Am J Physiol Cell Physiol 303: C475–C485, 2012.
First published June 13, 2012; doi:10.1152/ajpcell.00125.2012.

Review

The role of AMP-activated protein kinase in the coordination of skeletal muscle turnover and energy homeostasis

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Submitted 11 April 2012; accepted in final form 12 June 2012

Sanchez AM, Candau RB, Csibi A, Pagano AF, Raibon A, Bernardi H. The role of AMP-activated protein kinase in the coordination of skeletal muscle turnover and energy homeostasis. *Am J Physiol Cell Physiol* 303: C475–C485, 2012. First published June 13, 2012; doi:10.1152/ajpcell.00125.2012.—The AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that acts as a sensor of cellular energy status switch regulating several systems including glucose and lipid metabolism. Recently, AMPK has been implicated in the control of skeletal muscle mass by decreasing mTORC1 activity and increasing protein degradation through regulation of ubiquitin-proteasome and autophagy pathways. In this review, we give an overview of the central role of AMPK in the control of skeletal muscle plasticity. We detail particularly its implication in the control of the hypertrophic and atrophic signaling pathways. In the light of these cumulative and attractive results, AMPK appears as a key player in regulating muscle homeostasis and the modulation of its activity may constitute a therapeutic potential in treating muscle wasting syndromes in humans.

AMPK; autophagy; Ulk1; metabolism; ubiquitin-proteasome; FoxO

Structure and Regulation of AMPK

The AMP-activated protein kinase (AMPK) is a serine-threonine kinase highly conserved through evolution. AMPK is a heterotrimeric complex composed of a catalytic subunit (AMPK-α) and two regulatory subunits (AMPK-β and AMPK-γ) (Fig. 1). In humans, AMPK subunits are encoded by seven genes (α1, α2; β1, β2; γ1, γ2, γ3) that can form at least 12 αβγ heterotrimers, thus increasing the diversification of its functions (17, 109, 117). The most studied of these subunits is the catalytic subunit, which contains the threonine residue (Thr172) located in the activation loop of the kinase domain. Phosphorylation of Thr172 by upstream kinases leads to AMPK activation (47, 49). AMPK is considered as a key enzyme in conditions of cellular energy deficit and is able to inhibit metabolic pathways that consume energy and reciprocally to increase mechanisms that produce energy.

AMPK is activated by a large variety of cellular stresses that increase cellular AMP and decrease ATP levels such as electrical-stimulated muscle contraction (24, 120) and vigorous exercises (15, 20, 92, 125) and by hypoxia (79), ischemia (71), oxidative stress (18), metabolic poisoning (124), or nutrient deprivation (103). In response to energy depletion, AMPK activation promotes metabolic changes to maintain both bioenergetic state and cell survival. Moreover, by modulating multiple metabolic pathways and by regulating several transport proteins, AMPK potentially couples transport activity to cellular stress and energy levels. Pharmacological molecules such as 5-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside (AICAR), metformin, clozapine, or 2-deoxy-D-glucose (2DG) also lead to AMPK activation (21, 35, 48, 98, 102, 129, 130).

AMPK activity is modulated in an allosteric way by AMP that promotes the phosphorylation on Thr172 by the AMPK kinases (AMPKK), and by ATP that inhibits phosphorylation on this site (110). AMP and ATP competitively bind to AMPK γ subunits on four sites formed by cystathionine β synthase (CBS) domains (17). Moreover, binding of AMP to AMPK inhibits dephosphorylation of Thr172 by phosphatases, an effect that is antagonized by high concentrations of ATP (22). Interestingly, Xiao and colleagues (127) have recently determined the crystal structure of an active AMPK complex and have explored how the kinase region interacts with the regulatory nucleotide-binding site that mediates protection against dephosphorylation. They found that the binding of AMP or ADP to the regulatory domain protects AMPK dephosphorylation, although ADP does not lead to allosteric activation (127). Finally, it was shown that AMPK is inhibited by glycogen in an allosteric manner, leading to inhibition of Thr172 phosphorylation by upstream kinases (80). Thus, these data reveal that AMPK constitutes a sensor of the status of cellular glycogen reserves.

The phosphorylation of AMPK at Thr172 residue is regulated by three AMPKK identified to date. The serine threonine kinase LKB1, a tumor suppressor, and the Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ) were the primary characterized upstream kinases of AMPK (51, 60, 113, 131,

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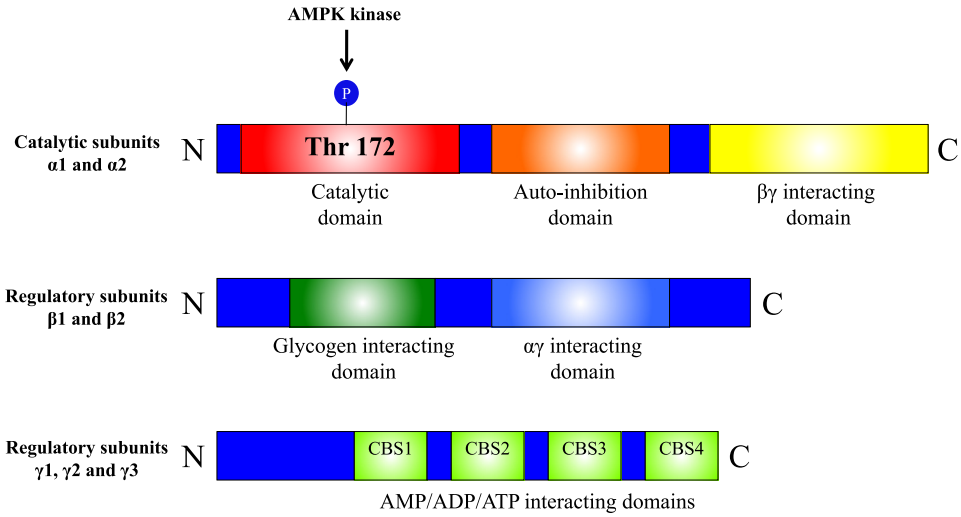

