Animal models to study AMPK

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Abstract

AMPK is an evolutionary conserved energy sensor involved in the regulation of energy metabolism. Based on biochemical studies, AMPK has brought much of interest in the recent years due to its potential impact on metabolic disorders. Suitable animal models are therefore essential to promote our understanding of the molecular and functionnal roles of AMPK but also to bring novel information for the development of novel therapeutic strategies. The organism systems include pig (*Sus scrofa*), mouse (*Mus musculus*), fly (*Drosophila melanogaster*), worm (*Caenorhabditis elegans*) and fish (*Danio rerio*) models. These animal models have provided reliable experimental evidence demonstrating the crucial role of AMPK in the regulation of metabolism but also of cell polarity, autophagy and oxidative stress. In this chapter, we update the new development in the generation and application of animal models for the study of AMPK biology. We also discuss recent breakthroughs from studies in mice, fly and worms showing how AMPK has a primary role in initiating or promoting pathological or beneficial impact on health.

Introduction

AMP-activated protein kinase (AMPK) is widely accepted as a sensor of cellular energy balance (Hardie 2014). At the cellular level, AMPK promotes ATP producing catabolic pathways, while simultaneously inhibiting ATP consuming anabolic pathways. At the organismal level, AMPK integrates stress responses such as exercise as well as nutrient and hormonal signals to control whole body energy expenditure and substrate utilization. As such a potent regulator of cellular and whole body metabolism, AMPK has become the focus of great deal of attention and appeared as an obvious target for treatment of metabolic disorders such as obesity and type 2 diabetes (Winder and Hardie 1999). In several rodents models of diabetes and obesity, pharmacological activation of AMPK results in the remodeling of a wide range of metabolic pathways and have led to substantial improvement of disease outcome (Buhl et al. 2002, Halseth et al. 2002, Song et al. 2002, Cool et al. 2006, Fullerton et al. 2013). In parallel with rapid scientific discovery in cell-free and cellular systems, the development of animal models has been instrumental to the expanding field of AMPK (Viollet et al. 2009). Over the last decade, knockout (KO) mouse models for the different AMPK subunit isoforms as well as transgenic mouse models overexpressing loss of function or gain of function AMPK mutants have been developed to better understand the impact of AMPK on metabolic health and disease and have largely contributed to expand our knowledge on the relevance of AMPK in human disease (**Figure 1**). Conditional targeting approaches are now at the forefront of mechanistic studies to investigate the relevance and specificity of AMPK in the homeostasis of multiple organs. These mouse models are also critically important for pre-clinical translational studies and early stage clinical investigation to progress. In addition, the use of model organisms such as *Saccharomyces cerevisiae* (yeast) [See chapter AMPK in yeast: the SNF1 (sucrose non-fermenting 1) protein kinase complex], *Drosophila melanogaster* (fly) [See chapter The role of AMPK in *Drosophila melanogaster*] and *Caenorhabditis elegans* (roundworm) [See chapter 5'-AMP-Activated Protein Kinase Signalling in *Caenorhabditis elegans*] has played a key role in delineating the physiological role of AMPK pathway and has been instrumental to provide novel information on the biology of AMPK system (**Figure 1**). These various models helped to expand the paradigm of AMPK as a metabolic sensor and showed that AMPK has broad

effects on cellular function, regulating cell growth, autophagy, oxidative stress and cell polarity.

1- Naturally occurring mutations

Many genetically engineered animal models have been generated to investigate the physiopathological role of AMPK, but understanding of AMPK function has been also greatly advanced by studies of naturally occurring mutations in AMPK genes (**Figure 1**). These mutations are apparently fairly rare but can result in pronounced pathological changes (Milan et al. 2000, Blair et al. 2001, Gollob et al. 2001, Arad et al. 2002). Naturally occurring mutations have been initially characterized in the pig *PRKAG3* gene and the human *PRKAG2* gene (encoding the γ3 and γ2-subunit of AMPK, respectively) and are associated with abnormally high glycogen accumulation that results in pathological changes to skeletal and cardiac muscle (Milan et al. 2000, Blair et al. 2001, Gollob et al. 2001, Arad et al. 2002). Later, a mutation in human PRKAG3 gene has been identified in association with an increase in skeletal muscle glycogen content and a decrease in intramuscular triglyceride (Costford et al. 2007).

• 1-1- Human and pig PRKGA3 gene mutations and related mouse models

In 2000, a naturally occurring mutation in the γ3-subunit of AMPK, primarily expressed in white skeletal muscle (glycolytic, fast-twitch type II), was identified in purebred Hampshire pigs by a positional cloning approach (Milan et al. 2000). A non-conservative substitution (R200Q) was the causative dominant mutation in RN^- (in French *Rendement Napole* for Napole yield) pigs. Animals carrying the RN^- mutation are characterized by a marked increase of the glycogen content in glycolytic skeletal muscle leading to low muscle pH 24h post mortem due to anaerobic glycogen degradation, poor water-holding capacity, and low processing yield in the production of cured and cooked ham (Enfalt et al. 1997). Glycogen accumulation in skeletal muscle is consistent with the upregulation in the activity of UDP-glucose pyrophosphorylase and glycogen branching enzyme, two key enzymes regulating glycogen synthesis (Estrade et al. 1994, Hedegaard et al. 2004). R200Q carriers are also characterized by a higher oxidative capacity in white skeletal muscle fibers (Estrade et al. 1994). Conversely, additional naturally occurring missense mutations (T30N, G52S and V199I) were also identified in the PRKAG3 gene from

Western and Chinese indigenous pig breeds associated with an opposite phenotype compared to the RN^- pigs, resulting in reduced skeletal muscle glycogen content and additive effect on meat quality traits (Ciobanu et al. 2001, Huang et al. 2004).

Strong support for the causative nature of the R200Q mutation in the elevated skeletal muscle glycogen content has been provided by transgenic mouse models overexpressing primarily in white skeletal muscle the mouse γ3 R200Q mutant form (in these studies, the R200Q missense mutation is designated R225Q as the start methionine codon proposed by Cheung *et al*. (Cheung et al. 2000) is used) (**Table 1**). Muscle-specific transgenic mouse lines overexpressing the AMPKγ3 R225Q mutant under the control of either the myosin light-chain or the muscle creatine kinase promoter/enhancer were generated and resulted in significant increases in skeletal muscle glycogen, replicating the pig RN^- phenotype (Barnes et al. 2004, Yu et al. 2006) (**Table 2**).

The R200Q missense mutation occurs within the cystathionine *β*-synthase domain 1 (CBS1) of the regulatory AMPKγ3 subunit, which is involved in the binding of the allosteric activator of the kinase, AMP. Originally, there was great controversy over the impact of the R200Q mutation on AMPK activity. It was initially reported that the activity of AMPK was reduced in muscle extracts from RN^- pigs both in the presence and absence of AMP (Milan et al. 2000). In resting muscle from AMPKγ3 R225Q mutant mice, AMPK activity was found to be decreased (Yu et al. 2006) or unaltered (Barnes et al. 2004). However, reduced AMPK activity could reflect the potential feedback inhibition of glycogen overload on AMPK activation (Milan et al. 2000, Jorgensen et al. 2004). Thus, to evaluate the impact of CBS domain mutation, activity of $\alpha 2\beta 2\gamma 3^{R225Q}$ trimer was measured from heterotrimeric complexes purified from transiently transfected COS cells and resulted in a higher basal AMPK activity and loss of AMP dependence (Barnes et al. 2004). Nevertheless, question remains on the mechanisms regulating AMP-independent activity of the AMPKγ3 R225Q mutant (Lindgren et al. 2007), indicating that more detailed functional studies are needed to precisely define the nature of this mutation in the CBS domain of AMPKγ3. Interestingly, a homologous mutation to the RN^- mutation found in pigs has been identified in the human PRKAG3 gene from genetic studies of lean and obese human populations (Costford et al. 2007). Subjects bearing the AMPKγ3 R225W mutation exhibit increased skeletal muscle glycogen content, indicating conserved AMPKγ3 function across mammalian species (Costford et al. 2007). The AMPK γ3 R225W mutation is associated with increased activity of AMPK in

