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# Targeting AMPK for Therapeutic Intervention in Type 2 Diabetes

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## 1. Introduction

This chapter begins with general information on the role of 5'-AMP activated kinase (AMPK) in human physiology and the molecular mechanisms that control this kinase. We discuss the functions of AMPK in different tissues and their relationship to type 2 diabetes. AMPK substrates in different subcellular organelles and compartments are described, and we speculate how the localized action of AMPK could help to control type 2 diabetes. Our review concludes with future directions that are based on the compartment-specific action of AMPK to develop new therapeutic strategies for patients with type 2 diabetes.

### 1.1 AMPK activity is critical to cell physiology in different tissues and organs

AMPK functions as a ser/thr kinase which provides an evolutionary conserved cellular energy sensor. This kinase is a focal point for metabolic control in all eukaryotes, where it regulates many aspects of physiology (Hardie, 2008a; Kim et al., 2009a; Li & McCullough, 2010; Lopaschuk, 2008; Ronnett et al., 2009; Steinberg & Kemp, 2009; Zhang, et al., 2009). It is well-established that AMPK and its yeast ortholog Snf1 control a large number of diverse processes; they include the response to nutrient limitation or other environmental changes, transcription, transport across the nuclear envelope, cell growth, cell cycle progression, mitosis, cell polarity, development, auto- and mitophagy (Amato et al., 2011; Bungard et al., 2010; Egan et al., 2010; Lee et al., 2007; Li & McCullough, 2010; Mirouse et al., 2007; Nagata & Hirata, 2009; Narbonne & Roy, 2009; Quan et al., 2007; Steinberg & Kemp, 2009; Viollet et al., 2009a; Wang et al., 2010; Witczak et al., 2008). As a result of these contributions, AMPK is vital to the function of several organs and tissues in metazoans (Fig. 1).

Owing to its pivotal role in the control of glucose homeostasis, carbohydrate, lipid and protein metabolism AMPK is a key player in many human diseases and disorders (Fogarty & Hardie, 2010; Lage et al., 2008; Towler & Hardie, 2007; Viollet et al., 2009b). In particular, the low activation state of AMPK could contribute to the increase in type 2 diabetes and obesity (Hardie et al., 2006). Moreover, as essential regulator of glucose homeostasis and lipid metabolism, AMPK has become an important therapeutic target in type 2 diabetes and obesity. This is exemplified by metformin and thiazolidinedione derivatives (TZDs); these drugs are used for therapeutic intervention in type 2 diabetes and lead to the activation of AMPK.

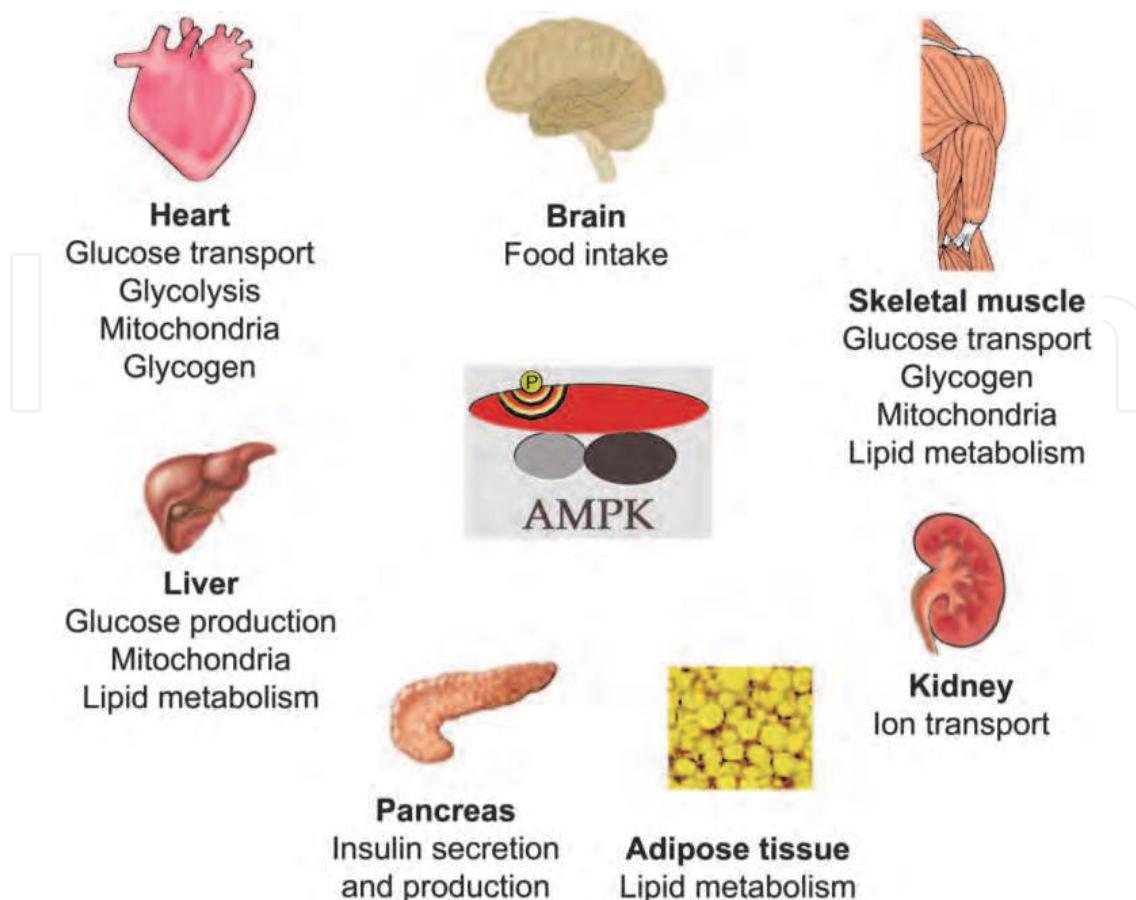


Fig. 1. The role of AMPK in different organs and tissues. AMPK controls the physiology of multiple organs which are critical to type 2 diabetes, obesity and other metabolic diseases. As such, AMPK regulates both anabolic and catabolic pathways as well as the function and biogenesis of organelles. See text for details.

## 2. Organization and activation of AMPK

AMPK senses a drop in cellular energy as it is induced by a reduction in glucose availability or other metabolic stresses. The overall consequence of AMPK activation is a change in metabolism; thus, when the AMP/ATP ratio increases AMPK becomes activated in order to rescue the energy balance. As a result of AMPK activation, the cellular metabolism switches from anabolic to catabolic processes. This metabolic shift is accomplished by the AMPK-dependent phosphorylation of multiple targets which are located in different cellular organelles and compartments (see below).

The heterotrimeric enzyme AMPK (Fig. 2;  $\alpha\beta\gamma$ ) contains one catalytic  $\alpha$  subunit that is encoded by two genes ( $\alpha 1$  and  $\alpha 2$ ). The regulatory  $\beta$  and  $\gamma$  subunits are encoded by two and three genes, respectively (Hardie et al., 2006). The two  $\beta$  subunits ( $\beta 1$ ,  $\beta 2$ ) can be myristoylated and phosphorylated, and these modifications may impact the activation and intracellular localization of AMPK (Oakhill et al., 2010; Warden et al., 2001; see below). The  $\gamma$  subunits ( $\gamma 1$ ,  $\gamma 2$ ,  $\gamma 3$ ) bind AMP and ATP in a mutually exclusive fashion, this AMP binding is important to the activation of the enzyme. The subunit composition of AMPK heterotrimers varies in different tissues and can affect the activation of the kinase (Canto & Auwerx, 2010; Cheung et al., 2000; Steinberg & Kemp, 2009; Viollet et al., 2010).