Fig. 1. Structure of AMP-activated protein kinase (AMPK). AMPK is composed of a catalytic subunit (AMPK-α) and two regulatory subunits (AMPK-β and AMPK-γ). AMPK-α contains a catalytic domain in the NH_2-terminal that can be phosphorylated by the AMPKK enzymes on the residue Thr172, an autoinhibition domain and a domain interacting with the subunits β and γ. The regulatory β subunits have interacting domains with glycogen and subunits α and γ. The regulatory γ subunits present four cystathionine β-synthase (CBS) domains interacting with AMP, ADP, and ATP.

132). More recently, the transforming growth factor β-activated kinase 1 (TAK-1) was also found to phosphorylate AMPK (52).

Analysis of AMPK substrates suggests a consensus recognition sequence in which the phosphorylated serine residue is close to a hydrophobic residue on the NH_2-terminal side (i.e., at −1) and at least one arginine residue at −2, −3, or −4. Substrates for cyclic AMPK which lack the hydrophobic residue at −1 are not substrates for AMPK (13).

Glossary

2DG	2-deoxy-D-glucose
ACC	acetyl-CoA carboxylase
AICAR	5-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside
AMPK	adenosine monophosphate-activated protein kinase
AMPKK	AMPK kinases
Atg	autophagy-specific gene
atrogin-1/MAFbx	muscle atrophy F-box
CaMKK	Ca^{2+}/calmodulin-dependent protein kinase kinase
CBS	cystathionine β-synthase
ChREBP	carbohydrate response element-binding protein
CPT-1	carnitine palmitoyltransferase 1
CREB	cAMP response element-binding
CRT	creatine transporter
eEF2	eukaryotic elongation factor-2
eIF4E	eukaryotic translation initiation factor 4E
4E-BP1	eukaryotic translation initiation factor 4E-binding protein 1
FAT/CD36	fatty acid translocase/cluster of differentiation 36
FIP200	focal adhesion kinase family interacting protein of 200 kDa
FoxO	Forkhead box O
GLUT-4	glucose transporter-4
GS	glycogen synthase
HK-2	hexokinase-2
HMGR-CoA	hydroxymethyl-glutaryl-coenzyme A reductase
HSL	hormone-sensitive lipase
LC3	microtubule-associated protein light chain 3
LCFA CoA	long-chain fatty acyl-CoA
LKB1	liver kinase B1
mLST8	mammalian target of rapamycin-associated protein LST8 homolog
mTOR	mammalian target of rapamycin
mtTFA	mitochondrial transcription factor A
mTORC1	mTOR complex 1
MuRF1	muscle RING finger 1
NRFs	nuclear respiratory factors
PDK1	phosphoinositide-dependent kinase 1
PFK-2	6-phosphofructo-2-kinase
PGC-1	peroxisome proliferator-activated receptor-γ coactivator 1
PPAR	peroxisome proliferator-activated receptor
PRAS40	proline-rich Akt substrate of 40 kDa
raptor	regulatory-associated protein of mTOR
Rheb	Ras homolog enriched in brain
rpS6	ribosomal protein S6
S6K1	ribosomal protein S6 kinase 1
SIRT1	sirtuin 1
SREBP1c	sterol regulatory element-binding protein 1c
TAK-1	transforming growth factor β-activated kinase 1
TSC1/2	tuberous sclerosis complex 1/2
Ulk1	unc-51-like kinase 1

Role of AMPK in Metabolic Regulations: AMPK Regulates Anaerobic Metabolism, Fatty Acid Oxidation, and Cholesterol Synthesis

AMPK is a regulator of anaerobic metabolism allowing the insulin-independent transport of glucose and its subsequent metabolism in skeletal muscle and in the heart (4, 50, 99) (Fig. 2). Activation of AMPK induces the expression and the translocation of the glucose transporter-4 (GLUT-4) to the plasma membrane, resulting in an increase in glucose uptake and blood glucose oxidation (53). AMPK regulates glycolysis through