differentiated muscle cells derived from *vastus lateralis* biopsies (Costford et al. 2007). Furthermore, it should be noted that introduction of the equivalent mutation R70Q in AMPKγ1 and R302Q in AMPKγ2 also caused a marked increase in AMPK activity (Gollob et al. 2001, Hamilton et al. 2001, Yavari et al. 2016). More recently, studies with exercise trained RN^- pigs have shifted towards the recognition that γ3 R200Q mutation is a gain-of-function mutation, which results in hyperaccumulation of glycogen due to increased influx of glucose and, enhanced endurance exercise capacity (Granlund et al. 2010, Granlund et al. 2011). Increased glycogen level is not due to impaired glycogen utilization as glycogen breakdown is similar after exercise in carriers and non-carriers of the RN^- mutation (Granlund et al. 2010). These results are consistent with the finding that pigs carrying the R200Q mutation show increased signaling response of Akt and phosphorylation of its substrate, AS160, a higher capacity for phosphorylation of glucose and, faster muscle glycogen resynthesis after exercise (Granlund et al. 2010, Essen-Gustavsson et al. 2011, Granlund et al. 2011). However, a slightly reduced basal and contraction-stimulated rates of glucose uptake was evidenced in transgenic AMPKγ3 R225Q mutant mice (Barnes et al. 2004, Yu et al. 2006), suggesting that enhanced glycogen accumulation is not due to a single mechanism. The possibility that increases in total glycogen synthase activity contribute to elevated glycogen concentrations cannot be excluded (Yu et al. 2006). Other factors that could influence glycogen synthesis are the difference in the gene regulatory responses that facilitate metabolic adaptations. It has been shown that overexpression of AMPKγ3 R225Q coordinates the transcription of genes important for glycolytic and oxidative metabolism (Nilsson et al. 2006) and leads to change in the adaptive metabolic response of glycolytic skeletal muscle by inducing mitochondrial biogenesis and function (Garcia-Roves et al. 2008). Accordingly, transgenic AMPKγ3 R225Q mutant mice display a greater reliance on lipid oxidation and are protected from high fat diet-induced insulin resistance in skeletal muscle through increased fat oxidation (Barnes et al. 2004, Barnes et al. 2005). One consequence of increased fatty acid oxidation in AMPKγ3 R225Q skeletal muscle could be the reduction of the demand for glucose oxidation via the Randle effect (Hue and Taegtmeyer 2009), inducing a glucose-sparing effect that drives glucose towards glycogen synthesis.

•1-2- Human PRKGA2 gene mutations and related animal models

Point mutations in the AMPKγ2 subunit, encoded by the *Prkag2* gene, have been associated with a rare autosomal-dominant genetic disease of the heart in humans (Blair et al. 2001, Gollob et al. 2001, Gollob et al. 2001). Genetic defects in the *Prkag2* gene are characterized by a cardiac glycogen overload, ventricular pre-excitation (Wolff-Parkinson-White syndrome) and cardiac hypertrophy. One of the first human mutation identified occurred at residue 302, resulting in change of arginine to glutamine (R302Q), which is homologous to the R200Q *Prkag3* gene mutation in pigs (Milan et al. 2000, Gollob et al. 2001). Subsequently, identification of additional dominant mutations of PRKAG2 gene has been reported in families coupled with congenital hypertrophic cardiomyopathy and familial pre-excitation syndrome. However, distinct clinical onset and variability in clinical manifestations exist between the various PRKAG2 mutations (Porto et al. 2016). This phenotypic disparity may be a result of the specific effects of individual mutations on AMPKγ2 activity and function. To date, missense mutations include G100S (Zhang et al. 2013), R302Q (Gollob et al. 2001, Arad et al. 2002), H383R (Blair et al. 2001), R384T (Akman et al. 2007), T400N (Arad et al. 2002), K485E (Liu et al. 2013), Y487H (Arad et al. 2005), N488I (Arad et al. 2002), E506K (Bayrak et al. 2006), E506Q (Kelly et al. 2009), H530R (Morita et al. 2008), R531G (Gollob et al. 2001), R531Q (Burwinkel et al. 2005), S548P (Laforet et al. 2006) and insertion of an additional leucine residue L351Ins (Blair et al. 2001). These mutations occur very selectively in strategic positions within the CBS motifs or in linker sequences between these motifs, but not in other parts of the AMPKγ2 subunit. One interesting exception is the G100S mutation mapped into a non-CBS domain. However, it remains unclear if a mutation outside the CBS domains can indirectly changes the binding ability of CBS domains to AMP and ATP. Collectively, these data strongly suggest a specific connection between AMP and ATP binding and the different PRKAG2 mutations, supporting the notion that dysregulation of AMPK activity (gain- or loss-of-function) contributes to the development of the cardiomyopathy.

Consistent with a causative role of PRKAG2 in Wolff-Parkinson-White syndrome, heterozygous mice overexpressing mutant PRKAG2 alleles (R302Q, T400N, N488I, R531G) under the cardiac-specific α-myosin heavy chain (α-MHC) promoter develop pathological cardiac changes resembling the human PRKAG2 cardiomyopathy with significant glycogen accumulation, ventricular preexcitation and cardiac hypertrophy (Arad et al. 2002, Arad et al. 2003, Sidhu et al. 2005, Davies et al. 2006, Banerjee et al.

2007) (**Table 2**). In addition, transgenic zebrafish with cardiac specific expression of the G100S and R302Q mutations in PRKAG2 exhibit typical features of the human disease with cardiac hypertrophy and increased glycogen storage in the heart (Zhang et al. 2014). The PRKAG2 cardiac phenotype has been attributed to alterations in AMPK activity resulting from the AMPKγ2 mutations. However, the action of the different PRKAG2 mutations on AMPK activity and sensitivity to AMP appears to be partially different, if not opposite (Daniel and Carling 2002, Scott et al. 2004). Transgenic mouse model with cardiac specific overexpression of the AMPKγ2^{N448I} and AMPKγ2^{T400N} mutations have been reported to increase AMPK basal activity (Arad et al. 2003, Banerjee et al. 2007), whereas the AMPKγ2^{R302Q} and AMPKγ2^{R531G} mutations results in the inhibition of AMPK (Sidhu et al. 2005, Davies et al. 2006). These discrepant findings could be related to a biphasic response of AMPK activity in response to the overexpression of PRKAG2 mutations (Banerjee et al. 2007, Folmes et al. 2009). Alteration in cardiac AMPK activity is possibly due to a feedback inhibition of the kinase activity by glycogen accumulation (Davies et al. 2006, Folmes et al. 2009), masking the consequences of human PRKAG2 mutations on AMPK activity. To confirm that increased AMPK activity is responsible for PRKAG2 cardiomyopathy, transgenic mice overexpressing AMPK γ2^{N448I} and γ2^{T400N} were crossbred with transgenic mice expressing a dominant negative form of the AMPKα2 subunit, AMPKα2^{D157A} (**Table 1**) in the heart to alter AMPK activity (Ahmad et al. 2005, Banerjee et al. 2007). Compound heterozygous mice have reduced cardiac AMPK activity and minimal cardiac dysfunction, demonstrating that activation of α2-containing AMPK heterotrimeric complexes rather than α1-containing complexes are responsible for the cardiac phenotype (Ahmad et al. 2005, Banerjee et al. 2007). Furthermore, studies using a transgenic mouse model expressing a human AMPKγ2^{N488I} mutant protein under transcriptional control of a tetracycline-repressible α-myosin heavy chain promoter (**Table 1**) provide evidence that clinical manifestations of PRKAG2 cardiomyopathy are significantly reversed by the suppression of mutant AMPK activity after the onset of the disease (Wolf et al. 2008). This finding suggests that the appropriate pharmacological targeting of AMPK or its downstream effectors may help to improve the phenotypic expression of the disease. Compelling evidence now exists that aberrant increase of basal AMPK activity by PRKAG2 mutations results in global remodeling of the metabolic network in favor of glycogen storage (Zou et al. 2005, Luptak et al. 2007) (**Table 2**).

