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### ► To cite this version:

Rémi Mounier, Marine Théret, Louise Lantier, Marc Foretz, Benoit Viollet. Expanding roles for AMPK in skeletal muscle plasticity. *Trends in Endocrinology and Metabolism = Trends in Endocrinology & Metabolism*, 2015, 26 (6), pp.275-286. 10.1016/j.tem.2015.02.009 . inserm-01171734

**HAL Id: inserm-01171734**

**<https://inserm.hal.science/inserm-01171734>**

Submitted on 6 Jul 2015

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## **Expanding roles for AMPK in skeletal muscle plasticity**

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## **Abstract**

Skeletal muscle possesses a remarkable plasticity and responds to environmental and physiological challenges by changing its phenotype in terms of size, composition, and metabolic properties. Muscle fibers rapidly adapt to drastic changes in energy demands during exercise through fine-tuning of the balance between catabolic and anabolic processes. One major sensor of energy demand in exercising muscle is AMP-activated protein kinase (AMPK). Recent advances have shed new light on the relevance of AMPK both as a multi-task gatekeeper and as an energy regulator in skeletal muscle. Here, we summarize recent findings on the function of AMPK in skeletal muscle adaptations to contraction and highlight its role in the regulation of energy metabolism and the control of skeletal muscle regeneration post-injury.

## **Keywords**

AMP-activated protein kinase; Exercise; Skeletal muscle; Regeneration; Muscular Diseases; Therapeutics.

## **Glossary**

**Acetyl-coenzyme A (CoA) carboxylase (ACC):** an enzyme that catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, a metabolic intermediate that inhibits fatty acid uptake into mitochondria. ACC exists as two isoforms, ACC1 and ACC2. ACC1 is highly expressed in liver, and brown and white adipose and ACC2 is principally expressed in skeletal and cardiac muscles. ACC phosphorylation by AMPK leads to inhibition of its activity.

**AMP-activated protein kinase (AMPK):** a serine/threonine protein kinase and sensor of cellular energy and nutrient status in eukaryotic cells.

**Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase  $\beta$  (CaMKK $\beta$ ):** a serine/threonine protein kinase that phosphorylates and activates AMPK when intracellular Ca<sup>2+</sup> is increased.

**Endurance exercise training:** generally encompasses exercise durations of several minutes up to several hours at various exercise intensities, incorporating repetitive exercise such as cycling, running, or swimming.

**Glucose transporter type 4 (GLUT4):** an insulin-regulated glucose transporter primarily found in adipocytes, and cardiac and skeletal muscles. In skeletal muscle, insulin, contraction, or AMPK activation induce the translocation of GLUT4 from intracellular vesicles to the plasma membrane for the delivery of glucose into the muscle cell.

**Liver kinase B1 (LKB1):** a serine/threonine protein kinase upstream of AMPK and other AMPK-related kinases. LKB1 activity does not change under conditions of metabolic stress such as during skeletal muscle contraction.

**M1 pro-inflammatory macrophages:** macrophages associated with acute inflammation that express specific pro-inflammatory cytokines such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) or IL-1 $\beta$ .

**M2 anti-inflammatory macrophages:** macrophages associated with healing that express specific anti-inflammatory cytokines such as transforming growth factor  $\beta$  (TGF- $\beta$ ) or IL-10.

**Mechanistic target of rapamycin complex (mTORC):** composed of two distinct complexes mTORC1 and mTORC2, that are involved in protein synthesis, metabolism, proliferation, growth and survival. The mTORC and AMPK signaling pathways are opposite in their function.

**Muscle glucose uptake:** the mechanism by which glucose is transported from the plasma into the skeletal muscle cells via three serial steps: delivery of glucose from the blood to the sarcolemma, transport across the sarcolemmal membrane by GLUT; and intracellular phosphorylation to yield glucose6-phosphate, effectively trapping the glucose inside the muscle cell.

**Resistance exercise training:** generally encompasses short-duration activity at high or maximal exercise intensity, and increases the capacity to perform a single or relatively few repetitions of high-intensity exercise.

**NAD<sup>+</sup>-dependent deacetylase silent mating-type information regulator2 homolog 1 (SIRT1):** an enzyme that deacetylates proteins that contribute to cellular regulation. SIRT1 acts as fuel sensor and plays a key role in the regulation of cellular stress responses..

**Skeletal muscle:** one of three types of muscle characterized by their connection to bone *via* tendons that enable voluntary bodily movements. Skeletal muscle comprises elongated, multinucleated cells termed myofibers that comprise organized, repeating cylinders of myofibrils. These contain the basic contractile unit, termed the sarcomere, which contains the filament, actin, and the motor protein, myosin. Skeletal muscle can be broken down into two main subtypes: red, slow, oxidative, type I and white, fast, glycolytic, type II muscles.

**Tre-2/BUB2/cdc 1 domain 1 and 4 (TBC1D) 1/4):** members of the Rab GTPase activating proteins (Rab-GAP) family of proteins that are phosphorylated by Akt and AMPK in response to insulin, muscle contraction and AICAR.

**Uncoordinated (unc)-51-like kinase 1 (ULK1):** a serine/threonineprotein kinase andhomolog of yeast Atg1 that plays a key role in the formation of the pre-autophagosome structures during autophagy induction.

## **Phenotypic plasticity of skeletal muscle**

- *Skeletal muscle adaptation to the functional demands of exercise*

Skeletal muscle(see glossary) is a dynamic tissue with considerable plasticity. This is well illustrated by the adaptive changes occurring in response to various external stimuli (contractile activity, loading conditions, substrate supply, hormonal profile, and environmental factors) to match structural, functional, and metabolic demands[1]. It is well established that physical exercise is a potent stimulus for the adaptation processes, and is known to remodel skeletal muscle to better respond to future challenges[2, 3]. Skeletal muscle is able to rapidly adapt to exercise interventions, and demonstrates remarkable malleability by changing its metabolic and contractile properties. Resistance exercise stimulates muscle protein synthesis and leads to growth of muscle fibers and hypertrophy. By contrast, endurance exercise leads to qualitative changes of muscle tissue by promoting phenotypic adaptations characterized mainly by fiber type transformation and increases in structures supporting oxygen delivery and consumption (mitochondrial biogenesis and angiogenesis), but no growth. Type IIb and type IIx fibers depend mainly on glycolytic pathways for ATP production, whereas type IIa and type I fibers rely predominantly on oxidative pathways.