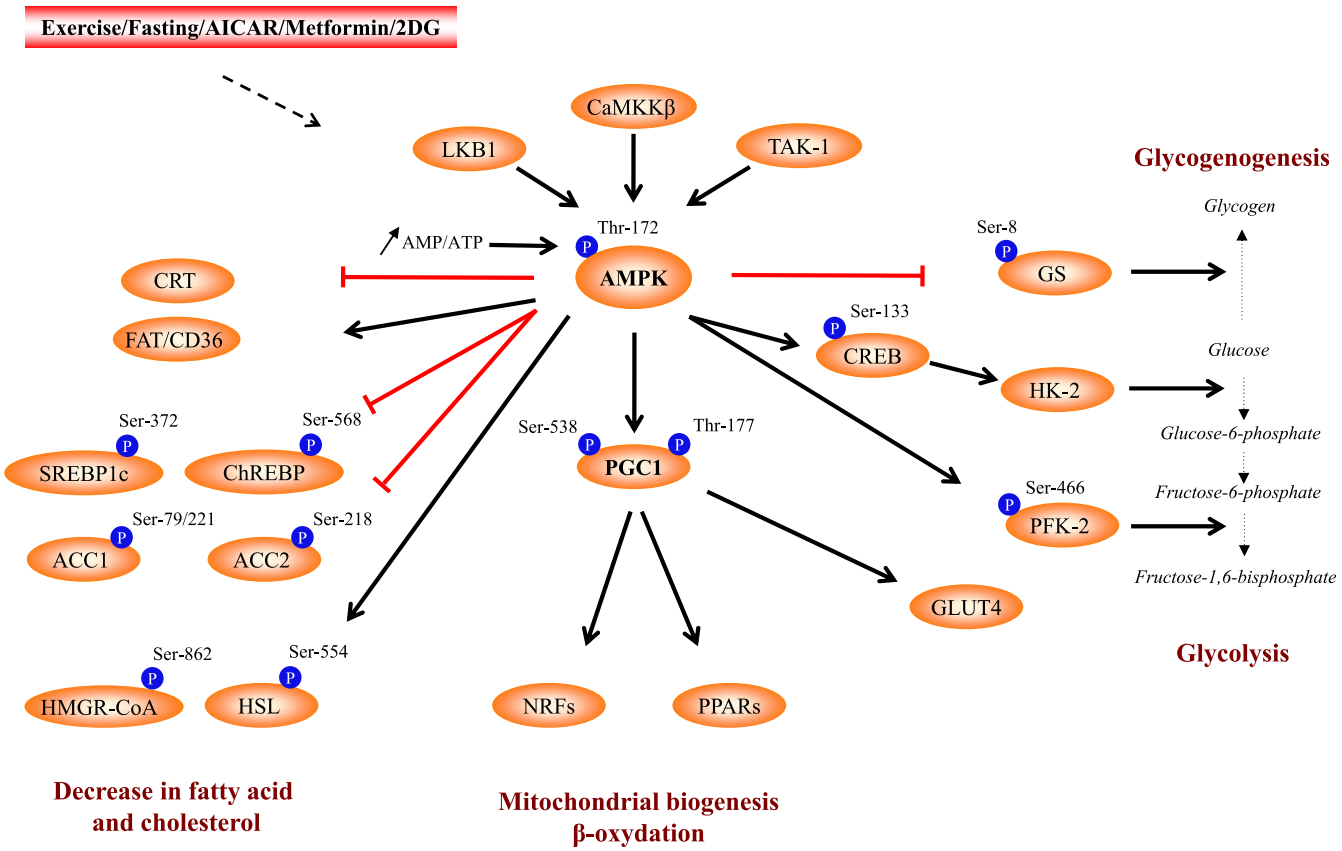

Fig. 2. AMPK regulates metabolic pathways in skeletal muscle. AMPK is directly activated by the enzymes LKB1, CaMKKβ, and TAK-1 upon high AMP levels, and indirectly by exercise, fasting, AICAR, metformin, or 2-deoxy-D-glucose (2DG) treatments. AMPK positively regulates glycolysis by phosphorylating 6-phosphofructo-2-kinase (PFK-2), by increasing hexokinase-2 (HK-2) transcription through cAMP response element-binding (CREB) phosphorylation, and by increasing the expression and translocation of the glucose transporters (GLUT4). AMPK inhibits the activities of the creatine transporter (CTR), glycogen synthase (GS), acetyl-CoA carboxylase (ACC), sterol regulatory element-binding protein 1c (SREBP1c), and carbohydrate response element-binding protein (ChREBP), and activates hormone-sensitive lipase (HSL) and hydroxymethyl-glutaryl-coenzyme A reductase (HMGR-CoA). AMPK increases fatty acid uptake through the translocation of fatty acid translocase (FAT)/CD36 to the plasma membrane. Activation of AMPK also leads to an increase of the transcriptional activity of the peroxisome proliferator-activated receptors and the nuclear respiratory factors (NRFs) via phosphorylation of PGC-1α.

phosphorylation of 6-phosphofructo-2-kinase (PFK-2), a key enzyme responsible for fructose 2,6-bisphosphate synthesis, a rate-limiting step in glycolysis (79). A dominant-negative form of AMPK could prevent both the activation and the phosphorylation of PFK-2 by oligomycin (79), which confirms the major role of AMPK in the regulation of PFK-2 activity. Moreover, AMPK increase hexokinase-2 (HK-2) transcription, an enzyme responsible for glucose-6-phosphate synthesis, by phosphorylating cAMP response element-binding (CREB) at Ser133 (111, 116). Furthermore, AMPK phosphorylates and inactivates glycogen synthase (GS), inhibiting glycogenogenesis (1, 13). This action seems to preferentially occur through the regulation of AMPK α2 isoform (62). In addition to the regulation of glycolysis and glycogenolysis, Li and colleagues (75) have recently shown that AMPK inhibits apical membrane creatine transporter (CRT) expression in kidney proximal tubule cells. CTR inhibition by AMPK is important because unnecessary creatine reabsorption and cellular energy expenditure are decreased under conditions of metabolic stress.

AMPK also modulates fatty acid and cholesterol metabolism in specialized tissues, such as adipose tissue, liver, and muscle (Fig. 2). AMPK increases fatty acid uptake due to translocation of the fatty acid translocase (FAT)/CD36 transporter to the cellular membrane (77). AMPK inhibits fatty acid and cholesterol synthesis through direct phosphorylation of the metabolic enzymes acetyl-CoA carboxylase 1 (ACC1), the hydroxymethyl-glutaryl-coenzyme A reductase (HMGR-CoA), and the hormone-sensitive lipase (HSL) (12, 14, 37). Furthermore, AMPK inhibits the expression and the activity of the transcription factors SREBP1c (sterol regulatory element-binding protein 1c) (33, 129) and ChREBP (carbohydrate response element-binding protein) (33, 68). The latter action represses the transcription of lipogenic genes and fatty acid synthesis (33). Furthermore, via phosphorylation and inhibition of ACC2, AMPK induces a drop in the production of malonyl-CoA, an allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT-1) (83, 122, 123). CPT-1 controls the transfer of cytosolic long chain fatty acyl CoA (LCFA CoA) into mitochondria (81) and represents the rate-limiting step of fatty acid oxidation. Thus, AMPK activation involves a reduction in the cytosolic concentration of malonyl-CoA and facilitates the penetration and the oxidation of fatty acids in mitochondria.