Thus, the metabolic consequences of chronic activation of AMPK in the absence of energy deficiency is distinct from those previously reported during stress conditions. In heart carrying a mutant PRKAG2 allele, inappropriate activation of AMPK triggers an increase in both glucose uptake and fatty-acid oxidation, inducing, via the Randle effect, an inhibition of glucose oxidation leading to the storage of the exceeding glucose into glycogen. The mechanism of increased glucose uptake has been attributed to the upregulation of the sodium-dependent glucose co-transporter (SGLT) isoform SGLT1 but not facilitated-diffusion glucose transporter 1 (GLUT1) or GLUT4 in AMPK $\gamma2^{T400N}$ transgenic mice (Banerjee et al. 2010). Confirmation of the role of SGLT1 in the phenotypic features of the PRKAG2 cardiomyopathy has been demonstrated by the knockdown of cardiac SGLT1 in transgenic mice overexpressing the *PRKAG2* T400N mutation, which attenuates the cardiomyopathy phenotype (Ramratnam et al. 2014). Due to increased glucose uptake, the transgenic mice overexpressing the AMPK $\gamma2^{N448I}$ mutation manifest high intracellular glucose-6-phosphate (G6P) levels, which contribute to the allosteric activation of glycogen synthase (GS) and enhanced glycogen synthesis (Luptak et al. 2007). By genetic inhibition of G6P–stimulated glycogen synthase activity, the pathological glycogen storage phenotype was rescued, providing definitive evidence for extensive remodeling of substrate metabolism and the causative role for high intracellular G6P in *Prkag2* cardiomyopathy (Kim et al. 2014). Surprisingly, elimination of excessive glycogen accumulation eliminated the ventricular preexcitation but not the cardiac hypertrophy phenotype, indicating that the abnormal cardiac growth is regulated by separate mechanisms. Recent studies indicate that AMPK$\gamma2^{N448I}$ and AMPK $\gamma2^{T400N}$ mutations stimulate hypertrophic signaling with activation of the transcription factors nuclear factor κB (NF-κB) and forkhead box O transcription factor (FoxO), and the mammalian target of rapamycin (mTOR) signaling pathway (Banerjee et al. 2010, Kim et al. 2014).

2- Genetically modified mouse models

Murine models have been widely used in biomedical research. Extensive similarities in anatomy, physiology and genetics have allowed numerous inferences about human biology to be drawn from mouse models. The recent development of conditional targeting approaches and the availability of numerous genetically modified mouse

models greatly facilitate functional studies. Important progress has been made during the last decade in the understanding of the pathophysiological function of AMPK, partly due to the generation of whole-body and conditional KO mouse models as well as tissue-specific transgenic mice (**Table 1**). These mouse models have made it possible to decipher the distinct physiopathological functions of the multiple AMPK isoforms and AMPK heterotrimer combinations.

•1-1- AMPK knock-out mouse models

Generation of whole-body deletion of each catalytic or regulatory AMPK subunits results in viable mice (**Table 1**). Of note, a mouse model of AMPKβ1 deletion using a gene trap approach resulted in severe brain developmental defect leading to postnatal death (Dasgupta and Milbrandt 2009). These mice express a fusion protein containing a AMPKβ1 N-terminal fragment (2-224) fused to β-galactosidase and may explain the abnormal brain development and not the loss of the AMPKβ1 subunit (Dzamko et al. 2010). Interestingly, targeted disruption of AMPK subunits is is associated with distinct phenotypic abnormalities (Viollet et al. 2003, Barnes et al. 2004, Jorgensen et al. 2004, Dzamko et al. 2010, Steinberg et al. 2010, Foretz et al. 2011, Dasgupta et al. 2012). These diverse phenotypes could simply reflect isoform-preferred substrate phosphorylation between the combination of AMPKα1- and AMPKα2-containing heterotrimeric complexes, but a recent phosphoproteomic approach in cancer cells fails to identify specific targets for AMPKα1 or AMPKα2 (Schaffer et al. 2015). These data are rather suggestive of distinct tissue-specific contribution of the different isoforms in the control of AMPK activity and function. This is in line with the description of specific pattern of expression for AMPKα, AMPKβ and AMPKγ isoforms between tissues (Stapleton et al. 1996, Thornton et al. 1998, Cheung et al. 2000). Indeed, the expression of AMPKβ1 and AMPKβ2 isoforms differ in a number of tissues, with AMPKβ1 highly expressed in liver and weakly expressed in skeletal muscle, whereas the opposite pattern is observed for AMPKβ2 (Thornton et al. 1998). Studies from AMPKβ1-/- and AMPKβ2-/- mice have highlighted the relative importance of these two isoforms in the liver and skeletal muscle, respectively (Dzamko et al. 2010, Steinberg et al. 2010). Another striking example of restricted expression of AMPK isoforms come from reports showing exclusive expression of AMPKα1, but not AMPKα2, in erythrocytes, macrophages and T cells (Tamas et al. 2006, Sag et al. 2008, Foretz et al. 2010). Mice lacking AMPKα1 are

anemic and had markedly enlarged spleens (Foretz et al. 2010). Similar phenotypes are observed in mice lacking AMPKγ1, the only AMPKγ isoform expressed in murine erythrocytes (Foretz et al. 2011). Studies of AMPKα1-/- and AMPKγ1-/- mice revealed that AMPK is required for erythrocyte homeostasis by regulating erythrocyte membrane elasticity (Foretz et al. 2010, Foretz et al. 2011) and autophagy-dependent mitochondrial clearance during erythrocyte differentiation (Wang et al. 2010). In addition, with the use of bone marrow-derived macrophages from AMPKα1-/- and AMPKγ1-/- mice, the regulatory role of AMPK in the macrophagic differentiation of monocytes (Obba et al. 2015) and the polarization of macrophages to an anti-inflammatory phenotype (Zhu et al. 2015) has been demonstrated. Support for a critical role of AMPK in the regulation of the pro-inflammatory/ anti-inflammatory balance in macrophage has been provided in previous studies using adoptive transfer of AMPKβ1-/- bone marrow in control recipient mice (Galic et al. 2011). It has been also established that AMPK is a key determinant of T cell effector responses by controlling T cell metabolism. AMPKα1-deficient T cells display reduced metabolic plasticity *in vitro* as well as *in vivo* during viral and bacterial infections (Blagih et al. 2015). However, with the exception of these studies on erythrocytes, macrophages and T cells, analysis of the consequence of genetic deletion of one catalytic isoform should be done with caution, since compensatory increase in expression and activity of the remaining isoform can mask a particular phenotype. This has been clearly evidenced in primary culture of mouse proximal tubules from AMPKα1-/- and AMPKα2-/- mice, where adaptive up-regulation of the other AMPKα isoform fully compensate and can substitute for the other for ameliorating the response to metabolic stress (Lieberthal et al. 2013). Similarly, it has been reported that AMPKα1 activity is higher in AMPKα2-/- muscle as compared to control muscles, suggesting a compensatory effect (Jorgensen et al. 2004) (see below). In addition, overexpression of AMPKα2 compensated for loss of AMPKα1 and may explain the lack of phenotype in chondrocyte-specific ablation of AMPKα1 (Yang et al. 2016). In attempt to generate full KO of AMPK, AMPKα1-/- and AMPKα2-/- or AMPKβ1-/- and AMPKβ2-/- mice have been crossed but combined disruption of AMPK isoforms is incombatible with life, indicating that AMPK is required during embryogenesis (Viollet et al. 2009, O'Neill et al. 2011). AMPK α1-/-α2-/- double knockout embryos die at ~10.5 days post-conception (B. Viollet, unpublished data). The recent development of tissue-

specific and conditional knockout technology has now allowed the generation of animal models completely lacking AMPK activity in a specific tissue (**Table 1**). Among the first tissues to be targeted, skeletal muscle-specific deletion of both AMPKα1/AMPKα2 and AMPKβ1/AMPKβ2 has been obtained by crossing AMPKα1$^{fl/fl}$α2$^{fl/fl}$ and AMPKβ$^{fl/fl}$β2$^{fl/fl}$ mice with transgenic mice expression the Cre recombinase under the control of the human skeletal actin and muscle creatine kinase promoter, respectively (O'Neill et al. 2011, Lantier et al. 2014).