**2.1 Control of AMPK activity by phosphorylation and changes in AMPK concentration**

The importance of AMPK as a key regulator in cellular metabolism requires a tight control of the enzyme. The rapid regulation of AMPK activity is based on at least three mechanisms that contribute to AMPK activation (Oakhill et al., 2010; Sanders et al., 2007; Shackelford & Shaw, 2009; Steinberg & Kemp, 2009). (a) The most important step for AMPK activation is the phosphorylation of Thr172 of the  $\alpha$  subunit which can be modified by the upstream kinases LKB1, CaMKK $\beta$  and TAK1 (Fig. 2). Thr172 is phosphorylated when the energy state of the cell is low, i.e. when the AMP/ATP ratio rises. Under these conditions, AMP binding to the regulatory  $\gamma$  subunit promotes the subsequent Thr172 phosphorylation. LKB1 is the major upstream kinase for this event in tissues like skeletal muscle. The effect of AMP binding depends on the type of  $\gamma$  subunit (Cheung et al., 2000). Specifically, AMP-binding to  $\gamma 2$  subunits leads to the largest increase in AMPK activity. By contrast, a relative small change is observed for the  $\gamma 3$  subunit which is mostly synthesized in glycolytic skeletal muscle. Recent data suggest that the  $\beta$  subunits also play a crucial role in AMPK activation. It was proposed that  $\beta$  subunit myristylation provides a switch that is a prerequisite for Thr172 phosphorylation (Oakhill et al., 2010). (b) Aside from changes in the AMP/ATP ratio, a rise in intracellular  $\text{Ca}^{2+}$  concentrations triggers Thr172 phosphorylation. This modification is mediated by CaMKK $\beta$  and particularly important in tissues where LKB1 is not the predominant kinase for Thr172. At present, the role of TAK1 in AMPK activation is not fully understood. (c) AMPK activation can be prolonged by preventing the dephosphorylation of Thr172, a process catalyzed by phosphatases PP2A and PP2C (Kim et al., 2009a; Nagata & Hirata, 2010).

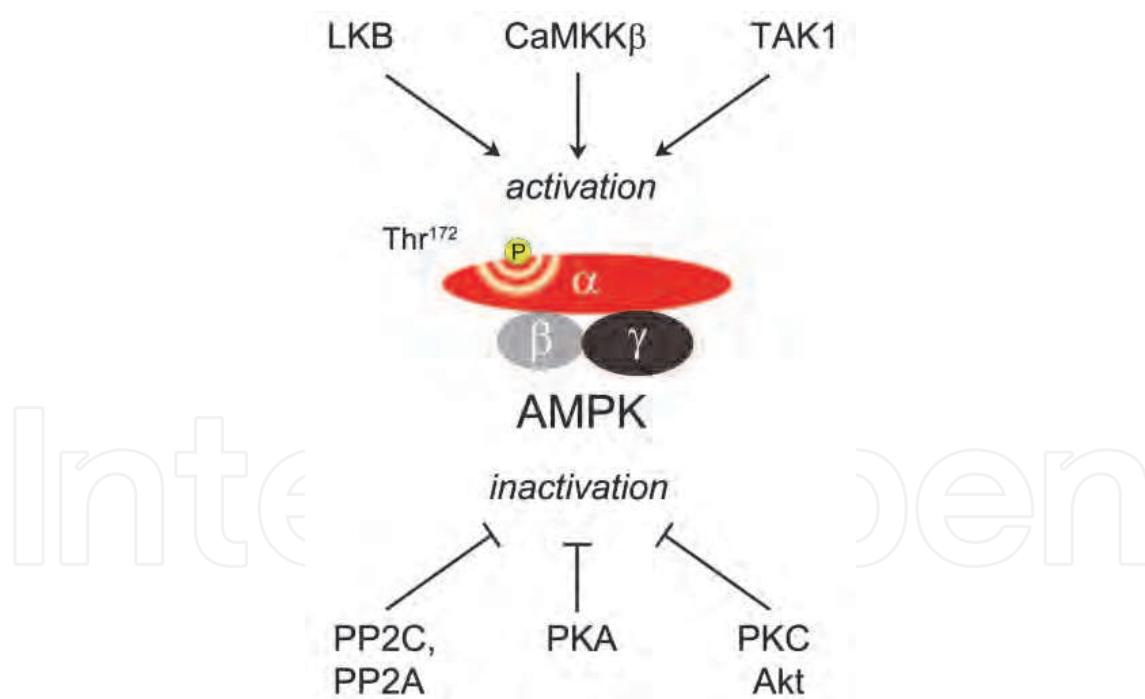


Fig. 2. Organization of AMPK and regulation of kinase activity by phosphorylation. AMPK is a heterotrimeric enzyme that is activated by phosphorylation on Thr172 of the  $\alpha$  subunit. Several upstream kinases can modify Thr172; they include LKB, CaMKK $\beta$  and TAK1. The activity of AMPK can be reduced by different mechanisms. For example, PP2A and PP2C mediate the dephosphorylation of phospho-Thr172. In addition, PKC and Akt phosphorylate Ser485/491 of the  $\alpha$  chain which decreases AMPK activity. Furthermore, PKA-dependent modification of Ser173 diminishes AMPK activity.

Aside from the dephosphorylation of phospho-Thr172, a negative regulation of AMPK involves the phosphorylation of Ser485/491 by PKC and possibly Akt, whereas the decline in activity by Ser173 phosphorylation was ascribed to PKA (Djouder et al., 2010). Such modification on Ser173 may help to fine tune lipid metabolism in adipose tissue.

The tissue-specific regulation of AMPK activity is likely achieved by the combined effects of upstream activating kinases, inactivating phosphatases as well as the synthesis and degradation of AMPK subunits. For example, LKB1 is particularly important to activate AMPK in skeletal muscle, whereas CaMKK $\beta$  is crucial in the brain (Ronnett et al., 2009). On the other hand, TNF $\alpha$  alters AMPK activation by modulating the synthesis of PP2C (Lu et al., 2010; Steinberg et al., 2006). Aside from the rapid control of AMPK activation by phosphorylation, changes in the expression of subunit genes or the turnover of AMPK subunits can help to fine tune AMPK activity in some tissues (Barry et al., 2010; Fukuyama et al., 2007; Hallows et al., 2006; Qi et al., 2008; Niesler et al., 2007; Steinberg et al., 2003).

## 2.2 Pharmacological compounds and other factors that alter AMPK activity

Previous work established the essential role of AMPK in the regulation of carbohydrate, protein and lipid metabolism; this made AMPK a key target for the treatment of type 2 diabetes, obesity and metabolic syndrome (Gruzman, Babai & Sasson, 2009; Hardie, 2008b; Steinberg & Kemp, 2009; Viollet et al., 2010; Viollet et al., 2009b). Indeed, in a clinical setting AMPK activity is altered with the anti-diabetic drug metformin and other biguanides. The drug-induced activation of AMPK has important consequences for the patient; among these is the improvement of insulin resistance.