The role of PPAR-γ coactivator 1α (PGC-1α) in adaptive responses after AMPK activation is now quite detailed (Fig. 2). PGC-1 is a master protein involved in the regulation of oxidative metabolism of brown fat and muscle that upregulates

mitochondrial respiration and biogenesis through an increase in the expression of enzymes implicated in the electron transport system and uncoupling proteins, and through regulation of the nuclear respiratory factors (NRFs) (94, 126). PGC-1 binds to and activates NRF-1 on the promoter of the mitochondrial transcription factor A (mtTFA), a direct regulator of mitochondrial DNA replication and transcription (126). In skeletal muscle, Canto and colleagues (11) have recently shown that inhibition of AMPK activity compromises the histone deacetylase sirtuin 1 (SIRT1)-dependent responses to exercise or fasting by decreasing cellular NAD^+ levels. This results in impaired PGC-1α deacetylation and blunted induction of mitochondrial gene expression (11, 60). The effects of AMPK on gene expression of GLUT-4, mitochondrial genes, and PGC-1α itself are almost entirely dependent on the function of PGC-1α protein (60). PGC-1α gene expression is increased with exercise, AICAR, and metformin treatments (54, 112, 113). AMPK directly phosphorylates PGC-1α at Ser538 and Thr177 and these phosphorylation events are required for induction of the PGC-1α promoter (60).

Role of AMPK in the Control of Skeletal Muscle Mass

The maintenance of muscle mass is controlled by a fine balance between catabolic and anabolic processes, which determine the level of muscle proteins and the diameter of muscle fibers. Skeletal muscle hypertrophy can be defined as an overall augmentation in muscle mass, as a result of an increase in the size of preexisting skeletal muscle fibers accompanied by enhanced protein synthesis without an apparent increase in the number of myofibers (39). Muscle hypertrophy is associated with a strong rate of protein synthesis more than with a change in protein degradation. This physiological process stimulates muscle growth in response to mechanical loading or nutritional stimuli (39). On the contrary, skeletal muscle atrophy can be defined as a decrease in muscle fiber diameter, protein content, force production, and fatigue resistance (34, 59). This process results from a plethora of causes including immobilization, denervation, aging, and neuromuscular diseases. Moreover, muscle atrophy can be secondary to some devastating pathologies or health problems, such as spinal cord injury (16), cancer cachexia, sepsis, diabetes, or AIDS, and exacerbated by microgravity, glucocorticoid treatment, and starvation (31, 73, 84).

When the cellular energy level is low, AMPK promotes ATP production by switching on catabolic pathways and conserves ATP levels by switching off ATP-consuming processes, including most biosynthetic pathways. In muscle, this action occurs when AMPK is activated because of energy deprivation, and it results in the arrest of protein synthesis and cell growth, with the stimulation of muscle proteolysis. Thus, AMPK, as the main energy sensor in muscle cells, modulates muscle turnover and skeletal muscle mass.

AMPK and inhibition of protein synthesis. Several data suggest that AMPK acts as a negative regulator of protein synthesis by reducing both the initiation and the elongation of ribosomal peptide synthesis. Thus, it was shown that AMPK activation leads to the Thr56 phosphorylation of the eukaryotic elongation factor-2 (eEF2) (55). Upstream of this phosphorylation, activation of AMPK inhibits the mammalian target of rapamycin complex 1 (mTORC1), a multiprotein complex composed of mTOR (also known as FRAP, RAFT1, or RAPT), the regulatory associated protein of mTOR (raptor), the proline-rich Akt substrate of 40 kDa (PRAS40), and the mTOR-associated protein LST8 homolog (mLST8) (69, 70, 119). This protein complex controls skeletal muscle hypertrophy (6, 97) through modulation of protein synthesis by phosphorylation toward its downstream effectors, the ribosomal protein S6 kinase 1 (S6K1) (2) and the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) (38). Its inhibition by AMPK results in decreased protein synthesis in both in vivo and in vitro models (7, 93). Phosphorylation of 4E-BP1 at Thr37/46 by mTOR promotes its dissociation from eIF4E bound to the mRNA 7-methylguanosine cap structure, allowing the assembly of the preinitiation complex (128). S6K1 activation needs initial phosphorylation by mTORC1 at Thr389 (101) and additional inputs on Thr229 for full activation by the phosphoinositide-dependent kinase 1 (PDK1) (95). S6K1-mediated regulation of translation is thought to occur in part through phosphorylation of the 40S ribosomal protein S6 (rpS6) at Ser235/236 (30). Several studies highlighted that AMPK activation by resistance exercise (27) or high-frequency electrical stimulation (114) leads to the inhibition of S6K1 and 4E-BP1. In vitro, pretreatment with AICAR completely inhibited the insulin-induced activation of mTORC1 and its downstream effectors (25). Two models have been proposed for the AMPK-mediated inhibition of mTORC1 activity (Fig. 3). The first involves the phosphorylation of the tuberous sclerosis complex 2 (TSC2) on residues Thr1227 and Ser1345, thus enhancing its GTPase activity towards the Ras homolog enriched in brain (Rheb), the direct activator of mTOR (58). The second model implicates the direct phosphorylation of raptor at Ser722/792, leading to the cytoplasmic retention of raptor by 14-3-3 binding (45).