•1-2- Insight from mouse models into AMPK-dependent stimulation of glucose uptake in skeletal muscle

Skeletal muscle contraction is associated with a dramatic increase in energy turnover rates that represents a major metabolic challenge. Since the first report on acute skeletal muscle AMPK activation in response to physical exercise in rodents (Winder and Hardie 1996) and later in humans (Chen et al. 2000, Fujii et al. 2000, Wojtaszewski et al. 2000), the role of AMPK in the adaptive changes in skeletal muscle has been a subject of intense research. During exercise, contracting skeletal muscle rapidly increases glucose uptake in an intensity-dependent manner to sustain the energy demand caused by increased ATP turnover. The idea that AMPK was involved in regulating glucose transport in skeletal muscle was supported by the observations that stimulation of glucose transport was achieved upon AMPK activation by AICAR (Merrill et al. 1997) and later by other pharmacological AMPK agonists such as Ex229/991 (Lai et al. 2014). The importance of AMPK in skeletal muscle glucose uptake has been investigated in genetically modified mouse models, including transgenic mice expressing naturally occurring mutation in the AMPKγ3 isoform (**Table 1**). Muscles from knock-out mice (AMPKα2-/-, AMPKβ2-/- and AMPKγ3-/-) or transgenic mice expressing a dominant negative form of AMPKα2 in skeletal muscle (AMPKα2-KD and AMPKα2i) completely abolished *ex vivo* AICAR- and EX229/991-stimulated glucose uptake (Mu et al. 2001, Barnes et al. 2004, Jorgensen et al. 2004, Fujii et al. 2005, Steinberg et al. 2010, Lai et al. 2014). From these data, it has been suggested that contraction-stimulated glucose uptake is dependent on AMPKα2β2γ3, the major heterotrimer activated by exercise in human muscle. However, the role of AMPK in contraction-stimulated glucose uptake remains controversial. In AMPKα2-/-, AMPKβ2-/-, AMPKγ3-/-, AMPKα2-KD and AMPKα2i mice *ex vivo* contraction-stimulated glucose uptake is normal or only moderately reduced (Mu et al.

2001, Barnes et al. 2004, Jorgensen et al. 2004, Fujii et al. 2005, Steinberg et al. 2010). Similarly, the role of AMPK in exercise-induced skeletal muscle glucose transport *in vivo* is not clear. While one study reported no alterations in exercise-induced glucose transport (Maarbjerg et al. 2009), a clear reduction was shown in another study (Lee-Young et al. 2009). These discrepancies are probably due to redundancy of signaling coming from residual AMPK activity in these mouse models where only a single AMPK isoform is genetically altered/deleted and is probably sufficient to increase glucose uptake during muscle contractions. AMPKα1 subunit is still present in AMPKα2-/- and AMPKα1 activity can be detected in AMPKα2-KD mice and may sustain alone the coordination of muscle metabolism and adaptation to exercise. To circumvent this problem, conditional muscle-specific knockout of both AMPKα and AMPKβ subunits have been generated. Deletion of AMPKβ1 and AMPKβ2 isoforms inhibited both contraction- (*ex vivo*) and exercise- (*in vivo*) induced glucose transport in skeletal muscle (O'Neill et al. 2011), while the deletion of both AMPKα1 and α2 showed reduced contraction-stimulated glucose uptake in soleus but not EDL muscle (Lantier et al. 2014). Consistent with the idea of functional redundancy between isoforms, it should be noted that mice lacking the AMPKβ2 subunit specifically in skeletal muscle had normal glucose uptake despite reductions in AMPK activity of more than 90% (O'Neill et al. 2011). Altogether, these findings support the notion that AMPK is necessary to achieve the effects of exercise on muscle glucose transport. The mechanism underlying AMPK-dependent contraction-induced stimulation of glucose uptake has been described to act through the phosphorylation of the Rab GTPase activating protein Tre-2/BUB2/ cdc 1 domain (TBC1D) 1. TBC1D1 has several common contraction and AICAR responsive phosphorylation sites that are blunted in AMPKα2-/-, AMPKγ3-/-, AMPKα2-KD and AMPKα2i mice (Treebak et al. 2006, Pehmoller et al. 2009, Vichaiwong et al. 2010). This has been further supported by recent findings showing a reduction in TBC1D1 phosphorylation in contracted muscle from skeletal muscle-specific AMPKβ1β2 KO mice concomitantly with decreased glucose transport (O'Neill et al. 2011).

3- Genetically modified fly models

AMPK system is highly conserved between insects and mammals. *Drosophila* AMPK contains three protein subunits, α, β and γ, which are encoded, in contrast to mammalian

AMPK, by single genes named dAMPKα, alicorn and loechrig (also known as SNF4Aγ), respectively (Pan and Hardie 2002) (**Figure 2**), making Drosophila an attractive animal model to study AMPK functions *in vivo.* However, Drosophila expresses several isoforms for each subunit generated from alternative splicing and differential transcription initiation (Tschape et al. 2002) and can presumably form a high number of different heterotrimeric complexes. Like mammalian AMPK, drosophila AMPK is allosterically activated by AMP and by treatments that depleted cellular ATP. This is associated with phosphorylation of Thr-184 within the activation loop of dAMPKα and of acetyl-CoA carboxylase at a homologous site also conserved between mammals and insects (Pan and Hardie 2002). Although mice have been the most commonly used animal model to decipher the biology of AMPK, genetically engineered fruit fly *Drosophila melanogaster* (**Tables 3 and 4**) has been particularly important in demonstrating the role of AMPK in the regulation of cell polarity during energy stress and in neuronal survival (Tschape et al. 2002, Lee et al. 2007, Mirouse et al. 2007, Spasic et al. 2008). Identification and generation of mutant AMPK drosophila has been realized by forward genetic screens using ethylmethanesulfonate (EMS) mutagenesis (Medina et al. 2006) or by the P-element transposon technology (Rubin and Spradling 1982). Germline transformation of drosophila can be realized via insertion of a single P transposable element or by imprecise mobilization of the P-elements (excision) to generate null mutants. In addition, creation of transgenic flies using different approaches, including the UAS/Gal4 and "tet-on" systems (Tower 2000), have been instrumental to complement more traditional genetics.