Pharmacological drugs have also been critical to define how AMPK mediates metabolic control (see Table 1). These compounds employ a variety of molecular mechanisms that culminate in AMPK activation (Gruzman et al., 2009; Hawley et al., 2010; Mantovani & Roy, 2011). For example, the kinase can be activated by a rise in the AMP/ATP ratio, generation of an AMP mimetic or increase in intracellular Ca $^{2+}$  concentrations (Hawley et al., 2010). Metformin impacts several biological processes that ultimately activate AMPK. These include changes in the respiratory chain, increased synthesis of the protein deacetylase SIRT1 (which activates LKB1) and activation of TAK1 (Caton et al., 2010; Hawley et al., 2002; Hawley et al., 2010; Xie et al., 2006). Phenphormin promotes the LKB1-dependent activation of AMPK by inhibiting mitochondrial complex I (Hawley et al., 2010). Resveratrol prevents the acetylation and concomitant inactivation of the upstream kinase LKB1, this compound also inhibits mitochondrial ATP synthase and may increase the concentration of adiponectin (Hawley et al., 2010; Wang et al., 2011). AICAR generates the AMP mimetic 5-amino-4-imidazolecarboxamide ribotide (ZMP) and causes a drop in cellular ATP and ADP, which leads to AMPK activation (Hawley et al., 2010). Aside from drugs that activate AMPK, compound C serves as an ATP-competitive inhibitor of AMPK that has been used widely. All of the compounds discussed here are established pharmacological tools that alter AMPK activation or enzymatic activity; they have been useful for the analysis of AMPK *in vitro*, in growing cells and in whole animals. The following table summarizes how AMPK activity can be affected *in vitro*, growing cells, organs or whole organisms.

Compounds, physiological processes and stress	Mode of action and effect on AMPK
<i>(A) Drugs and other compounds</i>	
AICAR (5-aminoimidazole-4-carboxamide-1-b-D-ribofuranoside)	Generation of ZMP, which functions as an analog of AMP; activation
Metformin (Biguanide)	Reduces mitochondrial ATP production; activation
Phenformin	Inhibition of respiratory chain, activation
Resveratrol	Change in ATP synthase activity; prevents acetylation of LKB1 via modulation of SIRT1; upregulation of adiponectin synthesis and multimerization; activation
Thiazolidinedione derivatives (TZDs, troglitazone; rosiglitazone, pioglitazone)	Stimulate the expression and secretion of adiponectin; increase in AMP concentration; activation of PPAR $\gamma$ ; AMPK activation
Antimycin A	Inhibition of respiratory chain; activation
Sodium azide	Inhibition of respiratory chain; activation
NO	Inhibition of respiratory chain; activation
Oligomycin	Inhibition of ATP synthase; activation
Dinitrophenol	Uncoupler of electron transfer/ATP synthesis; activation
2-Deoxyglucose	Inhibition of glycolysis; activation
Arsenite	Inhibition of TCA cycle; activation
$\beta$ -guanadinopropionic acid	Creatine analog; increases AMP/ATP ratio; activation
A23187	Activation by increase in cytosolic calcium ions
A769662	Direct AMPK activator
Compound C (dorsomorphin)	Reversible, ATP-competitive inhibitor
<i>(B) Hormones, cytokines, physiological processes and environmental stressors</i>	
Insulin	Inhibition of AMPK activation; mediated by Akt kinase
Ghrelin	Tissue-specific effects; activation in heart and hypothalamus; reduced activity in liver and adipose tissue
Adiponectin	Activation by increase in AMP concentration
Resistin	Tissue-specific effects; reduction of AMPK activity in skeletal muscle
Leptin	Tissue-specific effects; activates $\alpha 2$ heterotrimers; activation in muscle and fat tissue; reduces activity in hypothalamus
TNF $\alpha$	Acute and chronic effects; acute: activation; chronic: reduction in activity; increase in PP2C

Compounds, physiological processes and stress	Mode of action and effect on AMPK
IL-6	Increase in AMP/ATP ratio; activation (note that IL-6 can have different effects on insulin sensitivity)
CNTF (Ciliary neurotrophic factor)	Tissue-specific effects; activation in muscle; activity reduced in hypothalamus
UCP1, UCP3	Uncoupling proteins in mitochondria, change in energy status; activation
Reduction in glucose availability	Change in energy status; activation
Rise in $\text{Ca}^{2+}$ concentration, osmotic stress	CaMKK $\beta$ activation
Exercise	Skeletal muscle contraction; activation
Heat shock, oxidative stress	Environmental stressors; transient activation
Ischemia/hypoxia, reactive oxygen species	Metabolism/oxidative stress; activation

Table 1. Modulators of AMPK activity.

The data in Table 1 are compiled from several publications that describe the molecular mechanisms and tissue-specific effects on AMPK activity in detail (Caton et al., 2010; Dzamko & Steinberg, 2009; Hawley et al., 2010; Maeda et al., 2001; Nagata & Hirata, 2010; Steinberg et al., 2009; Viollet et al., 2010). It should be noted that although in most cases a correlation between treatment and changes in AMPK activity has been demonstrated, the molecular mechanisms are not always fully understood. For example, hormone or cytokine-dependent changes in AMP/ATP ratios may be secondary to other signaling events, such as changes in cAMP concentrations. For some of the treatments, it has yet to be established whether AMPK is *essential* for the downstream physiological effect. More recent experiments with knockout cells and animal models will help to fill these gaps (Viollet et al., 2009a).

### 3. AMPK functions in different tissues and organs

Although AMPK is present in different tissues and organs, the subunit composition varies, and changes in cell physiology can also alter the profile of expressed subunits (Mahlapuu et al., 2004; Pulinkkunnil et al., 2011; Putman et al., 2007; Quentin et al., 2011; Stapleton et al., 1996; Turnley et al., 1999). Of particular importance at the cellular, organ and organismal level is the ability of AMPK to switch from anabolic to catabolic processes when energy supplies are low. AMPK regulates metabolism and other aspects of cell physiology both under normal and disease conditions; studies with different cells or tissues emphasize the significance of AMPK for cellular metabolism and the response to various forms of stress. Thus, AMPK controls several metabolic pathways that are directly relevant to diabetes and other metabolic diseases or syndromes (Steinberg & Kemp, 2009; Viollet et al., 2010; Zhang et al., 2009). However, AMPK not only provides a sensor for nutrient availability, the kinase is also activated by hormonal signals in peripheral tissues and the hypothalamus (Jorda et al., 2010; Ronnett et al., 2009). Notably, this signaling in the central nervous system contributes to the regulation of food uptake. Research with hepatic, skeletal muscle, adipose,

pancreatic and kidney cells is particularly important to our understanding of type 2 diabetes as these cell types are crucial to the etiology or pathophysiology of the disease (Fig. 1). In general, the consequences of AMPK activation can be divided into acute and long-term effects (Mantovani & Roy, 2011; Viollet et al., 2010). Whereas the phosphorylation of key enzymes produces a fast downregulation of ATP-consuming metabolic pathways, long-term effects involve changes in the expression of target genes that control metabolism. Since several recently published excellent reviews covered these topics extensively, Table 2 only summarizes the impact of AMPK activation on tissues that are critical to type 2 diabetes.

Tissue or cell type	Physiological process	Enzyme or process affected by AMPK
Liver	activation of fatty acid oxidation, inhibition of lipogenesis	inhibition of acetyl-CoA carboxylase ACC (Acc1, Acc2)
	reduced cholesterol synthesis	HMG-CoA reductase
	stimulation of fatty acid uptake	CD36 (a fatty acid translocase) moves to the plasma membrane
	changes in lipogenesis and glycolysis due to reduced concentration of transcriptional regulators SREBP1 (sterol response element binding protein-1) and ChREBP (carbohydrate response-element binding protein)	inhibits ChREBP by phosphorylation, reduces the transcription of genes encoding SREBP1 and ChREBP
	increase in mitochondrial biogenesis	increased expression of PGC1 $\alpha$ and other genes required for mitochondrial biogenesis
	glycogen synthesis reduced	inhibition of glycogen synthase
Skeletal muscle	inhibition of gluconeogenesis and hepatic glucose production	changes in the activity, concentration or localization of key enzymes or transcriptional regulators; (phosphoenol pyruvate carboxy kinase, HNF4; TORC2, p300)
	stimulation of glucose uptake; fusion of GLUT4 (glucose transporter) containing vesicles with plasma membrane	phosphorylation of AS160 may promote trafficking of vesicles; increased transcription of GLUT4 gene by phosphorylation of HDAC5
	increase in mitochondrial biogenesis	increased expression of PGC1 $\alpha$ and other genes required for mitochondrial biogenesis
	increased fatty acid uptake and oxidation	inhibition of ACC
	reduction in protein synthesis	inhibition of mTOR pathway via modification of mTOR, TSC2 and eEF2 kinase
	control of glycogen metabolism	inactivation of glycogen synthase