Lantier and colleagues (72) reported that *AMPK* $^{-/-}$ mice exhibited a shift of muscular fiber size distribution toward higher values correlated with increased phosphorylation of S6K1 Thr389 and rpS6 Ser235/236 associated with a stimulation of protein synthesis. In this study, the size of AMPK-deficient myotubes was 1.5-fold higher than for controls and soleus mass was significantly higher by 42% in muscle AMPK-deficient mice compared with control mice. Another recent study performed by the same group demonstrates that AMPK-deficient mice exhibit enhanced hypertrophy after mechanical overloading, a phenomenon associated with increased activity of the mTORC1 signaling (87). Moreover, the overloading-induced hypertrophy was associated with a significant increase in AMPK α1 expression and activity after 7 and 21 days in non-transgenic muscle, probably to limit muscle mass growth (82). In the absence of AMPK α1, unphosphorylated eEF2 levels are higher in response to chronic overload with a parallel increase in mTORC1 signaling leading to greater muscle hypertrophy (87). Thus, these data show that AMPK α1 behaves as a negative effector required to limit mTORC1 activity and to inhibit overgrowth of skeletal muscle in response to hypertrophic stimuli.

In an aged rat model, Thomson and Gordon (115) showed that activation of AMPK is linked to the diminished overload-induced hypertrophy in fast-twitch skeletal muscle. An age-related increase in AMPK phosphorylation may partly contribute to the attenuated hypertrophic response observed in overloaded fast-twitch plantaris muscle. Furthermore, AMPK is upregulated with age in resting and overloaded fast-twitch skeletal

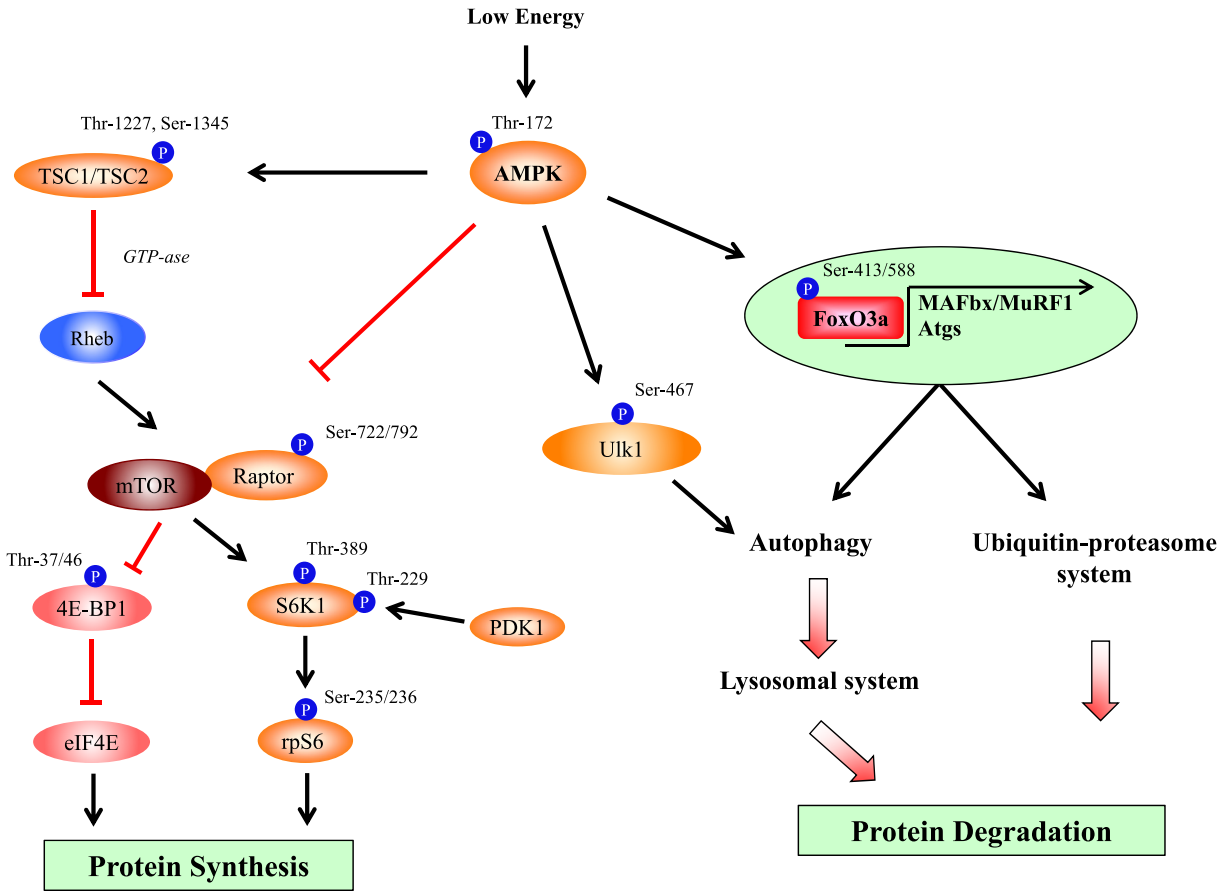

Fig. 3. AMPK regulates both anabolic and catabolic pathways in skeletal muscle. AMPK decreases protein synthesis by phosphorylating the tuberous sclerosis complex 1/2 (TSC1/2), an inhibitor of the mammalian target of rapamycin (mTOR) activator Ras homolog enriched in brain (Rheb), and by phosphorylating the regulatory associated protein of mTOR (raptor). mTOR modulates protein synthesis by phosphorylation toward its downstream effectors, the ribosomal protein S6 kinase 1 (S6K1) and the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). S6K1 activation needs initial phosphorylation by mTORC1 and additional inputs for full activation by the phosphoinositide-dependent kinase 1 (PDK1). S6K1-mediated regulation of translation occurs in part through phosphorylation of the 40S ribosomal protein S6 (rpS6). Phosphorylation of 4E-BP1 by mTOR promotes its dissociation from eukaryotic translation initiation factor 4E (eIF4E) and allows the assembly of the preinitiation complex. Activation of AMPK leads to an increase in Forkhead box O3a (FoxO3a) activity that induces the expression of the muscle atrophy F-box (MAFbx), the muscle RING finger 1 (MuRF1), and autophagy genes (Atgs). AMPK also interacts with, and phosphorylates, the unc-51-like kinase 1 (Ulk1) leading to autophagy induction.

muscle but not in slow-twitch muscle. Thus, the modulation of AMPK activity by pharmacological agents could constitute a relevant therapeutic tool to prevent sarcopenia.