The Drosophila *loechrig* (*loe*) mutant is characterized by degeneration and severe vacuolization (*loechrig* is the German word for full of holes) in the brain. The mutation in *loe* is caused by the insertion of a P-element that affects a neuronal isoform of the AMPKγ subunit (Tschape et al. 2002). Interestingly, among the six different Drosophila AMPKγ isoforms, this particular protein isoform strongly expressed in the nervous system contains a unique N-terminus and is the only isoform to rescue the *loe* phenotype when expressed in neurons, highlighting its specific role for the integrity of the central nervous system. The *loe/AMPKγ* mutation affects the regulatory function of AMPK on isoprenoid synthesis via the inhibition of its downstream target hydroxy-methylglutaryl (HMG)-CoA reductase and that changes play a pivotal role in the observed degenerative phenotype (Tschape et al. 2002). The loss of functional neuronal

AMPK upregulates the synthesis of isoprenoids leading to increased prenylation of the small GTPase Rho1, the fly ortholog of vertebrate RhoA, and thereby progressive neurodegeneration (Cook et al. 2012). Upregulation of RhoA signaling pathway induces changes in the actin cytoskeleton through an increase in phosphorylated cofilin and accumulation of F-actin, resulting in deleterious consequences on neuronal growth and impaired axonal integrity (Cook et al. 2014). Additional evidence for a role of AMPK in neuroprotective processes comes from the study of a P-element insertion line found to target *alicorn* (*alc*), encoding the Drosophila homolog of the regulatory AMPKβ subunit. Disruption of the *alc*/AMPKβ gene causes early-onset progressive retinal degeneration, characterized by extensive vacuolization and general structural disorganization (Spasic et al. 2008). The mechanism of progressive neuronal death observed as a consequence of loss of AMPK does not involve apoptosis (Tschape et al. 2002, Poels et al. 2012). It has been suggested that AMPK could contribute to the protection of neurons from increased metabolic activity by the regulation of the autophagic process. Accordingly, the *loechrig/ SNF4Aγ* gene has been identified in a genetic screen to select P-element insertion that affect autophagy in the larval fat body (Lippai et al. 2008). Surprisingly, in *alc* mutants, a severe induction of autophagy was reported (Poels et al. 2012). This is most likely a consequence of the absence of the negative regulatory feedback loop mediated by AMPK in condition of excessive autophagic induction (Loffler et al. 2011).

Parkinson's disease is one of the most common neurodegenerative diseases in the aging population. Recently, a *Drosophila* Parkinson's disease model was used as an initial system to evaluate the therapeutic potential of a number of candidate compounds (Ng et al. 2012). Epigallocatechin gallate (EGCG), a green tea-derived catechin, was found to provide the best protection against the loss of dopaminergic neurons and mitochondrial dysfunction, the essential pathological phenotypes of human Parkinson's patients. Importantly, the protective effects of EGCG are abolished when AMPK is knocked down, and loss of AMPK activity exacerbates neuronal loss and associated phenotypes (Ng et al. 2012), suggestive of a pathological role of AMPK in neurodegenerative diseases. In addition, it was demonstrated that genetic activation of AMPK also protects against neuronal loss and reproduces EGCG's protective effects, indicating that targeting AMPK will be useful therapeutically in the treatment of neurodegenerative disorders. Indeed, as mitochondrial dysfunction is currently widely accepted to be a key driver of neurodegeneration, activation of AMPK could preserve neuronal function by preserving

the energy balance and by restoring the clearance of damaged mitochondria via induction of mitophagy through the phosphorylation of the autophagy initiator autophagy-related gene 1 (Atg1)/ Unc-51-like kinase 1 (ULK1).

In agreement, with a role of AMPK in neural maintenance, lethal mutations in AMPKα, identified by forward genetic screen with EMS mutagenesis or generated by imprecise excision of P-elements, confirmed the importance for the kinase in the maintenance of cell integrity (Lee et al. 2007, Swick et al. 2013). AMPKα-null fly embryos showed severe abnormalities in cell polarity and disorganization of epithelial structures and lead to embryonic lethality (Lee et al. 2007). Moreover, AMPK-null embryos contained defective mitotic divisions with lagging or polyploid chromosomes. It was established that AMPK functions in mitosis and epithelial polarity by targeting myosin II regulatory light chain (MRLC), since phosphomimetic mutant of MRLC rescued the AMPK-null defects in cell polarity and mitosis (Lee et al. 2007). These findings uncovered a link between energy status and cell structures, revealing new pathophysiological functions for AMPK signaling pathway in the regulation of cellular structures.

Drosophila has been used as a model system to dissect possible roles of AMPK in aging and lifespan determination. Dietary restriction, a reduction in total food intake, has been shown to increase lifespan by modulating nutrient-sensing pathways in flies as well as in a wide range of organisms including nematodes and mammals (Fontana et al. 2010). However, question remains regarding the importance of cellular energy homeostasis and AMPK signaling as an evolutionary conserved determinant of life span control. For this purpose, to circumvent the lethality caused by AMPKα deletion in late larval stages (Lee et al. 2007), the consequences of tissue-specific knock-down of AMPKα and transgenic expression of AMPKα using the inducible GeneSwitch system (Poirier et al. 2008), which provided both temporal and spatial control, have been examined to influence drosophila life span. Different approaches were used including RNAi-mediated knock-down of AMPKα (Tohyama and Yamaguchi 2010) or AMPKγ (Johnson et al. 2010) and expression of a dominant negative construct ($AMPK\alpha^{K57A}$), in which the catalytic domain of the AMPKα subunit is inactive (Johnson et al. 2010) (**Table 4)**. Consistent with a causal role for AMPK in regulating energy homeostasis, reduced AMPK signaling leads to hypersensitivity to starvation conditions (Bland et al. 2010, Johnson et al. 2010, Tohyama and Yamaguchi 2010). Interestingly, a similar sensitivity to starvation conditions was observed with a selective loss of AMPK signaling in muscle (Tohyama

and Yamaguchi 2010) and was associated with a reduction in lifespan (Bland et al. 2010, Stenesen et al. 2013), suggesting potential tissue-specific requirements for AMPK-mediated lifespan extension. To study the impact of tissue-restricted upregulation of AMPK, pro-longevity effects were investigated in transgenic flies overexpressing AMPK in a subset of metabolic tissues. It was found that localized AMPK activation (e.g., by overexpressing wild-type AMPK in muscle, fat body, brain or intestinal epithelium) extends lifespan a non-cell-autonomous manner (Bland et al. 2010, Stenesen et al. 2013, Ulgherait et al. 2014). Activation of AMPK produces a non-cell autonomous induction of autophagy due to upregulation of Atg1/ULK1 signaling, which appears to be necessary and sufficient to slows systemic aging (Ulgherait et al. 2014). A recent study demonstrated that dietary application of β-guanidinopropionic acid (β-GPA), a creatine analog, can extend lifespan through AMPK-dependent induction of autophagy (Yang et al. 2015). Such findings may provide significant insight for pharmaceutical strategies to manipulate AMPK function in single tissue to prolong lifespan in mammals.

4- Genetically modified worm models

The nematode *Caenorhabditis elegans* has many excellent advantages as an *in vivo* model for detailed molecular analyses and functional genomics. It is a small nematode with a life cycle of 3.5 days and a lifespan of about 2-3 weeks. In addition, *C. elegans* displays conserved developmental programs, genetic tractability, and a fully sequenced genome, making an ideal model system to understand biological processes. To identify the biological function of specific genes in *C. elegans*, different methods have been developed using reverse and forward genetics or transgenesis techniques (Baylis and Vazquez-Manrique 2011, Boulin and Hobert 2012). *C. elegans* has many genes which are similar to those of other higher eukaryotes, suggesting many similar functions in cellular and molecular mechanisms. The *C. elegans* genome encodes the *aak-1* and *aak-2* genes, which are homologs of the catalytic α subunits of mammalian AMPK, the *aakb-1* and *aakb-2* genes, which are homologs of the regulatory β subunits of mammalian AMPK, and the aakg-1, aakg-2, aakg3, aakg-4 and aakg-5 genes, which are homologs of the regulatory γ subunits of mammalian AMPK (Apfeld et al. 2004) (**Figure 2**). Hence, a potential of 20 alternative heterotrimeric complexes can be formed in *C. elegans*.