- *Regulation of skeletal muscle energy balance during exercise*

Skeletal muscle contraction is associated with a dramatic increase in energy turnover and introduces a major energetic challenge to the muscle fiber. Change in the energy charge of the skeletal muscle leads to the activation of the AMP-dependent protein kinase (AMPK). This serine/threonine kinase, evolutionarily conserved in all eukaryotes, acts as an intracellular fuel sensor sensitive to the relative changes in the cellular AMP:ATP ratio and, to a lesser extent ADP:ATP ratio, which together signal a fall in cellular energy status[4]. AMPK functions as a signaling hub, coordinating anabolic and catabolic pathways to balance

nutrient supply with energy demand at both the cellular and whole-body levels. Exercise and skeletal muscle contraction are powerful physiological activators of AMPK as reported thoroughly in exercising human skeletal muscle and in isolated contracting rodent skeletal muscle [5]. Exercise-induced AMPK activation has been shown to have regulatory effects on selected skeletal muscle processes, such as glucose uptake, fatty acid oxidation, glycogen metabolism, and protein synthesis as well as mitochondrial biogenesis to promote an oxidative muscle phenotype [6, 7]. The action of AMPK is achieved through acute phosphorylation of key regulatory proteins in carbohydrate, lipid, and protein metabolism for short-term effects, as well as phosphorylation of transcription factors and co-activators for longer-term regulatory effects [7, 8].

### **AMPK: a safeguard of skeletal muscle energy metabolism**

- *AMPK structure and activation mechanism*

AMPK exists as a heterotrimeric complex comprising a catalytic subunit  $\alpha$ , a scaffolding  $\beta$  subunit, and a regulatory  $\gamma$  subunit (**Figure 1**). In mammals, each of the subunits occurs as multiple isoforms ( $\alpha 1$ ,  $\alpha 2$ ,  $\beta 1$ ,  $\beta 2$ ,  $\gamma 1$ ,  $\gamma 2$ , and  $\gamma 3$ ), encoded by separate genes, enabling the formation of a diverse collection of  $\alpha\beta\gamma$  heterotrimer combinations. The mechanism of AMPK activation involves two steps: a reversible phosphorylation at a conserved residue (Thr172 in rat  $\alpha 1/\alpha 2$ ) in the  $\alpha$  subunit, and a stimulatory allosteric binding of AMP within the C-terminal region of the  $\gamma$  subunit. The  $\beta$  subunit contains a central carbohydrate-binding module that causes AMPK to associate with glycogen particles and has inhibitory effects on AMPK activity [9] that prominently operate through  $\alpha 2$ -containing complexes [10]. In skeletal muscle, the major upstream kinase phosphorylating  $\alpha$  subunit Thr172 is liver kinase B1 (LKB1), as exercise-induced AMPK phosphorylation is prevented

in mouse models lacking LKB1[11, 12]. LKB1 is constitutively active, providing a continuous basal level of AMPK phosphorylation. AMPK activation is further enhanced by conformational changes imposed by binding of AMP and/or ADP to the  $\gamma$  subunit, which promotes phosphorylation of Thr172 and provides protection against dephosphorylation by protein phosphatases[13, 14](**Figure 2**). Of note, the nature of the  $\gamma$  subunit may determine the degree of allosteric activation, as  $\gamma 2$  complexes have greater AMP sensitivity than  $\gamma 1$  subunits, which have greater AMP sensitivity than  $\gamma 3$  subunits [15]. The combined effect of phosphorylation on Thr172 and allosteric regulation causes a >1000-fold increase in kinase activity, allowing high sensitivity in responses to small changes in cellular energy status [16]. The  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase kinase  $\beta$  (CaMKK $\beta$ ) has also been shown to phosphorylate and activate AMPK in response to an increase in intracellular  $\text{Ca}^{2+}$  concentration, independent of any change in cellular AMP:ATP or ADP:ATP ratios[17, 18]. CaMKK $\beta$  has been shown to activate AMPK during mild tetanic skeletal muscle contraction[19] and increase AMPK $\alpha 1$  activity in response to skeletal muscle overload in LKB1-deficient mice [20].

- *AMPK expression profile and regulation of activity*

In human skeletal muscle, only three complexes have been detected:  $\alpha 2\beta 2\gamma 1$  (65% of the total pool),  $\alpha 2\beta 2\gamma 3$  (20%), and  $\alpha 1\beta 2\gamma 1$  (15%) [21](**Figure 3**). The heterotrimer combination varies in mouse skeletal muscle, with the detection of five complexes, including  $\alpha 1$  and  $\alpha 2$ -associated AMPK complexes with both  $\beta 1$  and  $\beta 2$  isoforms [22](**Figure 3**). It also appears that the expression pattern of AMPK isoforms varies between muscle types [22](**Figure 3**). Of interest, the  $\gamma 3$  isoform seems to be exclusively expressed in the fast-twitch glycolytic *extensor digitorum longus* (EDL) mouse muscle and not in the slow-twitch oxidative soleus mouse muscle [23]. Interestingly, a mutation (R225W) occurring in the gene

encoding for the AMPK $\gamma$ 3 subunit PRKAG3 has been reported in pigs [24] and humans [25], causing increased deposition of glycogen in skeletal muscle. Recent studies have also revealed that each heterotrimer combination displays a distinct activation profile in response to physical exercise in human skeletal muscle, with  $\gamma$ 3-containing complexes ( $\alpha$ 2 $\beta$ 2 $\gamma$ 3 heterotrimer comprising 20% of total AMPK pool) predominantly activated [21], and  $\alpha$ 2 $\beta$ 2 $\gamma$ 1 and  $\alpha$ 1 $\beta$ 2 $\gamma$ 1 heterotrimers (comprising 80% of total AMPK pool) unchanged or activated only after prolonged exercise [26]. In addition, the specificity of  $\alpha$ 2-containing complexes to translocate to the nucleus in response to muscle contraction expands the consequences of AMPK activation on the transcriptional programs in exercised skeletal muscle [27]. Of note,  $\alpha$ 1-containing complexes are usually slightly activated during exercise compared with  $\alpha$ 2-containing complexes. An additional layer of complexity emerged from recent studies showing differential function of specific AMPK heterotrimers, as evidenced by a distinct phosphorylation signature in exercised human muscle [28]. Compartmentalization of AMPK complexes within the myofiber may provide clues for heterotrimer substrate selection and particular functional roles. Heterotrimer subcellular localization has been shown to be dependent on both myristoylation and phosphorylation of the  $\beta$  subunit [29] and the  $\gamma$ -associated isoforms in the heterotrimer complex [30].

