbohydrates have been classified into different families of carbohydrate binding modules (CBMs). In this

classification the CBM of the AMPKβ subunits now belongs to the CBM48 type, found also in amylolytic enzymes from several glycoside hydrolase families [19]. These CBMs contain well-conserved consensus residues involved in binding to carbohydrate. In the CBM of the AMPKβ subunits these residues correspond to W100, K126 and W133 [19]. These residues were defined previously by structural analysis which revealed, in addition, the importance of residues L146 and N150 in carbohydrate binding [20] (Fig. 1). This study also indicated that the CBM adopts a β-sandwich fold with a single carbohydrate-binding site formed by one of the β-sheets and a β-hairpin loop (from residues T143 to N150; see Fig. 1), that could accommodate five glucosyl units, suggesting that malto-pentaose is the smallest oligosaccharide that could prevent carbohydrates from binding to AMPK [20]. In agreement with these results, it has been recently described that AMPKβ CBMs bind maltohexose and maltoheptaose with a Kd <1 mM, whereas shorter oligosaccharides bind less strongly [21]. In addition, this study describes that binding was stronger with sugars containing α1-4 with a single glucose α1-6 branch, suggesting that AMPK may bind to partially degraded glycogen [21].

Among AMPKβ subunits, the β2 isoform binds carbohydrates more tightly than β1, probably as a result of the insertion of a Thr residue (T101) following the W100 site involved in carbohydrate binding, which may increase the flexibility of the β2-CBM ([22], [23]). This implies that AMPK complexes containing β2-subunit could interact with glycogen more efficiently than those containing β1-subunit [23].

The CBMs of the AMPKβ subunits are responsible for localizing AMPK to glycogen. It has been reported that this polysaccharide can inhibit AMPK activity. This regulatory mechanism was first observed in samples from skeletal muscle from individuals with high glycogen content which showed a reduced activation of AMPK in response to exercise ([24], [25]). Later on, it has been demonstrated that addition of glycogen to rat liver AMPK is inhibitory, and that this inhibition depends on the presence of the CBM of the β-subunit of the AMPK complex. Inhibition requires the presence of critical residues in the CBM involved in binding to carbohydrates (i.e., W100 and W133) and is mediated by

the presence of α1-6 sugar linkages in the carbohydrate molecule (i.e., isomaltose but not maltose was inhibitory) [26]. These results led to the conclusion that AMPK acts as a cellular glycogen sensor through its CBM-containing β-subunit [26]. However, at present, the molecular mechanism by which glycogen inhibits AMPK activity is still unclear.

The CBM of AMPKβ subunits seem to be dispensable for the formation of a stable heterotrimeric complex since truncated β-subunits lacking the entire CBM domain still form regular complexes with α- and γ-subunits, which are fully active [27]. However, a truncation of the first 65 residues (up to the CBM) [27] or the G2A mutant which blocks myristoylation (see below) [28], causes an increase in AMPK activity, which suggest that the very N-terminus of AMPKβ subunit either has a direct autoinhibitory role or has a recruiting function of an still unknown component to allow autoinhibition indirectly [29].

1.2.- $\alpha\gamma$-Subunit binding sequence ($\alpha\gamma$-SBS).

The $\alpha\gamma$-SBS domain is essential for activity. Deletion of small parts of this domain leads to forms that can no longer assemble in complexes and are inactive, indicating that the $\alpha\gamma$-SBS domain is required for the formation of a stable, active $\alpha\beta\gamma$ complex ([27], [30]). Structural analysis of this domain reveals the presence of two β-sheets that interact with another β-sheet from the N-terminus of the γ-subunit ([4], [31]) (Fig. 1 and Fig. 2). In the AMPKβ1 subunit, the C-terminal 85 residues (residues 186-270) are sufficient to form an active heterotrimer. The C-terminal 25 residues of β1-subunit (residues 246-270) still bind γ-subunits but not the α-subunits and deletion of only the C-terminal Ile270 precludes $\beta\gamma$ association in the absence of α-subunit ([32], [33]). The fact that only the C-terminal part of the β-subunit is involved in binding to the other AMPK subunits was confirmed in plants, where it has been found that a new β-subunit isoform (AKINβ3), that lacks the N-terminal domain and also the CBM domain, is still able to interact with α- and γ-subunits and form an active heterotrimeric complex [34].