During the last decade, *C. elegans* has been at the forefront as a model system to understand biological processes linking energetics to longevity. Limiting energy

availability through dietary restriction confers lifespan extension in organisms as diverse as yeasts, nematodes, and rodents (Fontana et al. 2010). It has been found that increases in cellular AMP/ATP ratio are associated with age in *C. elegans*, suggesting a link between genes known to affect lifespan and the control of energy metabolism (Apfeld et al. 2004). Thus, it is not surprising that AMPK has emerged as a key regulator of lifespan determination (Burkewitz et al. 2014). The use of *aak-2* mutants (**Table 5**) has highlighted the role of AMPK in the regulation of lifespan in response to environmental stress and insulin-like signaling (Apfeld et al. 2004, Narbonne and Roy 2006). *C. elegans* lacking *aak-2* failed to extend lifespan in response dietary restriction and low energy conditions (Greer et al. 2007, Schulz et al. 2007, Lee et al. 2008, Narbonne and Roy 2009, Fukuyama et al. 2012). Conversely, overexpression of aak-2 significantly promotes lifespan extension and mimics dietary restriction in well-fed wild-type animals (Apfeld et al. 2004). Similar increase in lifespan is observed in transgenic worms expressing an active form of the AMPKα (Mair et al. 2011) or the AMPKγ (Greer et al. 2007) subunits. *C. elegans* fed with the AMPK agonist metformin also display increase in lifespan that is dependent on *aak-2* (Onken and Driscoll 2010). The effect of metformin on extension of *C. elegans* lifespan can be direct by promoting resistance to biguanide toxicity through AMPK activation and indirect by impairing folate metabolism of *E. coli*, its trophic microbial partner (Cabreiro et al. 2013).

Under condition of energetic stress, the aak-2 subunit becomes phosphorylated at threonine 243 (Thr243), equivalent to Thr172 in the mammalian ortholog, and primarily takes part in its activity to phosphorylate and inhibits the CREB-regulated transcriptional coactivator CRTC-1, a cofactor involved in diverse physiological processes including energy homeostasis (Mair et al. 2011). Modulation of CRTC-1 phosphorylation status by AMPK activation in the central nervous system is required to locally and cell autonomously promote remodeling of mitochondrial metabolic networks and increase longevity (Burkewitz et al. 2015). Upon reduced AMPK activity, CRTC-1 modulates AMPK-mediated longevity cell nonautonomously via regulation of the neurotransmitter/hormone octopamine secretion, which drives mitochondrial fragmentation in distal tissues, and suppresses the effects of AMPK on systemic mitochondrial metabolism and longevity. These findings highlight the dominance of neuronal energy-sensing mechanisms and neuronal signals on systemic metabolic homeostasis and impact on aging process. This regulatory mechanism is consistent with

the cell-nonautonomous role for neuronal AMPK in modulating peripheral lipid storage in worms (Cunningham et al. 2014).

Concluding remarks

The AMP-activated protein kinase (AMPK) system was first discovered 35 years ago. Since that time, knowledge of the diverse physiological functions of AMPK has grown rapidly and continues to evolve. Most certainly, genetically modified mice have become instrumental to the study of AMPK. However, the use of fly and worm model organisms has also played a key role in delineating the physiological role of AMPK signaling pathway and will continue to contribute to the expanding field of AMPK.

Identifying the best animal model to study AMPK function or characterize AMPK agonists/ antagonists is an important consideration and requires a thorough understanding of the advantages and disadvantages of each animal model, as there are important factors to consider. Animal models with comparable AMPK heterotrimeric composition to human cells and tissues are typically relevant models to examine AMPK pathophysiological role and develop tissue-specific therapeutic interventions. However, although mouse models possess many advantages for biomedical research, it has been reported that the composition of AMPK heterotrimers differ between rodent and human hepatocytes (Stephenne et al. 2011, Wu et al. 2013), addressing a major challenge for future pre-clinical translational studies.

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Table 1. Genetically modified mouse models for the study of AMPK signaling pathway.

Knock-out and floxed mice			
Targeted gene	*Genetic modification*	*Viability*	*References*
AMPKα1	AMPKα1$^{-/-}$	Viable	(Jorgensen et al. 2004)
AMPKα2	AMPKα2$^{-/-}$	Viable	(Viollet et al. 2003)
AMPKα1/α2	AMPKα1$^{-/-}$α2$^{-/-}$	Embryonic lethal	unpublished data
AMPKα1	AMPKα1$^{fl/fl}$	Viable	(Nakada et al. 2010, Lantier et al. 2014)
AMPKα2	AMPKα2$^{fl/fl}$	Viable	(Viollet et al. 2003, Nakada et al. 2010)
AMPKβ1	AMPKβ1$^{-/-}$	Viable/ postnatal death	(Dasgupta and Milbrandt 2009, Dzamko et al. 2010)
AMPKβ2	AMPKβ2$^{-/-}$	Viable	(Steinberg et al. 2010, Dasgupta et al. 2012)
AMPKβ1/β2	AMPKβ1$^{-/-}$β2$^{-/-}$	Embryonic lethal	(O'Neill et al. 2011)
AMPKβ1	AMPKβ1$^{fl/fl}$	Viable	(O'Neill et al. 2011)
AMPKβ2	AMPKβ2$^{fl/fl}$	Viable	(O'Neill et al. 2011)
AMPKγ1	AMPKγ1$^{-/-}$	Viable	(Foretz et al. 2011)
AMPKγ1	AMPKγ1$^{fl/fl}$	Viable	(Foretz et al. 2011)
AMPKγ3	AMPKγ3$^{-/-}$	Viable	(Barnes et al. 2004)
Knock-in mice			
Targeted gene	*Genetic modification*	*Phenotype*	*References*
AMPKγ2	***AMPKγ2^{R299Q}***	obesity, hyperphagia, and impaired insulin secretion	(Yavari et al. 2016)
Transgenic mice			
Transgene	*Targeted tissue*	*Promoter*	*References*

AMPKα1^{K45R}	Skeletal and cardiac muscle cells	MCK promoter/enhancer	(Fujii et al. 2005)
AMPKα1^{D157A}	Skeletal muscle	HSA promoter	(Miura et al. 2009)
AMPKα1$^{1-312}$	Vascular endothelium	VE–cadherin promoter	(Li et al. 2012)
AMPKα1$^{1-312/T172D}$	Hepatocytes	human ApoE promoter	(Yang et al. 2008)
AMPKα1$^{1-312/T172D}$	Pancreatic β cells	RIP2 promoter	(Sun et al. 2010)
AMPKα2	Ubiquitous	CMV promoter	(Gong et al. 2011)
AMPKα2^{K45R}	Skeletal and cardiac muscle cells	MCK promoter/enhancer	(Mu et al. 2001)
AMPKα2^{D157A}	Skeletal and cardiac muscle cells	MCK promoter/enhancer	(Fujii et al. 2005)
AMPKα2^{D157A}	Pancreatic β cells	RIP2 promoter	(Sun et al. 2010)
AMPKα2^{D157A}	Cardiomyocytes	α-MHC promoter	(Xing et al. 2003)
AMPKγ1^{R70Q}	Skeletal muscle cells	α-actin promoter	(Barre et al. 2007)
AMPKγ1^{H151R}	Skeletal muscle cells	MLC1 promoter/enhancer	(Schonke et al. 2015)
AMPKγ2	Cardiomyocytes	α-MHC promoter	(Arad et al. 2003, Sidhu et al. 2005)
AMPKγ2^{R302Q}	Cardiomyocytes	α-MHC promoter	(Sidhu et al. 2005, Folmes et al. 2009)
AMPKγ2^{N488I}	Cardiomyocytes	α-MHC promoter α-MHC promoter/ tTA (Tet-off)	(Arad et al. 2003, Luptak et al. 2007) (Wolf et al. 2008)
AMPKγ2^{T400N}	Cardiomyocytes	α-MHC promoter	(Banerjee et al. 2007)
AMPKγ2^{R531G}	Cardiomyocytes	α-MHC promoter	(Davies et al. 2006)
AMPKγ3	Skeletal and cardiac muscle cells	MCK promoter/enhancer MLC1 promoter/enhancer	(Yu et al. 2006) (Barnes et al. 2004)
AMPKγ3^{R225Q}	Skeletal and cardiac muscle cells	MCK promoter/enhancer MLC1 promoter/enhancer	(Yu et al. 2006) (Barnes et al. 2004)
Tissue-specific deletion of AMPK subunits			
Targeted gene	*Targeted tissue*	*Cre-driven recombination*	*References*
AMPKα1	T cells	CD4-Cre	(Rolf et al. 2013)
	Myeloid cells	LysM-Cre	(Mounier et