Taken together, AMPK appears to be an essential contributor to the control of muscle cell size and adaptation to muscle hypertrophy. AMPK is involved in the cell size maintenance through the regulation of mTORC1 pathway and appears to play a major role in the metabolic program that controls muscle plasticity. The severe activation of AMPK in the overload model could contribute to restoring metabolic homeostasis that has been disturbed by the increased protein synthesis. Thus, AMPK seems to act as a homeostatic factor and to determine the extent to which protein translation is allowed under various energetic circumstances. Additionally, by limiting muscular hypertrophy, AMPK would act as negative feedback in the control of skeletal muscle mass.

AMPK and stimulation of protein degradation. In addition to the inhibitory effect of AMPK on protein synthesis, this enzyme has recently been associated with increased myofibrillar degradation in muscle cells. Nystrom and coworkers showed that AICAR and metformin treatments decreased protein synthesis and increased protein degradation in an AMPK-dependent manner in C2C12 myotubes (91).

AMPK regulates FoxO3a-dependent protein degradation. Nakashima and Yakabe (88) reported that AMPK activation stimulates myofibrillar protein degradation through increased expression of FoxO transcription factors. Protein degradation is mediated by two conserved pathways: the ATP-dependent ubiquitin-proteasome system and the autophagy-lysosomal pathway. The first one implicates a cascade of enzymatic reactions that labels substrate proteins with ubiquitin chains for degradation by the 26S proteasome. This system involves the activity of an E1 (ubiquitin-activating enzyme), an E2 (ubiquitin-conjugating enzyme), and an E3 ubiquitin ligase, which confers substrate specificity for ubiquitination. Two major E3 ligases have been described to be essential for muscle atrophy, atrogin-1/MAFbx (muscle atrophy F-box) and MuRF1 (muscle RING finger 1) (5, 32, 40). The second pathway implicates lysosomes and represents an important mechanism in the maintenance of protein turnover and cellular metabolism. This process requires Atg (autophagy-specific gene) proteins, which are necessary for the formation of autophagosomes (19). The

formation of these vesicles is required to drive substrates to lysosomes to achieve substrate degradation. mRNAs encoding Atg proteins are very abundant in skeletal muscle (86). This process is constitutively active in skeletal muscle and is enhanced in human myopathies caused by a genetic deficiency of lysosomal proteins, as evidenced by the accumulation of autophagosomes in Pompe's and Danon's diseases (89, 100, 121). Importantly, autophagy sequestration under starvation conditions requires the conjugation of LC3 (microtubule-associated protein light chain 3) with the phospholipids of the vacuolar membrane (65, 66).

Expression of a constitutively activated FoxO3a increases the transcription of many autophagy-related genes, including *LC3B, Gabarapl1, Beclin1, PI3KIII, Ulk2, Atg4b,* and *Atg12l* (78). FoxO proteins are an evolutionarily conserved subfamily of transcription factors involved in tumor suppression, regulation of protein degradation, and development in several tissues, and they are regulated by phosphorylation-dependent nuclear/cytoplasmic shuttling (9, 10). Besides its role in regulating the Atg, FoxO3, as FoxO1, positively controls the transcription of the E3 ligases MAFbx and MuRF1 (67, 107) and upregulation of MAFbx and MuRF1 expression leads to muscle atrophy. Akt phosphorylates FoxO1, FoxO3, and FoxO4 (Thr32, Ser253, and Ser315 in the FoxO3a sequence), leading to its inhibition by cytosolic retention via 14-3-3 (8).

The activation of FoxO3a by AMPK in skeletal muscle and its implication in regulating both autophagy and the ubiquitin-proteasome pathways are illustrated in Fig. 3. It was shown that AMPK activation by AICAR increases the mRNA and the protein content of MAFbx and MuRF1 in C2C12 and primary myotubes (88, 104). However, to the best of our knowledge, no supplementary data exist regarding the regulation of E1, E2, and proteasome subunits by AMPK in skeletal muscle.

Recently, we have described that activation of AMPK induces autophagy pathway in C2C12 cells and primary myotubes (104). The activation of FoxO3a by AMPK leads to an increase in the expression of Beclin, LC3-II and Gabarapl1, which are necessary for the promotion of autophagosome formation. AMPK phosphorylates FoxO3a at Ser413/588, residues known to lead to FoxO3a activation and protein degradation (41, 47). Nevertheless, no variations in nuclear content of FoxO3a were detected following AMPK activation after long treatments (i.e., 24 h), but an increase in the total protein level from 30 min was found. After a short time course (30 min–6 h) the activation of AMPK by AICAR induces accumulation of FoxO3a in the nucleus, consistent with the results of Tong and colleagues (118), who reported that AICAR treatment caused FoxO3a nuclear relocation correlated to a decrease in FoxO3a phosphorylation at Thr318/321. However, Greer and colleagues (41) have reported an increase of FoxO3a transcriptional activity without any change in the nuclear content of the factor after AMPK activation by 2-deoxyglucose in HEK293T cells. Nevertheless, these apparent divergent data strongly suggest that FoxO3a relocalization into the nucleus after AMPK activation is not necessarily required to increase its transcriptional activity. As demonstrated by Davila et al. in neurons, it can be suggested that AMPK activates FoxO3a directly into the nucleus (23) and may be implicated in the stability of the protein.