2.- Structural studies on AMPKβ subunits when forming part of heterotrimeric complex.

Recent reports on the structure of the heterotrimeric AMPK complex indicate that the C-terminus of the β-subunit forms a β-sheet structure that serves as a binding platform for both α- and γ-subunits ([4], [31]). Unfortunately, the structure of the other regions of mammalian AMPKβ subunits has not been defined yet. The only available structure containing the CBM domain is the one obtained from the *Saccharomyces cerevisiae* SNF1 complex (yeast orthologue of AMPK), containing a C-terminal part of Snf1 (aa 398 to 633) (AMPKα orthologue), a C-terminal part of Sip2 containing the CBM domain (aa 154 to 415) (AMPKβ orthologue) and the full length Snf4 (AMPKγ orthologue) [35]. In this structure, the C-terminal part of the Sip2 protein forms a two β-sheets fold that interacts with another β-sheet from the N-terminus of Snf4. Other regions from this C-terminus extend over the C-terminus of the α-subunit, although the crystal structure of this part is poorly defined. In addition, a clear CBM domain is observed, with the carbohydrate binding site facing towards Snf4. In fact, the β-hairpin loop [T143 to N150 (numbers corresponds to residues in AMPKβ1)] of the CBM faces on top of a hydrophobic patch formed by residues I172, F175 and L178 of the CBS2 of Snf4 (numbers corresponds to residues in AMPKγ1, which has an extra hydrophobic residue Phe179 not present in Snf4) ([29], [31], [35]) (Fig. 2). This is particularly interesting since CBS2 participates in the formation of site 2 of AMPKγ subunit, which is empty [31]. The interaction between the sugar binding β-hairpin loop of the β-subunit and the hydrophobic patch of the γ-subunit negatively regulates AMPK activation, since mutation of residues in these domains (i.e., F175Y, F175L, F179Y in AMPKγ1) increase the phosphorylation of Thr172 and AMPK activity [29].

Similar results were obtained in yeast where it was reported that mutations in different sites of the β-subunit (in this case Gal83, another β-subunit of *S. cerevisiae*), such as W184A (W100), R214Q (K126) and G235R (G147) (in brackets the number of the corresponding residue in AMPKβ1), which affect interactions between residues at the interface of CBM and γ-subunit (Snf4 in *S. cerevisiae*) [35], resulted in increased phosphorylation of

Thr210 and catalytic activity [36]. The authors also reported information about mutations in residues of the γ-subunit (Snf4) [C136 (V142), G145 (H151), R169 (R171) and N177 (F179); in brackets, the conserved residue in AMPKγ1], all located in CBS2, which produce similar enhanced effects on phosphorylation of Thr210 and catalytic activity [36]. All these results indicate that AMPKβ subunit, in addition to its function as scaffold to anchor the α- and γ-subunits through the αγ-SBS domain, may play an important role in the regulation of the activity of the heterotrimeric complex through the interaction of the CBMs with AMPKγ subunit.

Recently, our group has described that residue G147, in the sugar binding β-hairpin loop of the β-subunit (Fig. 2), participates in binding to R6, a regulatory subunit of protein phosphatase 1 (PP1) involved in the glucose-dependent dephosphorylation and inactivation of AMPK (a G147R mutation prevented the interaction of the β1-subunit with R6) [37]. This mutation did not affect the interaction between AMPKβ and AMPKα, but diminished its interaction with AMPKγ subunit. These results add an additional role of the sugar binding β-hairpin loop of the β-subunit in the regulation of AMPK activity by modulating the binding of specific phosphatases that dephosphorylate and inactivate Thr172 within the catalytic subunit.