			al. 2013)
	Chondrocytes	Col2α1-Cre	(Yang et al. 2016)
	White adipose tissue stromal vascular cells (*in vitro* deletion)	Gt(ROSA)26Sor-Cre tamoxifen-inducible	(Wang et al. 2015)
	Central nervous system	hGFAP-Cre	(Maixner et al. 2015)
	Muscle satellite cells	Pax7-Cre tamoxifen-inducible	(Fu et al. 2015, Fu et al. 2016)
	Adipocytes	Adipoq-Cre	(Wu et al. 2015)
AMPKα2	Hepatocytes	Alfp-Cre	(Andreelli et al. 2006)
	Hypothalamus AgRP neurons	AgRP-Cre	(Claret et al. 2007)
	Hypothalamus POMC neurons	POMC-Cre	(Claret et al. 2007)
	Endothelial cells	VE-cadherin-Cre	(Kohlstedt et al. 2013)
	Adipocytes	Adipoq-Cre	(Wu et al. 2015)
AMPKα1/α2	Hypothalamus POMC neurons	POMC-Cre	(Claret et al. 2007)
	Skeletal muscle cells	HSA-Cre	(Lantier et al. 2014)
	Hepatocytes	Alfp-Cre	(Guigas et al. 2006, Foretz et al. 2010)
	Pancreatic β cells	RIP2-Cre	(Sun et al. 2010)
	Pancreatic β cells	mIns1-Cre	(Kone et al. 2014)
	Pancreatic α cells	PPG-Cre	(Sun et al. 2015)
	Catecholaminergic cells	TH-Cre	(Mahmoud et al. 2015)
	Muscle satellite cells (*in vitro* deletion)	Gt(ROSA)26Sor-Cre (tamoxifen-inducible)	(Fu et al. 2013)
	Haematopoietic stem cells	Mx1-Cre (pIpC inducible)	(Nakada et al. 2010)
	proglucagon-expressing cells	proglucagon-Cre	(Sayers et al. 2016)
AMPKβ1	Skeletal and heart muscle cells	MCK-Cre	(O'Neill et al. 2011, Sung et al. 2015)
AMPKβ2	Skeletal and heart muscle cells	MCK-Cre	(O'Neill et al. 2011, Sung et al. 2015)

AMPKβ1/β2	Skeletal and heart muscle cells	MCK-Cre	(O'Neill et al. 2011, Sung et al. 2015)

Adipoq, adiponectin; AgRP, agouti-related protein; Alfp, albumin/ α-fetoprotein; ApoE, apolipoprotein E; CMV, cytomegalovirus; Col2α1, collagen type 2 α1; hGFAP, human glial fibrillary acidic protein; HSA, human α-skeletal actin; Ins1, mouse insulin 1; LysM, lysozyme M; MCK, muscle creatine kinase; MLC1, myosin light chain 1; α-MHC, α-myosin heavy chain; Mx1, myxovirus resistance protein 1; Pax7, paired box-containing 7; pIpC, polyinosine-polycytidine; POMC, pro-opiomelanocortin; PPG, preproglucagon; RIP2, rat insulin promoter 2; TH, tyrosine hydroxylase; tTA, Tetracycline-controlled transcriptional activator system; VE, vascular endothelial.

Table 2. Metabolic pathways dysregulated in transgenic mice overexpressing AMPKα and γ mutations in skeletal and cardiac muscles.

Organ	Pathway/function	Transgenic mice	References
Heart	Glycogen storage cardiomyopathy	AMPKγ2^{R302Q} AMPKγ2^{N488I} AMPKγ2^{R531G} AMPKγ2^{T400N}	(Sidhu et al. 2005) (Arad et al. 2003, Luptak et al. 2007) (Davies et al. 2006) (Banerjee et al. 2007)
	Ischemic/post-ischemic tolerance	AMPKα2^{R45A} AMPKα2^{D157A}	(Russell et al. 2004) (Xing et al. 2003)
	Glucose transport, glycogen metabolism, glycolysis	AMPKα2^{R45A} AMPKα2^{D157A}	(Xing et al. 2003, Russell et al. 2004)
	Mitochondrial biogenesis/ function	AMPKα2^{D157A}	(Gundewar et al. 2009)
Skeletal muscle	Glucose transport	AMPKα^{2R45A} AMPKα2^{D157A}	(Mu et al. 2001) (Xing et al. 2003)
	Contraction/exercise tolerance	AMPKα2^{R45A} AMPKα2^{D157A} AMPKγ1^{R70Q} AMPKγ3^{R225Q}	(Mu et al. 2001) (Fujii et al. 2007, Rockl et al. 2007) (Barre et al. 2007, Rockl et al. 2007) (Barnes et al. 2004, Barnes et al. 2005)
	Glycogen metabolism	AMPKγ3^{R225Q} AMPKγ1^{R70Q}	(Barnes et al. 2004, Yu et al. 2006) (Barre et al. 2007)
	Mitochondrial biogenesis/ function	AMPKα2^{R45A}	(Zong et al. 2002)

Table 3. AMPK mutations in *Drosophila*.

Gene	Allele	description	mutagenesis	References
dAMPKα	AMPKα^{D1} AMPKα^{D2} AMPKα^{1} AMPKα^{2}	Deletion 1,268,785–1,270,743 bp Deletion 1,269,080–1,270,246 bp Amino acid replacement (Y141) Amino acid replacement (S211L)	Imprecise excision P-element Imprecise excision P-element EMS mutagenesis EMS mutagenesis	(Lee et al. 2007, Schertel et al. 2013) (Mirouse et al. 2007) www.flybase.org www.flybase.org
dAMPKβ alicorn (alc)	Δ*12.125* *l(2)45Ad*^{2}	Deletion of 1718 bp (nonsense point mutation replacing codon 233/112 in *alc* transcripts RA/RB)	Imprecise excision P-element EMS mutagenesis	(Spasic et al. 2008) (Dockendorff et al. 2000)
dAMPKγ loechrig (loe)	*loechrig*	P-element insertion	P-element insertion	(Deak et al. 1997, Tschape et al. 2002)

Table 4. Transgenic *Drosophila* for the study of AMPK signaling pathway.