Tissue or cell type	Physiological process	Enzyme or process affected by AMPK
Adipose tissue	increase in fatty acid oxidation	inactivation of ACC
	inhibition of lipolysis	phosphorylation of HSL, reduced association of HSL with lipid droplets
Pancreas	inhibition of glucose-induced insulin secretion in $\beta$ cells	reduced trafficking of vesicles containing insulin
	inhibition of transcription of the preproinsulin gene in $\beta$ cells; stimulation of glucagon secretion in $\alpha$ cells	molecular mechanisms not fully understood
Heart	stimulation of glucose uptake by translocation of GLUT4 to the plasma membrane	fusion of GLUT4 containing vesicles with the plasma membrane
	stimulation of glycolysis	activation of 6-phosphofructo-2-kinase → enhances production of fructose 2,6-bisphosphate → stimulates 6-phosphofructo-1-kinase
	increase in fatty acid oxidation	inactivation of ACC
	control of glycogen metabolism	contributions of AMPK activity not completely understood at the molecular level
Kidney	ameliorates changes linked to diabetic nephropathy	inhibition of mTOR, inhibition of CFTR and other ion channels
Brain	food intake; multiple pathways affected in the hypothalamus; adiponectin, leptin, insulin and ghrelin control AMPK	control of neuropeptide synthesis

Table 2. AMPK-dependent modulation of cell physiology.

Some of the tissue-specific reactions regulated by AMPK and relevant to type 2 diabetes are listed. A comprehensive description of these processes can be found in several recent reviews (Hardie, 2008a; Ix & Sharma, 2010; Lieberthal & Levine, 2009; Steinberg & Kemp, 2009; Viollet et al., 2009a; Viollet et al., 2010) and original publications (da Silva Xavier et al., 2003; Leclerc et al., 2011; Takiar et al., 2011; van Oort et al., 2009).

#### 4. AMPK modulates targets in different subcellular organelles and compartments

##### 4.1 Subcellular distribution of AMPK substrates

The combination and integration of different subcellular events regulated by AMPK enables cells, tissues and organs to coordinate different metabolic pathways in order to achieve and maintain the proper energy balance of the whole organism. Fig. 3 depicts established AMPK

substrates according to their presence in different subcellular compartments. Table 3 expands this information and specifies how the AMPK-dependent phosphorylation of individual substrates alters their functions.

It is obvious from Fig. 3 that AMPK phosphorylates a large number of proteins that are associated with distinct organelles or subcellular compartments. Cytoplasmic and mitochondrial substrates of the kinase include enzymes that are involved in fat, protein, glucose and glycogen metabolism. Kinase targets in the plasma membrane consist of ion channels, carriers and receptors, whereas other substrates are linked to the function or trafficking of intracellular membranes. This includes the transport of vesicles containing the glucose transporter GLUT4, because GLUT4 translocation to the plasma membrane is a prerequisite for efficient glucose uptake in skeletal muscle and other tissues. AS160 and TBC1D1 likely play a role in these processes (Table 3). In the nucleus, the AMPK-mediated modification of transcription factors, transcriptional regulators and a subunit of RNA-polymerase I control the expression of genes that are implicated in specific anabolic and catabolic reactions. The phosphorylation of several of these targets is also critical to the biogenesis of mitochondria and the assembly of ribosomes.

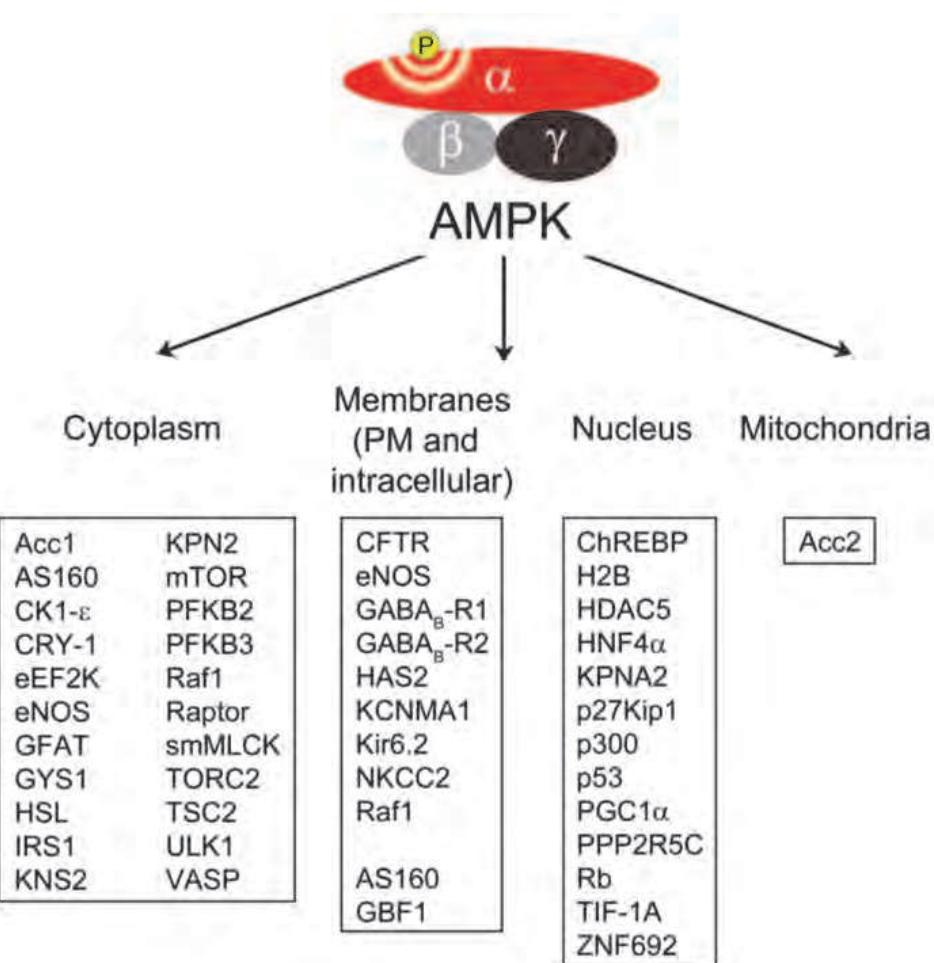


Fig. 3. AMPK affects functions in various cellular compartments and organelles. Examples are shown for the proteins that AMPK modifies in distinct sub-cellular locations. Some of the substrates are present in multiple compartments. PM, plasma membrane. See text and Table 3 for details.

Given the diverse types of AMPK substrates and their presence in different cellular locations, it is helpful to recapitulate their functions (Table 3). This knowledge is a prerequisite to understand how the dynamic association and action of AMPK in different compartments will impact downstream events.

Substrates that have been established for AMPK heterotrimers that contain the  $\alpha 1$  or  $\alpha 2$  subunit are shown. For different AMPK substrates the function, effect of AMPK-dependent phosphorylation and the major subcellular localization are depicted. For some substrates, there are cell-type specific differences, and the effect of AMPK-dependent phosphorylation may not be fully understood or controversial. The list of AMPK substrates was compiled from PhosphoSitePlus (phosphosite.org).