3.- Posttranslational modifications:

The function of AMPKβ subunits is regulated by different types of posttranslational modifications:

3.1.- Phosphorylation

AMPKβ subunits are phosphorylated at multiple sites. In the case of AMPKβ1 this process occurs at Ser24/25, Ser108 and Ser182 (Fig. 1); however as AMPKβ2 lacks the Ser24/25 site, it is only modified in the other two sites ([28], [38]). Mass spectrometry analysis indicated alternative residues phosphorylated in β1 (i.e., Ser96 and Ser101, [39]; Thr80, Thr158, Ser174, Ser177 [40]), however no functional studies were presented on the importance of these sites on AMPK function. Ser24/25 and Ser108 are autophosphorylated by the catalytic activity of AMPKα1, whereas Ser182 is phosphorylated by a still

unknown upstream kinase. It has been reported that phosphorylation of Ser24/25 and Ser182 residues, although it does not affect AMPK activity, is necessary for the nuclear exclusion of β1-subunit, whereas phosphorylation of Ser108, although it does not affect subcellular distribution of AMPKβ, increases AMPK activity [28]. Interestingly, residue S108 is located in the CBM domain of AMPKβ subunit (Fig. 1), pointing again to the importance of this region in regulating the activity of the heterotrimeric complex.

3.2.- Myristoylation

Myristoylation consists on the addition of myristic acid (a 14 carbon saturated fatty acid) to a Gly residue in position 2, following processing of N-terminal Met residue. The myristoyl group acts as a lipid anchor, recruiting modified protein to cellular membranes [41]. Human AMPKβ subunits contain the consensus motif for myristoylation (MGNXXS/T, [42]) as they have at their N-terminus the protein sequence MGNTSS (AMPKβ1) or MGNTTS (AMPKβ2). More importantly, by mass spectrometry analysis it has been demonstrated that both AMPKβ subunits are myristoylated in vivo at Gly2 ([38], [43]). It was also reported that the elimination of the myristoylation site (G2A mutant) results in a more homogeneous distribution of the AMPKβ subunits inside the cell and in an increase in AMPK activity [28]. To explain this effect, it has been proposed that myristoylation may cooperate with the N-terminal first 65 residues of AMPKβ subunits to inhibit AMPK activity. Without myristoylation, the inhibitory effect of the N-terminus would be lost, resulting in AMPK activation [28].

Recently, it has been reported that myristoylation of AMPKβ subunits increases the ability of AMP to allosterically activate AMPK and to promote phosphorylation of the Thr172 site of the catalytic subunit by upstream kinases [44]. In addition, these authors indicate that AMP and glucose deprivation promote membrane association of myristoylated AMPK, suggesting a preferential activation of AMPK at the cellular membrane in response to starvation conditions. They also indicate that myristoylation of AMPKβ subunits plays a regulatory function distinct from the classical role in membrane association; they suggest that when cellular ATP levels are replete, the

myristoyl-group of the β-subunit might interact with the AMPKα subunit suppressing Thr172 phosphorylation and maintaining it in the inactive state. Upon a shift to starvation conditions, a myristoyl-switch will occur: the AMPKβ subunit will expose the myristoyl group to the solvent, what would promote AMPK membrane association and Thr172 phosphorylation and activation [44].

3.3.- Ubiquitination

This modification consist on the addition of ubiquitin (a 76 amino acid protein) moieties to lysine residues of the target protein by a complex mechanism that involves the action of a E1-activating enzyme, a E2-conjugating enzyme and a E3-ubiquitin ligase, which helps in the transfer of the ubiquitin moieties to the target substrate. A single ubiquitin moiety can be attached to the target in one (monoubiquitination) o several (multiubiquitination) lysine residues, or new ubiquitin moieties can be added to inner lysine residues of the ubiquitin molecule (polyubiquitination), forming in this case ubiquitin chains whose topology will depend on the lysine residue involved in the modification. As ubiquitin contains six lysine residues, chain formation can proceed in any of these residues. Depending of the topology of the ubiquitin chains, the modified protein will be targeted to destruction by the proteasome (as in the case of K11- or K48-linked chains) or will be engaged in non-proteolytical functions (as in the case of K63-linked chains) (see [45], for review). In 2008, it was reported that AMPKβ subunits could be modified by ubiquitination [46]. These authors demonstrated that the cell-death-inducing DFFA-like effector A (CIDEA), promoted the ubiquitination of the C-terminal part of AMPKβ subunits by an unidentified E3-ubiquitin ligase. CIDEA interacted physically with AMPKβ subunits but not with AMPKα or AMPKγ, and when co-expressed with AMPKβ reduced its stability due to ubiquitin-dependent degradation [46]. Consistent with these results CIDEA-/- mice have an enhanced AMPK stability and activity, what confirms the hypothesis that CIDEA plays a negative role on AMPK activity, likely by targeting the AMPK complex or AMPKβ subunits for degradation by the proteasome [47] (Fig. 3). Although the topology of the ubiquitin chains that CIDEA promoted on AMPKβ was not reported, it is likely