Gene	Allele	description	References
dAMPKα	AMPKα$^{WT.Scer\UAS}$	Wilt-type AMPKα	(Lee et al. 2007, Schertel et al. 2013)
	AMPKα$^{KR.Scer\UAS}$	Kinase-dead form of AMPKα	(Lee et al. 2007)
	AMPKα$^{K56R.Scer\UAS}$	Kinase dead version of AMPKα with a K56R amino acid substitution	(Bland et al. 2010)
	AMPKα$^{K57A.Scer\UAS}$	Kinase dead version of AMPKα with a K57A amino acid substitution	(Johnson et al. 2010, Stenesen et al. 2013)
	AMPKα$^{TD.Scer\UAS}$	Constitutively active form of AMPKα	(Lee et al. 2007)
	AMPKα$^{T184D.Scer\UAS}$	Constitutively active form of AMPKα	(Mirouse et al. 2007)
	Scer\UAS- AMPKα^{RNAi}	AMPKα RNAi	(Dietzl et al. 2007)
	AMPKα$^{Scer\UAS.T:Avic\GFP}$	AMPKα GFP-tagged at the N-terminal end	(Kazgan et al. 2010)
	AMPKα$^{Scer\UAS.ORF.T:Ivir\HA1}$	Full-length AMPKα tagged at the C-terminal end with three copies of HA	(Schertel et al. 2013)
	AMPKα$^{\Delta C.Scer\UAS.T:Disc\RFP-mCherry}$	Truncated form of AMPKα lacking the C-terminal 22 amino acid residues (a stop codon has been introduced after Pro561) tagged at the N-terminal end with mCherry	(Mirouse et al. 2007)
	AMPKα$^{Scer\UAS.T:Disc\RFP-mCherry}$	AMPKα tagged at the N-terminal end with mCherry	(Kazgan et al. 2010)
AMPKγ loechrig (loe)	Scer\UAS- AMPKγ^{RNAi}	Scer\UAS- AMPKγ^{RNAi}	(Johnson et al. 2010)

Table 5. AMPK mutant strains for the study of AMPK signaling pathway in *C. elegans*.

Gene	Allele	description	References
aak	*aak-1(tm1944)*	Deletion of 618 bp	(Lee et al. 2008)
	aak-2(ok524)	Deletion of 408 bp (protein truncated after amino acid 164 lacking complete kinase domain as well as the inhibitory and AMPKβγ-binding domains)	(Apfeld et al. 2004)
	aak-2(rr48)	Point mutation H208Y predicted to disrupt the catalytic activity of the α subunit	(Narbonne and Roy 2006)
	aak-2(gt33)	Deletion of 606 bp generated by random UV-activated trimethyl-psoralen mutagenesis	(Jansen et al. 1997)
	Paak-2::gfp	Promoter GFP fusion	(Tullet et al. 2014)

aakb	aakb-1*(tm2658)*	Deletion of 146 bp (part of the glycogen-binding domain is missing)	(Narbonne and Roy 2009)
	Paakb-1::gfp	Promoter GFP fusion	(Tullet et al. 2014)
	aakb-2(rr88)	C262T transition introducing a premature stop codon	(Narbonne and Roy 2009)
aakg	aakg-4(tm5097)	Deletion of 231 bp + insertion of 24 bp	www.wormbase.org
	aakg-4(tm5269)	Deletion of 272 bp	www.wormbase.org
	aakg-4(tm5539)	Deletion of 405 bp (region encoding portions of both CBS3 and CBS4 domains)	(Tullet et al. 2014)
	Paakg-4::gfp	Promoter GFP fusion	(Tullet et al. 2014)
	Paakg-5::gfp	Promoter GFP fusion	(Tullet et al. 2014)

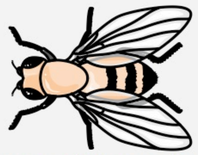
<u>Figure 1:</u> Timeline of animal models generated for the study of AMPK biology. Deletion of the AMPKα, β and γ genes in *Caenorhabditis elegans* (*aak-1*, *aak-2*, *aakb-1* and *aakb-2*), *Drosophila melanogaster* (*dAMPKα, dAMPKβ/Loechrig* and *dAMPKγ/Alicorn*), *mus musculus* (*AMPKα1*, *AMPKα, AMPKβ1*, *AMPKβ2*, *AMPKγ1* and *AMPKγ3*) are shown in the upper panel. Naturally occurring mutations in the human and porcine AMPKγ gene are shown in the middle panel. Transgenic mouse and fish models expressing AMPKα and γ mutations are shown in the lower panel. Refer to Tables 1, 3 and 5 for references.

<u>Figure 2:</u> Diversity in the genes encoding the AMPK subunits between animal species. Schematic representation of the different genes encoding the AMPKα, AMPKβ and AMPKγ subunits in *Caenorhabditis elegans* (aak-1/2, aakb-1/2 and aakg-1/2/3/4/5), *Drosophila melanogaster* (dAMPKα, dAMPKβ/Loechrig and dAMPKγ/Alicorn), *mus musculus* (AMPKα1/2, AMPKβ1/2 and AMPKγ1/2/3) and *Homo sapiens* (AMPKα1/2, AMPKβ1/2 and AMPKγ1/2/3).

Animal models deleted for the AMPKα, β and γ subunit genes

Mus musculus

AMPKα2 *AMPKα1* *AMPKγ3* *AMPKβ1* *AMPKβ2* *AMPKγ1* *AMPKβ2*



Drosophila melanogaster

dAMPKγ (loechrig) *dAMPKα* *dAMPKβ (alicorn)*

Caenorhabditis elegans

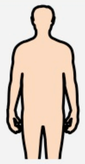
aak-2 *aak-1* *aakb-1* *aakb-2*

Identification of AMPK

1980s

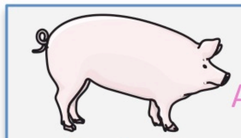
2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015

Naturally occurring AMPKγ mutations



Human mutations

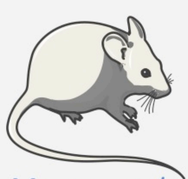
$AMPK\gamma2^{R302Q}$ $AMPK\gamma2^{R531G}$ $AMPK\gamma2^{T400N}$ $AMPK\gamma2^{N448I}$ $AMPK\gamma3^{R225W}$ $AMPK\gamma2^{G100S}$



Sus Scrofa

$AMPK\gamma3^{R200Q}$

Transgenic models for AMPKα and γ mutations

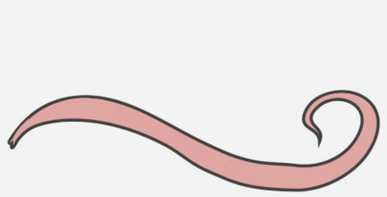
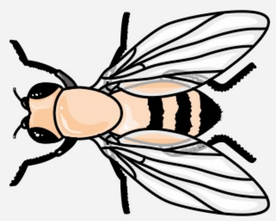
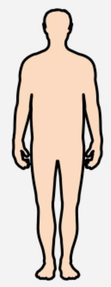


Mus musculus

$AMPK\gamma3^{R225Q}$ $AMPK\gamma2^{N448I}$ $AMPK\gamma2^{R302Q}$ $AMPK\gamma2^{R531G}$ $AMPK\gamma2^{T400N}$ $AMPK\alpha2^{R45A}$ $AMPK\alpha2^{D157A}$ $AMPK\alpha1^{R45A}$ $AMPK\gamma1^{R70Q}$ $AMPK\alpha1^{D157A}$ $AMPK\gamma1^{H151R}$

Danio rerio

$AMPK\gamma2^{R302Q}$ $AMPK\gamma2^{G100S}$

	Caenorhabditis elegans	*Drosophila melanogaster*	*Mus musculus*	*Homo sapiens*
				
AMPKα	*aak-1*, *aak-2*	*dAMPKα*	*AMPKα1*, *AMPKα2*	*AMPKα1*, *AMPKα2*
AMPKβ	*aakb-1*, *aakb-2*	*dAMPKβ* (*alicorn*)	*AMPKβ1*, *AMPKβ2*	*AMPKβ1*, *AMPKβ2*
AMPKγ	*aakg-1*, *aakg-2*, *aakg-3*, *aakg-4*, *aakg-5*	*dAMPKγ* (*loechrig*)	*AMPKγ1*, *AMPKγ2*, *AMPKγ3*	*AMPKγ1*, *AMPKγ2*, *AMPKγ3*