AMPK initiates autophagy by regulating Ulk1 complex. Another major signaling pathway has been identified in AMPK-induced skeletal muscle autophagy. It concerns the regulation of Ulk1 complex by AMPK (Fig. 4). This complex is composed of AMPK, mTORC1, Ulk1, FIP200, and Atg13 and has been identified in muscle cells. Ulk1, the homologue of yeast Atg1, is a serine/threonine-protein kinase that plays a key role in the initial stages of autophagy induction, particularly the nucleation and formation of the pre-autophagosome structures (36, 85, 90). Data obtained in skeletal muscle cells are consistent with the model described in HeLa, HEK293T, and mouse embryonic fibroblast cells and show that, under basal conditions, mTORC1 interacts with Ulk1 and prevents autophagy in opposition to energy stress conditions (56, 63, 64). Under nutrient-rich conditions, phosphorylation of Ulk1 by mTORC1 decreases Ulk1 kinase activity and its ability to interact with cofactors Atg13 and FIP200, a necessary interaction in coordinating the autophagic response (36, 46). It was reported in muscle cells that activation of AMPK by AICAR or inhibition of mTORC1 with either Torin1 or amino acid starvation leads to the dissociation of AMPK and mTOR/raptor from the Ulk1 complex (104). This process is thought to result in initiation of the Ulk1-dependent phosphorylation of Atg13 and FIP200 leading to the activation of autophagy (63).

A proteomic analysis of the autophagy system and a coimmunoprecipitation study performed in HEK293T cells have shown that AMPK interacts with Ulk1 and Ulk2 (3, 74). In muscle cells, Ulk1 was identified as a new interacting partner of AMPK that induces Ser467 phosphorylation (104). The phosphorylation of Ulk1 could lead to a conformational change and thereby disrupt the Ulk1-mTORC1 interaction, in line with the suppression of mTORC1 activity in the Ulk1 complex (56, 57, 64). Moreover, Ulk1 phosphorylation by AMPK may directly upregulate Ulk1 kinase activity. Indeed, it was shown in vitro that purified Ulk1 can phosphorylate itself and requires autophosphorylation for stability (26). In mammals, reconstitution of Ulk1-deficient cells with a mutant Ulk1 that cannot be phosphorylated by AMPK revealed that such phosphorylation is required for mitochondrial homeostasis and cell survival following starvation (28, 29). A lack of association between AMPK and Ulk1 resulted in an accumulation of abnormal mitochondria and cell death. Finally, AMPK controls Ulk1 activity by suppressing mTOR activity and by interacting and phosphorylating Ulk1.

Coimmunoprecipitation time course studies demonstrate that, following autophagy induction, AMPK dissociates from Ulk1 after 3 h of AICAR treatment (47). Shang and colleagues (108) reported that AMPK is associated with Ulk1 only under nutrient-rich conditions and dissociated from Ulk1 5 min after starvation in HeLa cells. Thus, altogether these data suggest that Ulk1 is associated with AMPK in normal conditions. However, upon AICAR-induced autophagy, the complex remains stable for 3 h and starts to dissociate later. It is conceivable, as suggested by Shang and colleagues (108), that Ulk1 dissociates from AMPK and thus becomes more active. Conversely, it is also possible that AMPK-Ulk1 dissociation is responsible for a negative regulatory feedback loop as described by Loffler and colleagues (76), who showed that Ulk1 could mediate phosphorylation of AMPK on its regulatory subunits. Additional experiments are necessary to define the molecular mechanisms for these events in skeletal muscle. We only conclude that AMPK takes part in the initiation of