that they are of the canonical K48-type, which targets proteins for proteasome-dependent degradation.

Our group reported recently the ubiquitination of AMPKβ subunits by the E3-ubiquitin ligase malin [48]. This E3-ubiquitin ligase forms a functional complex with laforin, a glucan phosphatase, being both involved in Lafora disease, a rare fatal type of progressive myoclonus epilepsy ([49], [50]). The topology of the polyubiquitin chain was studied being mainly of the K63-type. Perhaps, for this reason, the laforin-malin mediated ubiquitination of AMPKβ subunits did not change their protein stability. On the contrary, this modification increased the steady-state levels of AMPKβ subunits, possibly because it led to the accumulation of the proteins into cellular deposits [48].

Ubiquitination can be reversed by the action of specific deubiquitinases (DUBs), which eliminate ubiquitin moieties from the target protein (see [51] for review). In yeast, it has been described that the Ubp8 deubiquitinase mediates the deubiquitination of Snf1 (orthologue of AMPKα), improving this way the stability and phosphorylation status of Snf1 and the activation of SNF1 complex activity [51]. On the contrary, deletion of UBP8 gene causes a decrease in SNF1 activity, probably due to hyperubiquitination [51]. In addition, it was recently described that USP9X deubiquitinase was involved in the deubiquitination of AMPK-related kinases NUAK1 and MARK4. USP9X was able to remove the atypical K29/K33-kinked ubiquitin chains present in these kinases [52]. As deubiquitinases may exhibit ubiquitin linkage specificity [45], it remains to be clarified whether USP9X or the mammalian orthologue of Ubp8 (USP22) might regulate the ubiquitination status of AMPKβ subunits. Alternatively, other DUBs might be involved in the elimination of the ubiquitin chains depending on the corresponding topology (K48-linked, K63-linked) (Fig. 3).

3.4.- Sumoylation

Sumoylation consists on the addition of a member of the SUMO (small ubiquitin-like modifier) family of ubiquitin-related peptides to lysine residues in target proteins, in a process similar to ubiquitination (see [53], and [54] for review). In mammals three SUMO paralogues exist: SUMO1, SUMO2 and

SUMO3, although SUMO2 and SUMO3 are 96% identical to each other and are functionally redundant. The sumoylation reaction requires the participation of the E1 SUMO-activating enzyme AOS1/UBA2 and the E2 SUMO-conjugating enzyme UBC9/UBE2I. UBC9/UBE2I recognizes a consensus sumoylation motif in many known targets (ΨKXD/E, being Ψ any hydrophobic residue and X any residue) and although it may interact transiently with target proteins, often the efficient conjugation of SUMO moieties requires the participation of an E3 SUMO-ligase. Similarly to ubiquitination, a single SUMO moiety can be attached to the target protein in one (monosumoylation) or several sites (multisumoylation), or new SUMO moieties can be attached to inner lysine residues of the SUMO molecule (polysumoylation). The latter case is only possible with SUMO2/3, since SUMO1 lacks appropriate inner lysine residues. For this reason SUMO1 is only able to form monosumoylated derivatives, or it can be attached to the end of SUMO2/3 chains (see [53], and [54] for review). In contrast to ubiquitination, poly-SUMO modification does not usually trigger protein degradation. Instead, sumoylation may affect subcellular localization, protein-protein interaction and transcriptional activation of targeted proteins.