- *AMPK in the control of exercise-induced metabolic optimization*

The conversion of skeletal muscle fibers from the more glycolytic fiber (type IIb) to the more oxidative fibers (types IIa, IIx and I) is critical to optimize and enhance energy production during endurance exercise. This is consistent with the increase in the ratio of type IIa/x fibers in skeletal muscle from mice expressing an AMPK $\gamma$ 1 gain-of-function mutant (R70Q) [31] and from rats chronically treated with the AMP mimetic agent and AMPK activator 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside (AICAR) [32]. In addition,

using a transgenic mouse model with chronic inhibition of AMPK $\alpha$ 2 activity, Rockl *et al.* demonstrated that AMPK activation participates in the fiber type IIb to IIa/x transition during physical exercise training [31]. Interestingly, activation of AMPK has been recently involved in the control of fiber type specification by folliculin-interacting protein 1 (FNIP1) [33]. Loss of FNIP1 increases AMPK activation in skeletal muscle and results in a shift in muscle fibers from type IIb glycolytic fibers to type I and type IIa oxidative fibers, in a peroxisome proliferator-activated receptor  $\gamma$  co-activator-1 $\alpha$  (PGC-1 $\alpha$ )-dependent manner. Clearly, the negative interaction between FNIP1 and AMPK is an important area for future research, particularly in understanding the regulation of metabolic adaptation in skeletal muscle. Nevertheless, the role of AMPK in mediating glycolytic-to-oxidative fiber type transformation remains ambiguous, as AMPK $\gamma$ 3 gain-of-function (R225Q) mice [34] and AMPK $\beta$ 1 $\beta$ 2-deficient mice display normal fiber distribution [35], whereas a compensatory increase in the percentage of type I fibers has been reported in muscle AMPK $\alpha$ 1 $\alpha$ 2-deficient mice [36] at rest. Future investigations are needed to confirm the role of AMPK in fiber type specification by monitoring fiber type adaptation in AMPK $\alpha$ 1 $\alpha$ 2- and AMPK $\beta$ 1 $\beta$ 2-deficient mice following exercise training.

- *AMPK in the control of fatty acid oxidation*

A major catabolic pathway activated during exercise is fatty acid oxidation. Data from experiments using mouse models have provided compelling evidence for the important role of AMPK in regulating fatty acid metabolism in skeletal muscle. It has been shown that contraction-induced and pharmacological activation of AMPK stimulates fatty acid oxidation in skeletal muscle [37-40]. This was further supported by the increase in fatty acid oxidation in skeletal muscle from transgenic mice expressing a AMPK $\gamma$ 3 gain-of-function (R225Q) mutant [23]. It was also recently established that AMPK stimulates fatty

acid oxidation in skeletal muscle through the phosphorylation and reduction of the activity of acetyl-coenzyme A (CoA) carboxylase 2 (ACC2), the enzyme that catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, a metabolic intermediate involved in the regulation of fatty acid oxidation [41]. However, controversies exist about the role of AMPK in regulating fatty acid oxidation in skeletal muscle due to the dissociation between AMPK activation, ACC phosphorylation, and malonyl-CoA levels during prolonged exercise in humans [42], indicating that muscle lipid oxidation is not completely dependent on the AMPK/ACC pathway. Similarly, studies from transgenic mouse models with altered AMPK activity in skeletal muscle led to the conclusions that AMPK is not involved in the regulation of fatty acid oxidation during exercise [43-45], but the presence of residual ACC phosphorylation in the muscle of these animals may contribute to the normal metabolic adaptation to contraction and the contribution of the AMPK/ACC pathway cannot be definitively excluded. Thus, further studies involving the use of knockout mouse models are warranted in order to fully understand the contribution of muscle AMPK in the induction of fatty acid oxidation during exercise.

Another mechanism to enhance fatty acid utilization involves the translocation of the fatty acid transporter cluster of differentiation 36 (CD36) to the plasma membrane, to regulate fatty acid import into muscle cells. Although previous studies indicated that AMPK was not essential in the regulation of CD36 translocation and fatty acid uptake during contraction [35, 46], recent genetic evidence demonstrated that muscle AMPK $\alpha$ 1 $\alpha$ 2-deficient mice have impaired fatty acid utilization during *in vivo* exercise [47]. Absence of exercise-induced S237 phosphorylation of the Rab GTPase activating protein Tre-2/BUB2/cdc 1 domain (TBC1D) 1 in AMPK $\alpha$ 1 $\alpha$ 2-deficient muscle may provide a molecular mechanism explaining the defect in CD36 translocation and altered fatty acid uptake in response to contraction [47] (**Figure 4**).

• *AMPK in the regulation of exercise-induced glucose transport*

During exercise, contracting skeletal muscle rapidly increases glucose uptake in an intensity-dependent manner, to sustain the energy demand caused by increased ATP turnover. This increase in skeletal muscle glucose uptake is driven by increased capillary recruitment as well as translocation of glucose transporter type 4 (GLUT4) to the plasma membrane (**Figure 4**). This latter event is required for increased glucose uptake during exercise, in both humans and rodents. The mechanisms underlying contraction-induced stimulation of glucose uptake have garnered considerable interest, as skeletal muscle contraction is a more potent stimulus of skeletal muscle glucose uptake than maximal insulin, and the proximal molecular mechanisms regulating insulin and contraction-stimulated glucose transport are distinct [48]. However, recent findings have shown a convergence of these signals at TBC1D1 and TBC1D4, which are emerging as essential players in both insulin- and contraction-stimulated glucose uptake in skeletal muscle [49-51]. It is also important to note that, unlike insulin-dependent glucose uptake, AMPK-mediated glucose uptake is not impaired in type 2 diabetes during exercise [52] and therefore activation of AMPK represents an attractive target for intervention. Consistent with this notion, therapeutic doses of metformin increased AMPK activity in the skeletal muscle of type 2 diabetics, along with higher rates of glucose disposal [53].