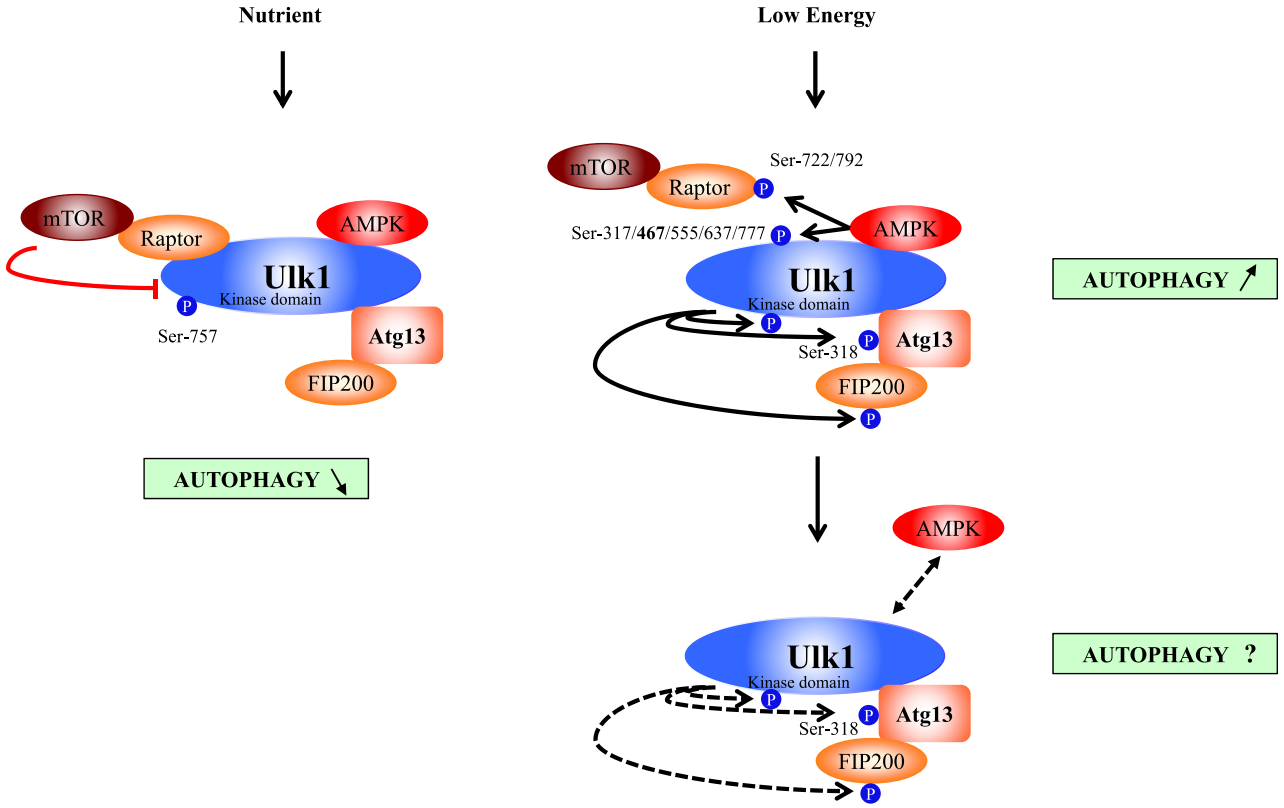

Fig. 4. Regulation of the unc-51-like kinase 1 (Ulk1) complex by AMPK in skeletal muscle. In basal condition, mTOR/raptor (mammalian target of rapamycin/regulatory associated protein of mTOR) interacts with, and phosphorylates, Ulk1 to inhibit its kinase activity and its ability to phosphorylate the autophagy protein 13 (Atg13) and the focal adhesion kinase family interacting protein of 200 kDa (FIP200). In this complex, AMPK also interacts with Ulk1. During cellular stress, AMPK phosphorylates raptor, leading to its dissociation from Ulk1, and phosphorylates Ulk1. Consequently, Ulk1 is activated and phosphorylates itself and its interacting partners Atg13 and FIP200. During autophagy process, AMPK dissociates from Ulk1 and two hypotheses can be formulated: Ulk1 dissociates from AMPK and thus may become more active, or, conversely, AMPK-Ulk1 dissociation may be responsible for a negative regulatory feedback loop. Further exploration will be necessary to better characterize these events. Note that Ulk1 phosphorylation at Ser467 has been identified in myoblasts while the other sites of Ulk1 and Atg13 site have been identified in other cell lines.

autophagosome formation by interacting with, and phosphorylating, Ulk1.

Physiological relevance. The physiological relevance of these findings is emerging. Sandri's group has provided evidence of the existence of an amplifying loop of mitochondrial fission in atrophying muscles. Romanello and coworkers (96) have shown that mitochondrial-dependent muscle atrophy requires AMPK activation and inhibition of AMPK restores muscle size in myofibers with altered mitochondria in a FoxO3a-dependent manner. Interestingly, data related to the role of autophagy during exercise are accumulating; thus Grumati and colleagues (44) have shown that autophagy is stimulated during endurance exercise. These authors found that exercise induced mitochondria and myofibrillar degeneration in type VI collagen knockout mice. This deleterious effect was explained by autophagy failure in this model (42–44). He and coworkers have reported that autophagy is required for beneficial metabolic effects of exercise in skeletal and cardiac muscle of fed mice (51). Indeed, BCL2 AAA mice, which have mutations in BCL2 phosphorylation sites preventing stimulus-induced disruption of the BCL2-beclin-1 complex and autophagy activation, presented a decreasing endurance and altered glucose metabolism during acute exercise. The authors also showed chronic exercise-mediated protection against high-fat-diet-induced glucose intolerance. These studies support a new and essential role of muscular autophagy in the adaptation to exercise. However, excessive autophagy can also lead to atrophy and muscular diseases (105, 106). Lastly, in humans, Jamart and coworkers (61) have found that AMPK and FoxO3a regulate autophagy and ubiquitin proteasome-mediated proteolysis in a coordinated way during ultra-endurance exercise. Thus, AMPK promotes availability of internal energy sources and enhances cellular survival under conditions of energy stress.

Summary and Conclusion

In skeletal muscle, AMPK appears to be a master regulator of metabolism; it contributes to decreasing protein synthesis through inhibition of mTORC1 activity, and it plays a role in protein turnover through increased activity of the ubiquitin-proteasome and autophagy-lysosomal pathways. Accumulating data suggest that modulation of activity of AMPK substrates will constitute good candidates for muscle wasting therapy. In Pompe's disease, like in many myopathies, autophagy levels are modified and contractile proteins can be degraded. In this context, it appears important to understand the precise mechanisms that regulate autophagy to develop and improve therapeutic strategies against muscular dystrophies. Among the signaling pathways that control proteolysis and especially

autophagy, AMPK may play a key role that should be further addressed. In addition, attempts to evaluate the effects of physical exercise should be considered. AMPK has become a target for the development of new drugs for the treatment of type II diabetes, obesity, and even cancer, and we can assume that in the near future it will become a target for fighting myopathies.

ACKNOWLEDGMENTS

We thank Serge A. Leibovitch and Guillaume Py for critically reading the manuscript.

GRANTS

This work was supported by INRA's PHASE division and by the Université de Montpellier 1, Faculté des Sciences du Sport. A. M. J. Sanchez held a graduate fellowship from the Ministère de la Recherche et de la Technologie (MRT).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.M.J.S. and H.B. conception and design of the research; A.M.J.S. prepared the figures; A.M.J.S. and H.B. drafted the manuscript; A.M.J.S., R.B.C., A.C., A.F.P., A.R., and H.B. approved the final version of the manuscript; R.B.C., A.C., and A.R. edited and revised the manuscript.

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