Our group has recently reported that AMPKβ2 subunit is able to be posttranslationally modified by sumoylation [55]. This reaction requires the participation of the E3 SUMO-ligase PIASy (protein inhibitor of activated STAT) (Fig. 3). AMPKβ2 interacts physically con PIASy and, as a result of this interaction, AMPKβ2 is modified by the attachment of poly-SUMO2 chains. Since in previous results we found a strong yeast two-hybrid interaction between AMPK subunits and the E2 SUMO-conjugating enzyme UBC9/UBE2I [56], this reinforces our results of AMPKβ2 being modified by sumoylation. Interestingly, AMPKβ1 is not a target of PIASy, suggesting that the modification occurs on lysine residues present in AMPKβ2 but not in AMPKβ1. Sumoylation of AMPKβ2 enhances the phosphorylation status of Thr172 in the catalytic subunit and the activity of the AMPK complex, probably because this modification either improves the phosphorylation of AMPK by upstream kinases or because it prevents the dephosphorylation of AMPK by making it a poorer substrate for specific phosphatases (Fig. 3). Finally, we also demonstrated that

sumoylation and ubiquitination of AMPKβ2 subunit are competitive and antagonistic processes: the promotion of one of the modifications prevents the other, and the final consequence of one of them is different from the other: sumoylation activates AMPK complex whereas ubiquitination promotes a reduction in AMPK activity since it targets AMPK for degradation (Fig. 3).

Sumoylation is a reversible process. Specific proteases (SENPs, sentrin-specific proteases) remove the SUMO moieties from target proteins [57] (Fig. 3). However, at present no information has been reported about the putative role of these enzymes in regulating the sumoylation status of AMPKβ subunits.

4.- Differences between AMPKβ subunits.

Although AMPKβ1 and AMPKβ2 are highly similar in protein sequence, several reports suggest that each of these subunits can be affected by different mechanisms.

4.1.- Sensitivity to AMPK activators.

In 2006, a new activator of AMPK, namely A769622, was described [58]. A769622 activates AMPK both allosterically and by inhibiting the dephosphorylation of AMPK on Thr172 by specific phosphatases. Its mechanism of action does not involve binding to the γ-subunit, in contrast to other AMPK activators (AMP, AICAR, etc.). On the contrary, its effect depends on the presence of the β-subunit. The CBM of the β-subunit plays a key role in the mechanism of action of A769622 as its effect is abolished in an AMPK complex where the β-subunit lacks the carbohydrate binding module. It was also reported that A769622 does not interfere with the binding of the CBM to carbohydrates and that Ser108 residue plays a role in the activation of AMPK by A769622, since a S108A mutation in the β-subunit almost completely abolishes activation ([29], [59], [60]). As it has been suggested that AMPKβ subunits play a negative role in the regulation of AMPK activity (see above), all these results suggest that A769622 acts by reversing the autoinhibitory role of the β-subunits on AMPK activity. Interestingly, it has been demonstrated that A769622 is selective for β1-subunit isoform and does not activate AMPK

heterotrimers containing β2-subunits [29]. At present no explanation has been proposed for this selective effect of the A769622 compound.

A recent study indicates that salicylate activates AMPK through binding to the same β-subunit site that interacts with A769622. This compound also causes allosteric activation and inhibition of the dephosphorylation of the Thr172 site in the catalytic subunit on AMPK complexes containing β1, but being inactive on β2-containing ones [61]. So, both A769622 and salicylate activate AMPK in an AMP-independent manner [62], and their effect depends on still unknown modifications in the AMPKβ1 subunit that avoid the negative role that this β-subunit has on the activity of the AMPK complex. In the same way, GlaxoSmithKline company has recently presented pyrrolo-pyridine derivatives with preference for AMPKβ1 subunit, but again the mechanism of action of these compounds is still unknown [63].

4.2.- Sensitivity to undergo sumoylation

As reported above, only AMPKβ2 can be modified posttranslationally by PIASy-dependent sumoylation, leading to the activation of the AMPK complex. The basis for this effect is still unknown. Perhaps this modification induces changes in the conformation of the β-protein that can be transmitted to the whole AMPK complex, making it more active. This opens a way to selectively activate AMPK complexes containing β2-subunit, as those present in skeletal and cardiac muscle.