In recent years, the importance of AMPK in skeletal muscle glucose uptake has been examined extensively in genetically modified mouse models. Studies using the AMPK activators AICAR [22, 37, 54, 55] and ex229, a cyclic benzimidazole derivative and potent direct AMPK activator [56], have shown that pharmacological activation of AMPK is sufficient to cause an increase in glucose transport in resting rodent muscle. Intriguingly, although AMPK activation by the direct AMPK activator A-769662 is associated with

increased glucose uptake in isolated mouse muscle, its action appears to be mediated through a phosphoinositide 3-kinase (PI3K)-dependent pathway, indicating potential off-target effects[22]. There is genetic evidence to support an important role for AMPK in regulating TBC1D1 and TBC1D4 phosphorylation in response to exercise[35, 57, 58]. Consistent with these results, AICAR-mediated glucose uptake is blunted in skeletal muscle from TBC1D1- and TBC1D4-deficient mice[49-51]. Interestingly, in exercised human skeletal muscle, TBC1D4 phosphorylation is significantly correlated with an increase in  $\alpha 2\beta 2\gamma 1$  heterotrimer activation, whereas TBC1D1 phosphorylation is correlated with the activity of the  $\alpha 2\beta 2\gamma 3$  heterotrimer[28]. However, it remains unclear whether AMPK is necessary for contraction-stimulated glucose transport, as various transgenic AMPK-deficient mouse models display decreased, normal, or increased glucose transport, depending on the study [35, 36]. Muscle-specific knockout of both  $\beta$ -AMPK subunits inhibited contraction-stimulated glucose uptake [35], while muscle-specific AMPK- $\alpha$  deficient mice showed reduced contraction-stimulated glucose uptake in *soleus* but not *EDL* muscle [36]. These conflicting results could be due to redundancy of signaling or diversity in contraction protocols and genetic models, but also highlight the extremely complex regulation of contraction-induced glucose uptake. It seems unwise to expect a single pathway to account for the full control of contraction-induced muscle glucose uptake. Muscle contraction is a multifactorial process and potential signals include changes in cellular energy status, reduced creatine phosphate and glycogen levels, increases in sarcoplasmic  $\text{Ca}^{2+}$  levels, generation of reactive oxygen species, and altered redox state. Based on the finding that specific  $\text{Ca}^{2+}$ -activated proteins are necessary for the translocation of vesicles to the plasma membrane, a direct stimulatory effect of  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum during contraction is believed to act as a direct feedforward mechanism to facilitate GLUT4-mediated glucose transport independently of AMPK activation [59]. However, Jensen *et al.* have recently reported the contrasting finding, that both

AMPK and mechanical stress are required for increased glucose transport in skeletal muscle, and are sufficient to recruit the full contraction-sensitive glucose transport response, while sarcoplasmic  $\text{Ca}^{2+}$  release is neither sufficient nor required, challenging the working model whereby skeletal muscle glucose transport is directly stimulated by  $\text{Ca}^{2+}$  released from the sarcoplasmic reticulum [60].

- *AMPK-dependent long-term control of exercise-induced metabolic adaptations*

AMPK has emerged as a key mediator of the long-term adaptation to training, with the ability to regulate the transcription of the glucose transporter GLUT4 and genes involved in mitochondrial biogenesis [61] (**Figure 4**). Enhanced GLUT4 gene expression is dependent on an AMPK-dependent phosphorylation of the transcriptional repressor histone deacetylase 5 (HDAC5) following exercise [8]. The transcriptional control of mitochondrial biogenesis is mediated through the regulation of PGC-1 $\alpha$ , a transcriptional co-activator that promotes the expression of mitochondrial genes encoded in both nuclear and mitochondrial DNA. AMPK activation causes the stimulation of PGC-1 $\alpha$  by direct phosphorylation [62] but also involves deacetylation through the silent mating-type information regulator 2 homolog 1 (SIRT1), which is downstream of AMPK, for the triggering of exercise-induced skeletal muscle adaptations [63]. AMPK also controls the expression of PGC-1 $\alpha$  by phosphorylating HDAC5, leading to the release of myocyte enhancer factor 2 (MEF2) from repression by the histone deacetylase and the activation of PGC-1 $\alpha$  gene transcription [61, 64]. Interestingly, the regulation of HDAC5 phosphorylation can be maintained during exercise despite the absence of AMPK by a compensatory activation of protein kinase D (PKD), an alternative HDAC5 kinase, to control PGC-1 $\alpha$  gene expression [65]. These results highlight the importance of the adaptive response in maintaining the integrity and metabolic capacity of skeletal muscle. In the same line, PGC-1 $\alpha$ -deficient mice remain able to adapt to exercise training in a normal

fashion, suggesting that other, currently unidentified factors are implicated in exercise-induced adaptations [66].

The regulation of PGC-1 $\alpha$  by AMPK is consistent with the critical role of AMPK in initiating mitochondrial biogenesis in response to increases in the intramuscular AMP:ATP ratio by the creatine analogue  $\beta$ -guanidinopropionic acid ( $\beta$ -GPA) [67]. In addition, reduced muscle AMPK activity has been associated with decreased mitochondrial content during aging [68] and in skeletal muscle from AMPK $\beta$ 1 $\beta$ 2- and LKB1-deficient mice [35, 69]. However, there are some controversies regarding the role of AMPK in muscle mitochondrial biogenesis, as data from AMPK $\alpha$ 1 $\alpha$ 2-deficient mice indicate that AMPK is dispensable for the regulation of baseline mitochondrial muscle content [36]. Nevertheless, loss of AMPK activity alters skeletal muscle basal metabolic function through the reduction in the expression of mitochondrial proteins and mitochondrial respiratory capacity [36, 70, 71] and may contribute to the exercise intolerance observed in muscle-specific AMPK-deficient mice [35, 36, 72].