Substrates for AMPK $\alpha 1$	Function	Effect of phosphorylation	Primary intracellular localization	References
Acc1; Subunit of acetyl-CoA carboxylase, ACC	Carboxylates acetyl-CoA, thereby generating malonyl-CoA; this step is rate-limiting for FA biosynthesis.	inhibition	cytoplasm	Sun et al., 2006
Acc2; Subunit of acetyl-CoA carboxylase, ACC	Carboxylates acetyl-CoA, thereby generating malonyl-CoA; this step is rate-limiting for FA biosynthesis.	inhibition	mitochondria (outer mitochondrial membrane)	Reihill et al., 2007
AMPK $\alpha 1$	Catalytic subunit of AMPK	potential autoregulation	cytoplasm, nucleus	Stein et al., 2000
AMPK $\beta 1$	Regulatory subunit of AMPK	enzymatic activity, localization	cytoplasm, nucleus	Warden et al., 2001
CFTR	Cystic fibrosis transmembrane conductance regulator, chloride channel	inhibits PKA-dependent stimulation of CFTR	plasma membrane, ER, cytoplasmic vesicle; early endosome	King et al., 2009; Kong-suphol et al., 2009; Takiar et al., 2011
ChREBP	Transcription factor; repressor	inactivation of DNA binding	nucleus, cytoplasm	Kawaguchi et al., 2002
CK1- $\epsilon$ (CK1 epsilon)	Ser/thr kinase; member of the casein kinase 1 (CK1) family; phosphorylates mPer2	increase of CK1- $\epsilon$ activity; control of circadian clock	cytoplasm, nucleus, cell junction, centrosome	Um et al., 2007
CRY1 (crypto-chrome)	DNA photolyase, regulates circadian rhythm	phosphorylation leads to destabilization	nucleus, mitochondria, cytoplasm	Lamia et al., 2009

Substrates for AMPK $\alpha 1$	Function	Effect of phosphorylation	Primary intracellular localization	References
CRTC-1	CREB-regulated transcriptional coactivator	phosphorylation causes cytoplasmic retention	nucleus, cytoplasm	Mair et al., 2011
eEF2K (eEF2 kinase)	Protein kinase; eEF2K modifies and thereby inactivates eEF2	activation of kinase activity	cytoplasm	Brown et al., 2004
eNOS	Endothelial nitric oxide synthase (constitutive), EC 1.14.13.39; regulation of cytoskeletal reorganization	activation, promotes deacetylation by SIRT1	plasma membrane, Golgi	Chen et al., 2010; Sun et al., 2006
GABA <sub>B</sub> -R1	GABA <sub>B</sub> receptor subunit; G-protein coupled receptor	increase in receptor function, promotes functional coupling to K <sup>+</sup> channels	plasma membrane, ER, dendrite, axon, postsynaptic membrane	Kuramoto et al., 2007
GABA <sub>B</sub> -R2	GABA <sub>B</sub> receptor subunit; G-protein coupled receptor	increase in receptor function, promotes functional coupling to K <sup>+</sup> channels	plasma membrane, ER, dendrite, axon, postsynaptic membrane	Kuramoto et al., 2007
GBF1; Golgi-specific brefeldin A resistance factor	Guanine nucleotide exchanger for ARF5; mediates ARF5 activation; Golgi to ER trafficking	triggers disassembly of Golgi apparatus	Golgi membrane	Miyamoto et al., 2008
GFAT	glutamine-fructose-6-phosphate transaminase 1; EC 2.6.1.16; regulates glucoseflux to hexosamine pathway	1.4 fold increase in enzymatic activity	cytoplasm	Li et al., 2007
GYS1	Glycogen synthase; EC 2.4.1.11	inhibits enzymatic activity	cytoplasm	Skurat, et al., 2000
H2B	Histone H2B	activates stress-induced transcription	nucleus	Bungard et al., 2010

Substrates for AMPK $\alpha$ 1	Function	Effect of phosphorylation	Primary intracellular localization	References
HDAC5	Histone deacetylase 5; EC3.5.1.98; transcriptional regulator, cell cycle progression, development	reduced binding to GLUT4 promoter → enhanced GLUT4 expression	nucleus (cytoplasm)	McGee et al., 2008
HNF4 alpha	Hepatocyte nuclear factor 4 alpha; transcription factor, control of gene expression in hepatocytes	reduced DNA-binding	nucleus	Hong et al., 2003
HSL	Hormone sensitive lipase; EC 3.1.1.79; involved in triglyceride lipolysis	inhibition; change in the association with lipid droplets	cytoplasm, lipid droplets	Garton et al. 1989; ; Watt et al., 2006
IRS1; adaptor protein	Insulin receptor substrate 1, adaptor protein	modulation of PI3 kinase signaling	cytoplasm	Jakobsen et al., 2001; Tzatsos & Tsichlis, 2007
KCNMA1 iso4; $\alpha$ subunit of BK <sub>Ca</sub> channel	Potassium channel, activated in response to membrane depolarization, oxygen sensing in carotid body	inhibition of potassium currents	integral membrane protein, plasma membrane, axon	Ross et al., 2011
Kir6.2 (KCNJ11)	Potassium channel, voltage dependent, inward rectifying	might play a role in insulin secretion	plasma membrane	Chang et al., 2009
KNS2	Kinesin 2; kinesin light chain 1, motor protein	biological role of modification not known	cytoplasm, microtubules	McDonald et al., 2009, 2010
KPNA2, importin- $\alpha$ 1	Adaptor for classical nuclear import	interferes with nuclear import of HuR	cytoplasm, nucleus, nuclear envelope	Wang et al., 2004
mTOR	Ser/thr kinase; EC 2.7.11.1; catalytic subunit of mTORC1 and mTORC2	links nutrient supply to translation	cytoplasm	Cheng et al., 2004
NKCC2 (SLC12A1)	Electroneutral transporter; reabsorption of Na <sup>+</sup> and Cl <sup>-</sup> ; controls cell volume	regulation of transporter activity	plasma membrane	Fraser et al., 2007

Substrates for AMPK $\alpha 1$	Function	Effect of phosphorylation	Primary intracellular localization	References
p27Kip1 (CDKN4)	Cyclin-dependent kinase inhibitor 1B, controls cell cycle progression at G1	stabilization of p27; linked to autophagy	nucleus	Liang et al., 2007
p300	Protein (histone) acetyltransferase; EC2.3.1.48; transcriptional co-activator	inhibits interaction with nuclear receptors, such as PPAR $\gamma$	nucleus	Yang et al., 2001
p53	Tumor suppressor, transcription factor, cell cycle arrest, DNA repair, apoptosis	stabilization of p300-p53 interaction; controls cell cycle progression	nucleus	Dornan & Hupp, 2001
PFKFB2	6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2; EC 2.7.1.105 or EC 3.1.3.46; glycolysis	activation; stimulation of glycolysis	cytoplasm	Marsin et al., 2000
PFKFB3; iPFK2	similar to PFKFB2; inducible in monocytes	activation	cytoplasm	Marsin et al., 2002
PPP1R3C (R5/PTG)	Regulatory subunit of protein phosphatase PP1; controls PP1 activity; targets PP1 to glycogen; stimulation of glycogen synthase	promotes ubiquitination and thereby degradation	glycogen granules	Vernia et al., 2009
PPP2R5C (B56 $\gamma$ )	Regulatory subunit of protein phosphatase PP2A; possible role in the regulation and targeting of PP2A	increases PP2A activity	nucleus, chromosomes	Kim et al., 2009b
Raf1	Ser/thr kinase; EC 2.7.11.1; component of Ras→Raf→MEK1/2→ERK 1/2 signaling pathway		cytoplasm, plasma membrane	Sprenkle et al., 1997
Raptor	Regulation of mTORC1 (mammalian target of rapamycin complex 1); functions as scaffold	inhibition of mTORC1; cell cycle arrest	cytoplasm	Gwinn et al., 2008
Rb	Retinoblastoma protein; regulates cell cycle progression at G1; functions as transcriptional co-regulator; tumor suppressor	control of brain development	nucleus	Dasgupta & Milbrandt, 2009