5.- Concluding remarks

Having in mind all the evidence presented in this review, it is clear that AMPKβ subunits have, in addition to their scaffold function in the formation of the AMPK complex, alternative functions in the regulation of AMPK activity. Mutational analysis of specific residues in the CBS2 domain of AMPKγ in both yeast and mammals and the modification of AMPKβ subunits, either by myristoylation or by mutation of critical residues of the CBM, support the idea that AMPKβ subunits play a critical negative role in the regulation of the activity of the AMPK complex. This role may be due, at least in part, to the ability of

AMPKβ subunits to interact with the CBS2 domain of the AMPKγ subunit. Perhaps A769622 and salicylate operate at this interface to activate AMPK activity, since they do not affect the binding of AMP to the different AMP-binding sites of the AMPKγ subunit. However, more work needs to be done to characterize molecularly the mechanism of action of these compounds. Interestingly, AMPKβ subunits offer selectivity in the activation of the AMPK complex. Both A769622 and salicylate are only able to activate β1-containing AMPK complexes, whereas sumoylation affects only AMPK complexes containing β2-subunit. This selective behavior opens the possibility to design specific drugs that may activate one particular type of AMPK complexes in specific tissues.

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FIGURE LEGENDS

Fig. 1: Structural domains in AMPKβ subunit. A diagram of human AMPKβ1 subunit is shown. The CBM (Carbohydrate Binding Module) (aa 68 to 163) and αγ-SBS (αγ-Subunit Binding Sequence) (aa 184 to 270) domains are drawn. The position of the myristoylation (G2) and the phosphorylation sites (S24/25, S108, S182) is also indicated. On the left, the crystal structure model of CBM domain of the rat β1-subunit (residues 68 to 163) (PDB, 1Z0M) [20] was modeled using the PyMol programme (DeLano Scientific LLC, San Carlos, CA). Residues involved in carbohydrate binding (W100, K126, W133, L146 and N150) are colored in green. The position of T143 in the β-hairpin loop and S108 (in red) is indicated. The cyclodextrin ring is also drawn. On the right, the crystal structure model of the C-terminus of rat β1-subunit (residues 235 to 270) (PDB, 4EAK) [4] was also modeled.

Fig. 2: Structure model of the heterotrimeric AMPK complex. On the left, crystal structure of the heterotrimeric core of the *Saccharomyces cerevisiae* SNF1 complex (PDB, 2QLV) [35] was modeled using the PyMol programme (DeLano Scientific LLC, San Carlos, CA). The C-terminal part of Snf1 (AMPKα) (residues 398 to 633) is colored in blue, the C-terminal part of Sip2 (AMPKβ) (residues 154 to 415) is colored in green and the full length Snf4 (AMPKγ) is colored in yellow (N-terminus), orange (CBS1), dark brown (CBS2), violet (CBS3) and magenta (CBS4). On the right, a zoom of the interface between the β- and γ-subunits is shown. Residues forming part of the hydrophobic patch [I172 in cyan, F175 in blue and L178 in red; numbers correspond to residues present in AMPKγ1, which has in addition a F179 residue (in light blue) instead of Asn] in the CBS2 of Snf4, and those forming part of the sugar-binding β-hairpin loop of Sip2 (T143 to N150, in green; numbers correspond to the residues present in AMPKβ1) are indicated. The position of residue G147 is also indicated.

Fig. 3: Sumoylation and ubiquitination produce antagonist effects of AMPKβ function. AMPKβ subunits can undergo CIDEA-dependent ubiquitination, leading to inhibition of AMPK complex activity, or PIASy-dependent

sumoylation, leading to an enhancement of AMPK complex activity. These modifications can be reversed by the action of deubiquitinases (DUBs) or desumolyases (SENPs) respectively. U, ubiquitin; S, SUMO.

AMPKβ1

G2 S24/25 S108 S182

CBM αγ-SBS

1 68 163 184 270

L146
N150
T143
W133
W100
K126
S108

H235
I270

S
S
S
S
AMPKβ

PIASy
Sumoylation

SENPs

AMPKβ

CIDEA
Ubiquitination

DUBs

U U
U
U
AMPKβ

Activation of
AMPK complex

Inhibition of
AMPK complex