- *AMPK in the regulation of skeletal muscle exercise-induced autophagy*

Recent studies have documented the role of autophagy in the plasticity of skeletal muscle in response to exercise [73, 74]. Autophagy is the process by which organelles and macromolecular complexes are engulfed into double-membraned vesicles, called autophagosomes, and delivered to lysosomes for digestion. During contraction, activation of autophagy ensures optimal energy utilization for maintaining cellular energy homeostasis, as well as the efficient turnover of damaged organelles following strenuous physical activity. AMPK has been shown to play a fundamental role in the stimulation of autophagy in skeletal muscle cells by phosphorylating the transcription factor Forkhead box protein O3a (FoxO3a), and the autophagy-related protein uncoordinated(unc)-51-like kinase 1 (ULK1), which participates in the initiation of autophagosome formation [75]. It has been proposed that

autophagy may function in a feed-forward manner to activate AMPK [74], but studies in mice deleted of the autophagy-related gene autophagy-related protein 7 (*atg7*) specifically in skeletal muscle challenged this hypothesis. Lo Verso *et al.* showed that inhibition of autophagy has no impact on AMPK $\alpha$ 1 activation and energy provision to fuel contraction [76]. However, autophagy appears to be critical for the maintenance of mitochondrial quality control during damaging physical activity [76].

### **AMPK: a new player in skeletal muscle regeneration**

- *AMPK, inflammation, and skeletal muscle regeneration*

Adult skeletal muscle possesses a remarkable capacity to regenerate after injury, exercise, or disease. Recent studies revealed that skeletal muscle regeneration (**Box 1**) requires the migration, proliferation, and fusion of myoblasts to form multinucleated myotubes as well as interaction with other cell types within the muscle, including immune cells [77-79]. The inflammatory response during skeletal muscle regeneration is a spatially and temporally coordinated process. While the first steps of the inflammatory response are associated with M1 macrophages, which secrete pro-inflammatory cytokines, resolution of inflammation is associated with M2 macrophages, which exhibit an anti-inflammatory phenotype. The inflammatory resolution is a critical step and its alteration impairs skeletal muscle regeneration. In parallel of its role as an energy sensor, AMPK activation is associated with a decrease in the inflammatory response. On bacterial lipopolysaccharide (LPS) stimulation *in vitro*, while AMPK activation decreases the secretion of pro-inflammatory compounds by macrophages, blocking of AMPK action induces an enhanced pro-inflammatory response [80-82]. *In vivo*, the administration of AICAR attenuates LPS-induced acute lung injury [82], whereas the inhibition of AMPK leads to the opposite effect [83].

Recent evidence demonstrated that macrophage AMPK $\alpha$ 1 is crucial for the resolution of inflammation during skeletal muscle regeneration [84]. Specific deletion of AMPK $\alpha$ 1 in macrophages induces a delay in macrophage skewing (M1-to-M2 transition), impairing skeletal muscle regeneration. Inversely, rescue experiments with wild type bone marrow transplantation in AMPK $\alpha$ 1-deficient mice improve skeletal muscle regeneration. During the regeneration process, AMPK $\alpha$ 1 activation and macrophage skewing are linked to the phagocytosis of apoptotic/necrotic myofibers. In this context, AMPK $\alpha$ 1 activation in macrophages is dependent on phosphorylation by CaMKK $\beta$  but not LKB1 [84] (**Figure 5**).

• *Energy and muscle stem cell fate choice*

During post-natal skeletal muscle regeneration, muscle stem cells activate and recapitulate the myogenic program to repair damaged myofibers. Muscle stem cells are capable of both differentiation to repair muscle tissue after injury, and self-renewal to replenish the stem cell pool. Control of the return to quiescence is crucial for skeletal muscle homeostasis, in order to maintain the pool of satellite cells for future needs. Molecular mechanisms that control muscle stem cell self-renewal are starting to be understood and some factors involved in this process have now been identified [85, 86]. Interestingly, the AMPK upstream kinase LKB1 was shown to limit satellite cell proliferation through the AMPK/mechanistic target of rapamycin complex 1 (mTORC1) pathway [87]. In addition, FoxO3, an AMPK-regulated transcription factor, has recently been involved in muscle stem cell self-renewal by pushing cells to quiescence [88]. Moreover, it has been established that endothelial cells trigger expansion cues to muscle stem cells through the release of vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and fibroblast growth factors (FGFs) [89], while perivascular cells promote muscle stem cells self-renewal and quiescence through the Angiopoietin-1/Tie-2/extracellular signal-related kinase (ERK)1/2 axis [90]. The

observation of increased VEGF expression in LKB1-deficient mice[91] reinforces the putative link between the LKB1/ AMPK pathway and the control of muscle stem cell self-renewal. However, further studies in mice lacking AMPK activity in vascular cells are needed to validate this hypothesis.

It has recently been claimed that regulation of stem cell fate depends on the metabolic state of the cell and the surrounding stem cell niche[92, 93]. Recent work from Ryall *et al.* investigated how metabolic reprogramming promotes the activation of satellite cells and modulates myogenic cell fate[94]. Muscle stem cell activation is associated with an increase in glycolysis that induces a decrease in intracellular  $\text{NAD}^+$  and a decrease in SIRT1 expression. This leads to an increase in acetylated H4K16, an epigenetic mark of transcriptional activity, and the derepression of several muscle developmental genes. These findings reveal a tight link between the metabolic state of satellite cells and epigenetic modifications that regulate their myogenic commitment. Thus, manipulating metabolic pathways might aid in regulating stem cell fate between self-renewal and differentiation. Although the importance of AMPK in stem cells has not yet been extensively studied, it has been shown that AMPK activation inhibits mesenchymal stem cells proliferation[95] and fosters the differentiation of endothelial cells progenitors[96].

Very recently, Rodger *et al.* highlighted the capacity of quiescent muscle stem cells to enter cell cycle into a “ $G_{\text{Alert}}$ ” phase ( $G_0$ -to- $G_1$  pre-transition) [97]. “ $G_{\text{Alert}}$ ” transition of quiescent stem cells allows a rapid response to injury, by priming the cells for cell cycle entry. This transition into the alert state is induced by the activation of mechanistic target of rapamycin complex 1 (mTORC1) signaling, and by increases in mitochondrial number and function. Because mTORC1 and AMPK are two distinct and opposite signaling pathways[98-100], the contribution of the opposing functions of mTORC1 and AMPK in this adaptive response to skeletal muscle injury remains to be explored.