Substrates for AMPK $\alpha$ 1	Function	Effect of phosphorylation	Primary intracellular localization	References
smMLCK	Smooth muscle myosin light chain kinase; ser/thr protein kinase; EC2.7.11.18	reduces activity of smMLCK	cytoplasm	Horman et al., 2008
TBC1D1	GTPase activating protein Rab family members; regulates glucose transport	induces binding to 14-3-3 proteins	ER	Chen et al., 2008
TIF-IA	Transcription initiation factor for RNA-pol I	reduced rDNA transcription	nucleolus	Hoppe et al., 2009
TORC2 (CRTAC2)	Transducer of regulated CREB protein 2; transcriptional regulator	phosphorylation causes cytoplasmic retention	nucleus, cytoplasm	Koo et al., 2005
TSC2 (tuberin)	Generates heterodimer with hamartin (TSC1); TSC1/TSC2 functions as GTPase activator of Rheb	enhances TSC2 activity → inhibition of mTOR	cytoplasm	Inoki, et al., 2003
ULK1	Ser/thr kinase; EC 2.7.11.1; binds to mTORC1 via raptor, binding controlled by nutrient supply	control of autophagy	cytoplasm	Egan et al., 2011
VASP	Vasodilator-stimulated phosphoprotein; actin regulator	impairs endothelial actin assembly	cytoplasm, cytoskeleton	Blume et al., 2007
ZNF692 (AREBP)	Transcriptional regulator, contains zinc finger	reduction in DNA binding	nucleus	Inoue & Yamachi, 2006

Substrates for AMPK $\alpha$ 2	Function	Effect of phosphorylation	Primary intracellular localization	References
ACC1	See above description of AMPK $\alpha$ 1 targets		cytoplasm	
AS160 (TBC1D4; Akt substrate of 160k)	GTPase activating protein for Rab; implicated in GLUT4 exocytosis in skeletal muscle	not fully understood; may regulate glucose uptake	cytoplasm	Eguez et al., 2005; Treebak et al., 2010
HAS2	Hyaluronic acid synthase 2; EC 2.4.1.212; integral membrane protein	inhibition of enzymatic activity	plasma membrane	Vigetti et al., 2011

HDAC5	See above description of AMPK $\alpha$ 1 targets		nucleus	
p53	See above description of AMPK $\alpha$ 1 targets		nucleus	
PGC1 $\alpha$	PPAR $\gamma$ coactivator-1; transcriptional co-activator; association with PPAR $\gamma$ ; binds to CREB and nuclear respiratory factors; controls mitochondrial biogenesis	phosphorylation alters activity as transcriptional regulator	nucleus	Jager et al., 2007
PLD1	Phospholipase D1 phophatidylcholine specific; EC 3.1.4.4; linked to Ras signaling; involved in membrane trafficking	activation of enzymatic activity	Golgi, ER	Kim et al., 2010

Table 3. Substrates of AMPK heterotrimers containing the  $\alpha$ 1 or  $\alpha$ 2 subunit

#### 4.2 Subcellular distribution and trafficking of AMPK

AMPK is associated with different organelles and subcellular compartments; however, little is known about the dynamic nature of this distribution. Analyses of other signaling pathways have demonstrated that the subcellular localization of kinases is critical for the proper response to extra- and intracellular stimuli, and it is likely that the same scenario applies to AMPK. We will therefore briefly review what is currently known about the subcellular localization and trafficking of AMPK.

AMPK is found in the nucleus and cytoplasm which reflects functions for the enzyme in both locations (Kodiha et al., 2007; Witczak et al., 2008). Although the kinase is associated with multiple compartments,  $\alpha$ 1 and  $\alpha$ 2 subunits differ in their nucleocytoplastic distribution. Under normal growth conditions,  $\alpha$ 1 is predominantly in the cytoplasm, whereas  $\alpha$ 2 locates to both the nucleus and cytoplasm (Salt et al., 1998). It was further shown that the nuclear localization sequence (NLS) present in the  $\alpha$ 2 subunit is required for AMPK nuclear translocation (Suzuki et al., 2007), suggesting that the catalytic  $\alpha$  subunit is essential for the proper intracellular localization of the holoenzyme. Our current model of AMPK trafficking proposes that the kinase shuttles between the nucleus and the cytoplasm. Data from our group and others support this idea, as they demonstrated that the nuclear carrier Crm1 is essential for AMPK export from the nucleus to the cytoplasm (Kazgan et al., 2010; Kodiha et al., 2007). How the  $\beta$  subunit impacts the proper targeting of the holoenzyme is at present not entirely clear. The  $\beta$ 1 subunit can be modified posttranslationally, both by phosphorylation and myristoylation, and these modifications were linked to the subcellular targeting of the  $\beta$ 1 subunit (Suzuki et al., 2007; Warden et al., 2001). It was proposed that AMPK concentrates in the cytoplasm when the heterotrimeric enzyme contains the  $\beta$ 1 subunit (Suzuki et al., 2007). However, this model is difficult to reconcile with the fact that both  $\beta$ 1 and  $\beta$ 2 subunits can be detected in the nucleus (Kodiha et al., 2007). Furthermore, recent studies suggest that the myristoylation of  $\beta$ 1 and  $\beta$ 2 subunit is particularly important for AMPK activation, as AMP-dependent myristoylation provides a switch that triggers Thr172 phosphorylation (Oakhill et al., 2010). Although the contribution of  $\beta$  subunits to nuclear and membrane targeting of the holoenzyme is not completely understood at this point, the importance of the  $\beta$  subunit for glycogen binding is well established (Polekhina et al., 2003).

In contrast to the  $\alpha$  and  $\beta$  subunits, little is known about the trafficking of AMPK  $\gamma$  subunits. In *Drosophila*, the single  $\gamma$  subunit migrates into the nucleus of the fat body with the onset of autophagy during normal development, and a potential NLS was detected in this subunit (Lippai et al., 2008). It was speculated that this nuclear accumulation contributes to the expression of genes that are necessary for autophagy.

Several studies support the model that the intracellular distribution of AMPK in human and other cells is dynamic. This is particularly important in the context of disease, because the distribution of AMPK can be modulated by physiological and environmental stimuli. For example, the  $\alpha 2$  subunit translocates to the cell nucleus upon exercise or environmental stress (Kodiha et al., 2007; McGee et al., 2003), indicating that the adaptation of skeletal muscle during exercise or metabolic stress is at least in part mediated by the subcellular relocation of AMPK. Examples of the relocation of AMPK  $\alpha$  subunits in human cells exposed to oxidative stress or depleted for energy are shown in Fig. 4 (Kodiha et al., 2007). The relocation of AMPK subunits in response to physiological changes is not restricted to the  $\alpha$  subunits; our previous experiments demonstrated that AMPK  $\beta$  subunits accumulate in the nucleus as well when cells are exposed to oxidative and other forms of stress (Kodiha et al., 2007). Moreover, AMPK localization could be regulated by the circadian rhythm. Specifically, changes in the expression of AMPK subunits may depend on the circadian rhythm; this change in expression will then alter the intracellular distribution of AMPK (Lamia et al., 2009). Since these studies were carried out with mice, it has yet to be shown whether the same applies to humans.