## **Skeletal muscle plasticity as a therapeutic target**

### *•Myokines and whole-body adaptations to exercise*

Muscle contraction has emerged as an important activator of the synthesis and the release of several secretory cytokines and other peptide molecules, known as myokines, that can exert either autocrine, paracrine, or endocrine effects. These myokines have been proposed to mediate whole-body adaptations to exercise through the regulation of skeletal muscle metabolism and signaling to distant metabolic organs. The cytokine interleukin-6 (IL)-6 has been recognized as the prototypical myokine, contributing to muscle metabolic adaptation and the beneficial systemic effects of exercise training [101]. In the past decade, an abundance of evidence has emerged demonstrating that IL-6 stimulates glucose transport and fatty acid oxidation in skeletal muscle in an AMPK-dependent fashion [102]. Other myokines, such as IL-8 and IL-15, have also been shown more recently to increase glucose transport through an increase in AMPK phosphorylation [103]. Similarly, irisin, a newly discovered myokine mediating the effects of exercise on adipocyte browning, exerts a direct action on skeletal muscle metabolism through AMPK activation [104]. Interestingly, it appears that skeletal muscle irisin expression is controlled by an AMPK-PGC-1 $\alpha$  signaling cascade, suggesting the potential existence of a positive feedback loop [105]. AMPK activation has also been proposed to be involved in the mechanism governing IL-6 production and release. However, although AMPK was shown to regulate IL-6 release from isolated skeletal muscle in response to pharmacological activators of AMPK [106], recent evidence indicates that AMPK $\alpha$ 2 is dispensable for contraction-stimulated IL-6 release [107].

### *•Engineering skeletal muscle for repair*

Degenerating myopathies are characterized by chronic inflammation associated with permanent attempts at regeneration and persistent inflammation. Chronic activation of AMPK in the dystrophic mdx mouse, a murine model of Duchenne myopathy, with AICAR, improves the metabolic function of the skeletal muscle (mainly mitochondrial activity) and leads to an improvement of the histological pattern of the muscle by a reduction of both sarcolemma damage and regenerating events [108]. Besides the role of AMPK in myofiber and mitochondrial metabolism, AMPK also seems to act on the regulation of inflammation in the myopathic muscle. Fibrosis and IL-6 and IL-10 levels are reduced in the muscle of AICAR-treated mdx mice [109]. Therefore, knowledge of the molecular regulation of the macrophage inflammatory profile may be useful in understanding how the inflammation balance is disrupted in these diseases. In fibrotic mdx muscle, the myeloid population encompasses various macrophage subsets [110]. The balance between these various populations is currently unknown, but analysis of the involvement of AMPK in the balance between the macrophage subsets in fibrotic mdx may be relevant to improving myopathic muscle. Thus, novel knowledge of the mechanisms of skeletal muscle regeneration obtained through satellite cells and non-satellite cells holds great therapeutic potential for the treatment of muscle disorders.

- *Towards exercise mimetics: myth or reality?*

Physiological adaptations in skeletal muscle are widely recognized to improve whole-body performance and metabolism in health and disease. Several epidemiological and interventional studies have demonstrated that regular physical exercise is of great benefit in both the treatment and prevention of clinical conditions including obesity and type 2 diabetes. By contrast, physical inactivity is a recognized risk factor for these diseases. Several studies have reported an increased proportion of type IIb fibers and decreased numbers of type I

fibers with obesity and insulin resistance[111, 112]. However, endurance exercise has been reported to cause a switch away from glycolytic type IIb fibers towards more oxidative type I and IIa fibers, and is therefore likely to be beneficial[113]. In addition, regular moderate-intensity exercise induces favorable changes in glucose intolerance and decreases mean plasma glucose levels in type 2 diabetic patients through increased muscle glucose disposal [114]. Thus, beyond human performance, there is great interest in promoting regular exercise, combined with an improved diet, not only for protection against the development of obesity and type 2 diabetes, but also as a first line of treatment[115].

Building on the observation that metabolic reprogramming, which favors oxidative over glycolytic metabolism, has a beneficial effect on skeletal muscle, several AMPK activators have been proposed as endurance exercise mimetics. For example, resveratrol, a natural polyphenolic compound mainly found in red wine and an AMPK activator, increases oxidative metabolism and endurance capacity in mice [116]. The effects of resveratrol are mediated by AMPK, as skeletal muscle mitochondrial biogenesis and endurance performance fail to increase in AMPK-deficient mice [117]. Similarly, AICAR also promotes a high-endurance phenotype in mice[118]. The question remains as to what extent these data can be extrapolated to humans. The first clues will probably come from drug abuse by athletes, as AICAR has been recently added to the prohibited list of the World Anti-Doping Agency [119].

### **Concluding remarks**

Recent major scientific advances have begun to unravel the signaling pathways as well as the genetic, biochemical, and metabolic properties of skeletal muscle through which the beneficial effects of physical exercise arise. Studies have highlighted the potential of AMPK as an important integrator of cell signaling pathways that mediate the phenotypic

plasticity of skeletal muscle, but some specific questions have not been fully explored(**Box 2**). AMPK activation is triggered by sustained contraction, and appears to be particularly important in the metabolic changes that occur during physical exercise. Mice lacking AMPK $\alpha$  or AMPK $\beta$  subunits in skeletal muscle have a dramatically impaired ability to perform skeletal muscle contractions and forced treadmill running, and also display reduced voluntary wheel running[35, 36]. Exercise-induced AMPK activation acutely activates glucose uptake by promoting translocation of the transporter GLUT4 to the membrane. While further studies are warranted to fully understand the mechanism by which AMPK promotes GLUT4 translocation, likely candidates are TBC1D1 and TBC1D4 [28], which have been proposed to be the converging point with the insulin signaling pathway and endothelial Nitric Oxide Synthase (eNOS), which generates nitric oxide in blood vessels and is involved in regulating vascular tone[71]. Understanding how AMPK and other signals are integrated to elicit specific changes in glucose transport depending on muscle fiber type and exercise intensity remains a challenge that the field still needs to address. Another important metabolic pathway targeted by AMPK during contraction is fatty acid oxidation. Recent studies from muscle-specific AMPK $\alpha$ 1 $\alpha$ 2-deficient mice demonstrated the essential role of AMPK in enhancing fatty acid utilization during exercise. Prolonged AMPK activation also causes some of the chronic adaptations to endurance exercise, such as increased GLUT4 expression and mitochondrial biogenesis, through the modulation of PGC-1 $\alpha$  and SIRT1 activities. Lastly, AMPK appears to be a crucial contributor to phagocytosis-induced macrophage skewing during the regeneration of damaged skeletal muscle.