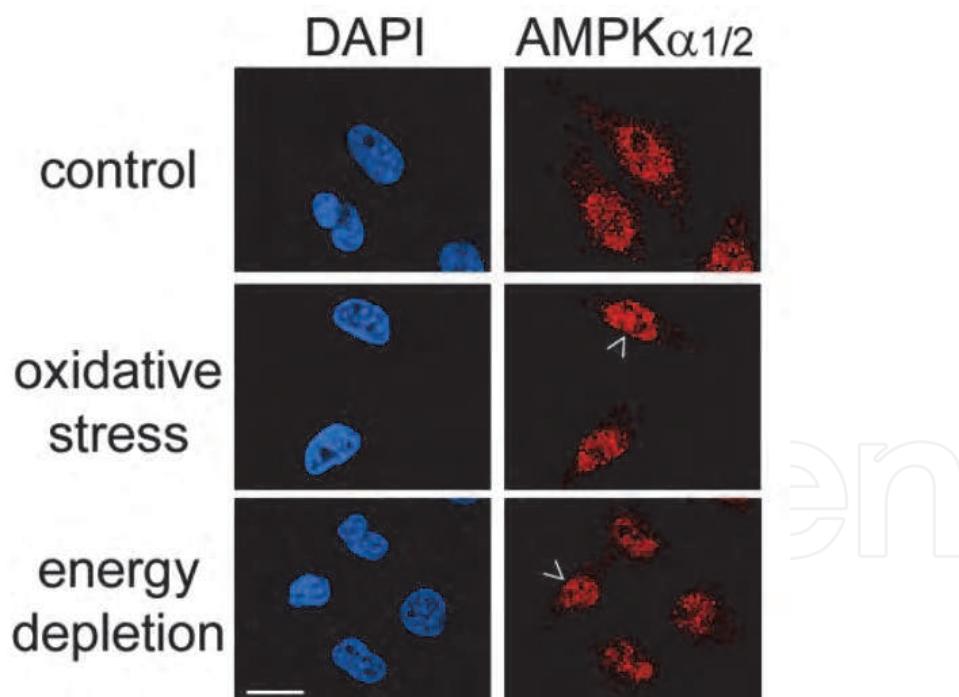


Fig. 4. AMPK $\alpha$  concentrates in nuclei when cells are exposed to oxidative stress or depleted for energy. HeLa cells were treated with diethyl maleate to induce oxidative stress or with a combination of sodium azide and 2-deoxyglucose for energy depletion. The distribution of AMPK $\alpha 1$  and  $\alpha 2$  subunits was examined by indirect immunofluorescence; DNA was stained with DAPI (Kodiha et al., 2007). Note that the  $\alpha$ -subunits are more concentrated in nuclei of stressed cells. Several nuclei are marked with arrowheads. Size bar is 20  $\mu\text{m}$ .

Studies from several laboratories, including our group, defined the signals and mechanisms that determine the trafficking and intracellular distribution of AMPK. Our work also suggested crosstalk between other signaling cascades and the localized action of AMPK (Kodiha et al., 2007 and unpublished). Ultimately, such crosstalk will add to the complexity of downstream events that are modulated by AMPK. Taken together, previous research suggests that AMPK subunits move between different subcellular locations, and it can be expected that the compartment-specific actions of the kinase are linked to the physiological response of cells and tissues.

#### 4.3 How does the compartment-specific action of AMPK impact cellular functions that are relevant to type 2 diabetes?

Type 2 diabetes is associated with the increased risk of a growing number of diseases and pathologies. This is exemplified by renal nephropathy, myocardial disease, stroke, Alzheimer's and Parkinson's disease (Almdal et al., 2004; Biessels et al., 2006; Burdo et al., 2009; Hallows et al., 2010; Hu et al., 2007; Maher & Schubert, 2009; Schernhammer et al., 2011). Several drugs are currently used in the clinical setting to activate AMPK in patients suffering from type 2 diabetes or obesity. However, it should be kept in mind that AMPK activation can be beneficial as well as harmful in the ischemic heart, and AMPK activation may be linked to neurodegeneration (Lopaschuk, 2008; Spasic, Callaerts & Norga, 2009; Thornton et al., 2011; Vingtdeux et al., 2011). Thus, activation of AMPK throughout the whole organism or the entire cell of a particular tissue may not always be advantageous. As an alternative approach, we put forward the concept of a compartment-specific modulation of AMPK action. Since AMPK activation can be damaging in the context of some of the complications associated with type 2 diabetes, our approach applies both to the localized activation as well as inhibition of the kinase. We believe that the confined action of AMPK will provide a better therapeutic approach in the future that could reduce the side-effects of AMPK modulators. The simplified model in Fig. 5 summarizes the possible changes of cellular functions that will be induced by targeting AMPK in different subcellular compartments.

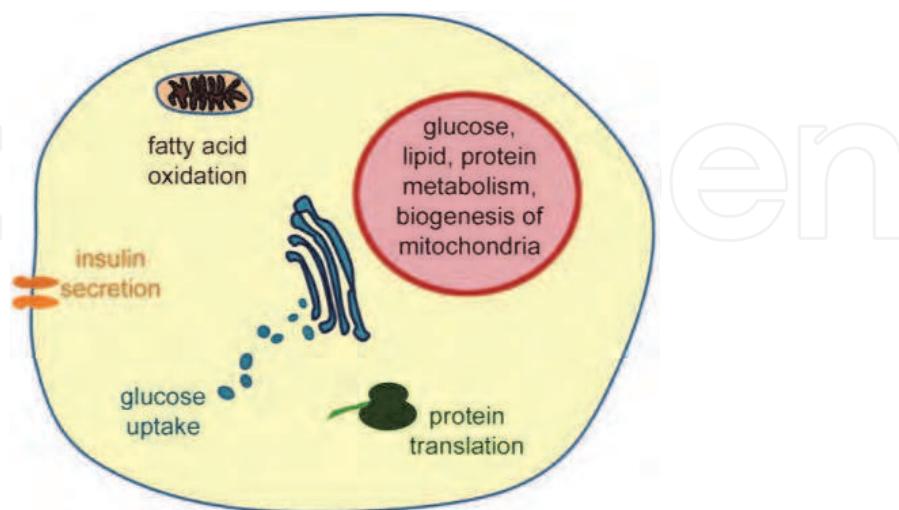


Fig. 5. Localized modulations of AMPK activity. The possible changes induced by the compartment-specific alteration of AMPK activity are depicted. Note that there are cell-type dependent differences for the processes regulated by AMPK.

In the past few years, significant progress has been made with the identification of AMPK substrates and their links to human disease. As shown in Fig. 3 and Table 3, AMPK modifies targets in different subcellular compartments or organelles. We propose that the modification of these substrates relies on (a) the amount of active AMPK and (b) the intracellular distribution of AMPK. The combination of AMPK activation and subcellular localization will then determine the level of phosphorylation of its substrates and the subsequent changes in cell physiology. Such changes will affect both the rapid response to specific stimuli as well as the long-term modification of metabolism and other processes. In the following, we focus on some of the processes that are controlled by AMPK and linked to type 2 diabetes or the complications associated with the disease.

#### **4.3.1 AMPK targets the protein synthesis apparatus in the cytoplasm**

Several of the cytoplasmic substrates of AMPK are essential to promote a fast cellular response to changes in nutrient supply. For example, AMPK phosphorylates cytoplasmic targets, such as eEF2 kinase and mTOR, which regulate protein translation. AMPK-dependent modification of these substrates results in downregulation of protein synthesis. Under some conditions, it could be desirable to preferentially modulate these processes that are associated with cytoplasmic AMPK targets. A possible example of such a scenario is the diabetic kidney (Cammisotto et al., 2008; Hallows et al., 2010; Lee et al., 2007; McMahon et al., 2009). Diabetes-induced renal hypertrophy correlates with diminished AMPK activity and, at the same time, with increased protein synthesis under high glucose conditions (Hallows et al., 2010). AICAR and metformin reduce protein synthesis triggered by high glucose (Lee et al., 2007), but these compounds also produce effects that are unrelated to AMPK activation (Mantovani & Roy, 2011). Thus, stimulating AMPK in the cytoplasm could provide a more focused approach to reduce damage in the diabetic kidney.