Given the various degrees of beneficial phenotypic plasticity demonstrated by chronic AMPK stimulation in pre-clinical animal models, the potential of pharmacological activation of AMPK in humans represents a significant challenge. A better understanding of the molecular mechanisms involved in the control of skeletal muscle plasticity is important for the opening

of new avenues for pharmacological targets in type 2 diabetes and obesity as well as in muscle disorders such as muscular dystrophy. Interestingly, changes in the AMPK heterotrimer composition and AMPK activation are attenuated with age in response to endurance-type muscle contraction and may contribute to skeletal muscle metabolic dysfunction in aged sarcopenic muscle [68, 120]. However, reductions in skeletal muscle AMPK activity are not sufficient to promote the development of aging-induced insulin-resistance [121], indicating that AMPK activation may lead to improved strategies for preventing the reduced mitochondrial function and dysregulated intracellular lipid metabolism in skeletal muscle associated with aging.

### **Box 1: Skeletal muscle regeneration**

Skeletal muscle regeneration relies on resident adult stem cells, named satellite cells, that undertake a series of cell-fate decisions to ensure efficient repair of the damaged muscle fibers. Briefly, these cells remain quiescent under the basal lamina of myofibers and are activated after injury. Once activated, these precursor muscle cells proliferate, differentiate and fuse to reform new myofibers. A small subset of these precursor muscle cells do not differentiate, and return into quiescence to reform a sufficient pool of muscle stem cells for further needs [86]. However, to be complete, skeletal muscle regeneration also needs the involvement of other cells such as perivascular cells and immune cells like macrophages [79]. After injury, circulating monocytes enter into the damaged muscle and differentiate into pro-inflammatory (M1) macrophages. These M1 macrophages secrete pro-inflammatory cytokines, phagocytose debris/necrotic myofibers, and activate muscle precursor cell proliferation. These macrophages then skew their phenotype and become anti-inflammatory (M2) macrophages [77]. At this time, M2 macrophages secrete anti-inflammatory cytokines and sustain myogenesis; that is, the differentiation and fusion of muscle precursor cells [77].

### **Box 2: Outstanding questions**

- What is the role of AMPK and its various complexes in mediating glycolytic-to-oxidative fiber-type transformation?
- What is the specific mechanism by which AMPK promotes GLUT4 translocation during exercise?
- Does AMPK regulate muscle stem cell-fate choice by way of a “metabolic switch”?
- How is AMPK involved in the balance between different types of macrophages in dystrophic muscle?

## Figure legends

### **Figure 1: Schematic representation of AMP-activated protein kinase (AMPK) subunit isoforms.**

AMPK is a heterotrimeric complex comprising a catalytic subunit ( $\alpha$ ) and two regulatory subunits ( $\beta$  and  $\gamma$ ), existing as multiple isoforms ( $\alpha 1$ ,  $\alpha 2$ ,  $\beta 1$ ,  $\beta 2$ ,  $\gamma 1$ ,  $\gamma 2$ , and  $\gamma 3$ ). AMPK $\alpha$  contains a kinase domain phosphorylated by upstream kinases on the residue Thr172, an autoinhibition domain (AID), and a domain that interacts with the  $\beta$  subunits [ $\beta$  subunit interacting domain ( $\beta$ -ID)]. AMPK $\beta$  subunit presents domains that interact with glycogen [carbohydrate-binding module (CBM)] and the  $\alpha$  and  $\gamma$  subunits [ $\alpha\gamma$  subunits interacting domain ( $\alpha\gamma$ -ID)]. AMPK $\gamma$  contains cystathionine  $\beta$ -synthase (CBS) domains binding AMP, ADP, and ATP, but only three of the four potential nucleotide-binding sites contribute to nucleotide regulation, with CBS-2 always unoccupied, CBS-4 permanently bound to AMP and CBS-1 and -3 binding AMP, ADP, and ATP interchangeably.

### **Figure 2: Regulation of AMP-activated protein kinase (AMPK) by exercise.**

Following skeletal muscle contraction, AMP and ADP cellular concentrations and  $\text{Ca}^{2+}$  levels increase. Binding of ADP and AMP to the  $\gamma$  subunit of AMPK triggers Thr172 phosphorylation by the upstream kinase liver kinase B1 (LKB1) protects against dephosphorylation, maintaining the kinase in an active conformation. After exercise, AMPK is returned to an inactive form by dephosphorylation catalyzed by the action of protein phosphatases (PP) 1A, 2A, and 2C).

### **Figure 3: AMP-activated protein kinase (AMPK) heterotrimer composition in skeletal muscle.**

A cartoon of the predominant AMPK heterotrimeric complexes in human (*quadriceps*), murine glycolytic (EDL, *extensor digitorum longus*) and oxidative (*soleus*) muscles is shown.

**Figure 4: Schematic overview of AMPK targets in the regulation of skeletal muscle adaptation to exercise**

Following exercise, AMPK is activated by low energy status and regulates glucose uptake (TBC1D1/ TBC1D4), fatty acid uptake (TBC1D1), fatty acid oxidation (ACC2), mitochondrial biogenesis (PGC-1 $\alpha$ ), mitophagy (ULK1), and gene transcription (HDAC5, PGC-1 $\alpha$ , FoxO3).

Key to abbreviations: ACC, acetyl CoA carboxylase; FoxO3, Forkhead box protein 3; HDAC5, histone deacetylase 5; PGC-1 $\alpha$ , peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$ ; SIRT1, silent mating-type information regulator 2 homolog 1; TBC1D1/4, Tre-2/BUB2/cdc domain family domain family, member1/4; ULK1, uncoordinated-51-like kinase 1.