#### **4.3.2 AMPK targets associated with the plasma membrane and vesicular trafficking**

Several channels and transporters in the plasma membrane are phosphorylated by AMPK and control the secretion of insulin, hyperpolarization of  $\beta$ -cells under low glucose concentration and the response to hypoxia (Beall et al., 2010; Chang et al., 2009; Düfer et al., 2010; Evans et al., 2009; Hallows, 2005; Zheng et al., 2008). Although details of the molecular mechanisms are not always clear and in part controversial, altering the AMPK activity at the plasma membrane has the potential to modify  $\beta$ -cell function. The same principle could also apply to the heart and kidney, where several integral proteins of the plasma membrane are modified by AMPK.

#### **4.3.3 AMPK substrates in the nucleus**

In the nucleus, AMPK directly regulates the transcription of genes that control metabolism as well as the biogenesis of mitochondria and ribosomes. As such, AMPK modifies HNF4 $\alpha$ , HDAC5, p300, histone H2B, the tumor suppressor p53, PGC1 $\alpha$ , and TIF-1A (see Fig. 3 and Table 3). AMPK-dependent phosphorylation of these targets is critical to alter the transcriptional profile, which in turn is necessary to adjust metabolic activities in skeletal muscle, heart, liver and adipose tissues in response to changes in glucose availability. It is reasonable to assume that the modification of transcription factors and transcriptional regulators will rely to a large extent on AMPK in the nucleus. This model is supported by a recent study that shows AMPK to move along genes together with the transcriptional

machinery (Bungard et al., 2010). Activation of AMPK in the nucleus could enhance the effects of AMPK on the transcription of several target genes. One of the possible benefits of the activation of nuclear AMPK will be the increase in mitochondrial biogenesis. In the long-term, this could help to stimulate the oxidation of fatty acids and limit the lipotoxicity that is linked to type 2 diabetes (Schrauwen & Hesselink, 2004).

#### 4.3.4 AMPK targets associated with mitochondria

Acc2 is associated with mitochondria and important for the synthesis of malonyl-CoA, an intermediate of fatty acid biosynthesis. AMPK phosphorylates and thereby inactivates Acc, which leads to a reduction in malonyl-CoA concentration. As a consequence, *de novo* fatty acid synthesis is reduced and fatty acid oxidation is upregulated. It is conceivable that the localized Acc2 inhibition by AMPK could stimulate CPT-1 (carnitine palmitoyltransferase-1) dependent transport of fatty acids into mitochondria for subsequent degradation. This in turn could reduce the load of peroxidation products of fatty acids in the cytoplasm and the subsequent damage to mitochondria (Schrauwen & Hesselink, 2004).

### 5. Development of drugs that alter the compartment-specific activity of AMPK

In order to modulate AMPK activity in a fashion that is more localized as compared to the currently used drugs, a number of questions will have to be addressed. This includes the ability to regulate AMPK (a) in different organs or tissues and (b) in specific subcellular locations. Oral administration of metformin is believed to preferentially alter liver metabolism, whereas TZDs and their derivatives affect adipose tissue, skeletal muscle and probably  $\beta$ -cells (Gruzman et al., 2009). One possibility to enhance the tissue-specific action of AMPK will rely on the development of drugs that *directly* bind to AMPK; indeed such compounds have been described (Hawley et al., 2010). Since tissue-specific differences in AMPK subunits have been established, developing compounds that preferentially interact with individual subunits or specific subunit combinations of heterotrimers could provide a means to increase the specificity of AMPK action. For example, the  $\gamma 3$  subunit is predominantly synthesized in glycolytic skeletal muscle and could therefore serve as a target to alter AMPK in this tissue.

Taking into account differences in AMPK subunits could further be exploited to regulate the kinase in different subcellular locations (see section 4.2). Thus, it is believed that the  $\alpha 2$  subunit is more concentrated in the nucleus as compared to the  $\alpha 1$  subunit; this difference could help to activate mainly nuclear or cytoplasmic pools of the kinase. This line of reasoning could be expanded to the posttranslational modifications of the  $\beta$  subunit, as phosphorylation and myristylation of the  $\beta$  subunits are implicated in the subcellular distribution of the kinase.

In addition to taking advantage of the differences in AMPK heterotrimers, developing AMPK modulators that accumulate in distinct subcellular compartments will be useful. This strategy could be based on the generation of combimolecules with multiple properties (Rachid et al., 2007). Combimolecules that combine DNA-binding with AMPK activation could enhance the modification of nuclear substrates and thereby alter the gene expression profile. On the other hand, such combimolecules could exploit the differences in lipid composition of intracellular membranes to control the AMPK-dependent phosphorylation of mitochondrial Acc2 or channels residing in the plasma membrane.

## 6. Conclusions

AMPK and its substrates are critical to the etiology and pathology of type 2 diabetes and other metabolic diseases. Several of the current therapeutic regimens for type 2 diabetes alter AMPK activity, either by affecting the cellular energy status or the concentration of AMPK modulators. More recent studies led to the identification of compounds that directly bind AMPK and change its enzyme activity. We propose that the future design of drugs that takes into account the dynamic subcellular distribution of the kinase and its substrates will help to regulate AMPK not only in different tissues and organs, but also at the subcellular level. In the long term this approach will help to fine-tune AMPK action and the downstream events that rely on the phosphorylation of its targets.

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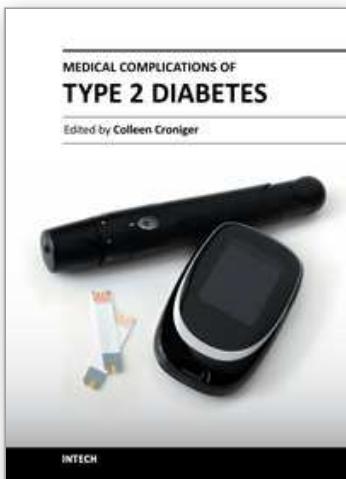
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## Medical Complications of Type 2 Diabetes

Edited by Dr. Colleen Croniger

ISBN 978-953-307-363-7

Hard cover, 412 pages

**Publisher** InTech

**Published online** 12, September, 2011

**Published in print edition** September, 2011

Obesity and type 2 diabetes are increasing worldwide problems. In this book we reviewed insulin secretion in both healthy individuals and in patients with type 2 diabetes. Because of the risk associated with progression from insulin resistance to diabetes and cardiovascular complications increases along a continuum, we included several chapters on the damage of endothelial cells in type 2 diabetes and genetic influences on endothelial cell dysfunction. Cardiovascular complications occur at a much lower glucose levels, thus a review on the oral glucose tolerance test compared to other methods was included. The medical conditions associated with type 2 diabetes such as pancreatic cancer, sarcopenia and sleep disordered breathing with diabetes were also discussed. The book concludes with several chapters on the treatments for this disease offering us hope in prevention and successful alleviation of the co-morbidities associated with obesity and type 2 diabetes.

### How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mohamed Kodiha and Ursula Stochaj (2011). Targeting AMPK for Therapeutic Intervention in Type 2 Diabetes, Medical Complications of Type 2 Diabetes, Dr. Colleen Croniger (Ed.), ISBN: 978-953-307-363-7, InTech, Available from: <http://www.intechopen.com/books/medical-complications-of-type-2-diabetes/targeting-ampk-for-therapeutic-intervention-in-type-2-diabetes>

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