**Figure 5: Roles of AMPK in skeletal muscle regeneration.**

After an injury of skeletal muscle by cardiotoxin injection, muscle stem cells activate, proliferate and enter into the terminal differentiation program to fuse and to form new myofibers. A small subset of muscle cells do not differentiate but return to quiescence to reform a pool of muscle stem cells (*i.e.* self-renewal). AMPK implication in these steps remains unknown. In parallel, monocyte/macrophages infiltrate the place of injury, acquire pro-inflammatory (M1) phenotype and promote myoblast proliferation. Then, pro-inflammatory (M1) macrophages skew into anti-inflammatory (M2) macrophages, by an activation of the CaMKK $\beta$ /AMPK pathway, linked to the phagocytosis of apoptotic/necrotic

myofibers. These anti-inflammatory (M2) macrophages sustain myoblast differentiation and fusion [84].

Key to abbreviations: CTX: cardiotoxin, Mo : monocytes, MPs : macrophages

### **Acknowledgements**

The work of B.V.'s group is performed within Département Hospitalo-Universitaire (DHU) AUTOimmune and HORmonal diseaseS and was supported by grants from the INSERM, the CNRS, the Université Paris Descartes, the Agence Nationale de la Recherche, the Association Française contre les Myopathies, and aEuropean Commission integrated project (LSHM-CT-2004-005272/ exgenesis).The work of R.M.'s group is supported by grants from the INSERM, the CNRS, the Université Claude Bernard Lyon1, theAssociation Française contre les Myopathies, the Ligue Nationale Contre le Cancer, the Agence Française de Lutte Contre le Dopage,and the Société Française de Myologie.

The authors have no conflict of interest related to this work.

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Figure 1

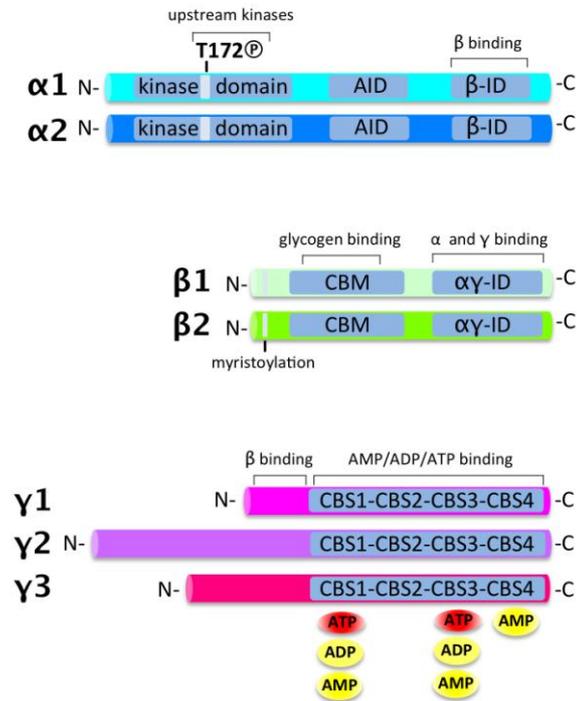


Figure 2

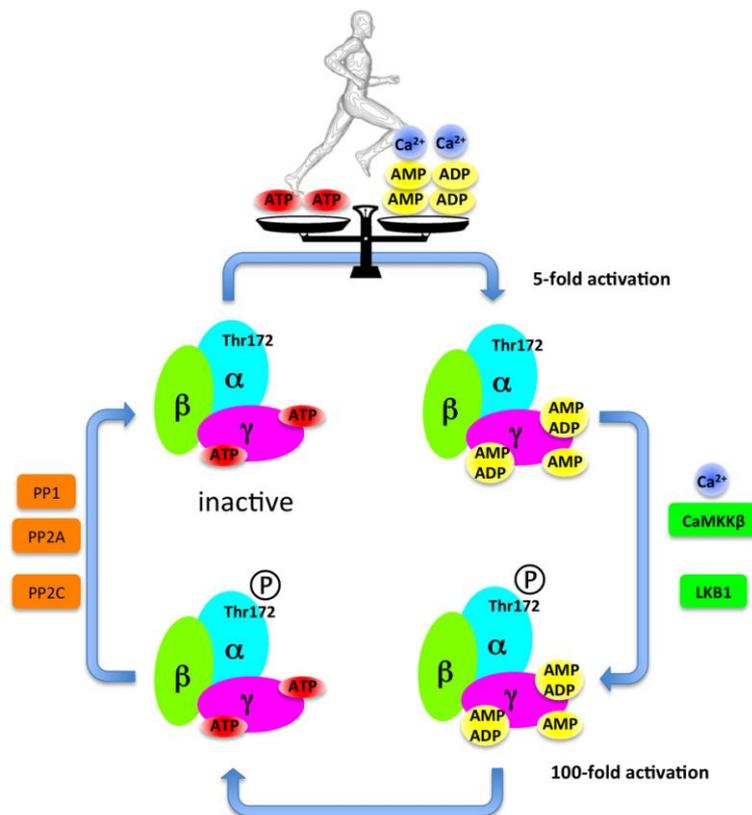


Figure 3

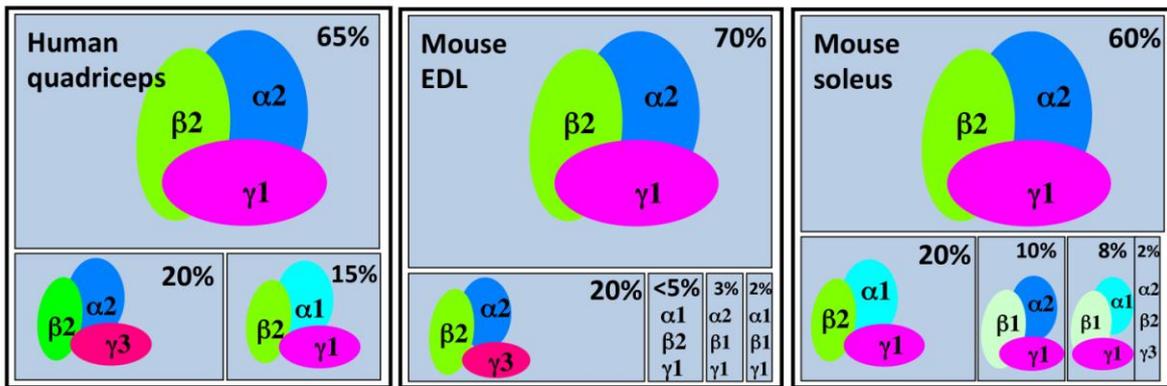


Figure 4

