

# AMPKs

## Key References

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## Overview

The AMP-activated protein kinase (AMPK) acts as a sensor of cellular energy status. AMPK exists as heterotrimeric complexes comprising a catalytic  $\alpha$  subunit and regulatory  $\beta$  and  $\gamma$  subunits. In mammals, each of these subunits are encoded by multiple genes and at least 12 possible combinations of subunit isoforms are possible. The  $\alpha$  subunits ( $\alpha$ 1,  $\alpha$ 2) contain the kinase domain at the N-terminus followed by a C-terminal region that is required for formation of the  $\alpha\beta\gamma$  complex. The  $\beta$  subunits ( $\beta$ 1,  $\beta$ 2) contain short, variable N-terminal regions followed by two more highly conserved regions. The first is now recognized to be a glycogen-binding domain (related to N-isoamylase domains that are found in enzymes that metabolize the  $\alpha$ 1 $\rightarrow$ 6 branches in  $\alpha$ 1 $\rightarrow$ 4 linked glucans such as glycogen) that causes AMPK to associate with glycogen particles inside the cell. The C-terminal conserved region is required for the formation of the  $\alpha\beta\gamma$  complex. The  $\gamma$  subunit isoforms ( $\gamma$ 1,  $\gamma$ 2,  $\gamma$ 3) contain variable N-terminal regions of unknown function, followed by four tandem repeats of a sequence termed a CBS motif. These motifs, which also occur in a small number of other proteins, act in pairs to form two domains that bind the regulatory nucleotides, AMP and ATP, in a mutually exclusive manner. Binding of AMP to the two sites is highly co-operative. Mutations within the AMP binding sites of the  $\gamma$ 2 and  $\gamma$ 3 isoforms cause glycogen storage disorders in cardiac muscle in humans and skeletal muscle in pigs, respectively.

The AMPK system is activated by cellular stresses that cause a drop in the cellular ATP:ADP ratio either by interfering with ATP synthesis (e.g. metabolic poisons, hypoxia, glucose starvation) or by increasing ATP consumption (e.g. contraction in muscle).

An increase in the cellular ADP:ATP ratio is amplified into a much larger increase in AMP:ATP by adenylate kinase. AMP binds to the two sites on the  $\gamma$  subunit (an effect antagonized by high ATP). This promotes phosphorylation within the  $\alpha$  subunit by the upstream kinase, which is essential for AMPK activity. The major form of the upstream kinase is a complex between the tumor suppressor LKB1, and two accessory subunits, STRAD and MO25. AMP binding also allosterically activates the phosphorylated AMPK complex. Dissociation of AMP both reverses the allosteric activation and also promotes dephosphorylation to switch the kinase off again.

Once activated, AMPK switches on catabolic processes that generate ATP, such as the uptake and oxidation of glucose and fatty acids. It also switches off processes that consume ATP that are not essential for the short-term survival of the cell. This includes the biosynthesis of fatty acids, cholesterol, glycogen and protein. AMPK switches off protein biosynthesis and cell growth in part by down-regulating the TOR (target-of-rapamycin) pathway. AMPK causes both short-term effects via direct phosphorylation of metabolic enzymes, and longer-term effects by modulating gene expression.

AMPK is now a prime target for drugs aimed at treatment of obesity and Type 2 diabetes. It can be activated in intact cells and *in vivo* using the nucleoside 5-aminoimidazole-4-carboxamide riboside (AICAR), which is taken up by cells and converted to the equivalent monophosphorylated nucleotide, ZMP, which mimics all of the effects of AMP. AMPK is also activated in intact cells and/or *in vivo* by two major classes of anti-diabetic drugs, i.e. the biguanides (metformin and phenformin) and

the thiazolidinediones (rosiglitazone and pioglitazone). These drugs appear to act indirectly on AMPK, possibly via inhibition of the respiratory chain, and it remains uncertain to what extent their therapeutic benefits are mediated by AMPK.

# AMPK $\alpha$ s

ISOFORMS	$\alpha$ 1	$\alpha$ 2
OTHER NAMES	—	—
MOLECULAR WEIGHT/ STRUCTURAL DATA	62.8 kDa 550 aa	62.3 kDa 552 aa
SPECIES	Human	Human
DOMAIN ORGANIZATION	Kinase domain, complex formation	Kinase domain, complex formation
PHOSPHORYLATION SITES	Thr <sup>172</sup> (by LKB1, CaMKKs), Thr <sup>258</sup> , Ser <sup>485</sup>	Thr <sup>172</sup> (by LKB1, CaMKKs), Thr <sup>258</sup> , Ser <sup>491</sup>
TISSUE DISTRIBUTION	Ubiquitous	Muscle, liver
SUBCELLULAR LOCALIZATION	Cytoplasmic	Nuclear, cytoplasmic
BINDING PARTNERS/ ASSOCIATED PROTEINS	$\beta$ and $\gamma$ subunits	$\beta$ and $\gamma$ subunits
UPSTREAM ACTIVATORS	LKB1/STRAD/MO25 Calmodulin-dependent protein kinases (CaMKKs)	LKB1/STRAD/MO25 Calmodulin-dependent protein kinases (CaMKKs)
DOWNSTREAM ACTIVATION	Not known	Not known
ACTIVATORS <sup>a</sup>	AICAR ( <b>A9978</b> ), metformin ( <b>D5035</b> ), rosiglitazone, pioglitazone ( <b>P4120</b> )	AICAR ( <b>A9978</b> ), metformin ( <b>D5035</b> ), rosiglitazone, pioglitazone ( <b>P4120</b> )
INHIBITORS <sup>b</sup>	Compound C <sup>2</sup>	Compound C <sup>2</sup>
SELECTIVE ACTIVATORS	Not known	Not known
PHYSIOLOGICAL FUNCTION	Catalytic	Catalytic
DISEASE RELEVANCE	Not known	Mouse KO: insulin resistant, glucose intolerant

## FOOTNOTES

<sup>a</sup> These activators only work in intact cells and require an intact  $\alpha\beta\gamma$  complex.

<sup>b</sup> Compound C<sup>2</sup> may inhibit the isolated kinase domain of the  $\alpha$  subunit but has only been tested on the intact  $\alpha\beta\gamma$  complex; see Zhou, et al., *J. Clin. Invest.*, **108**, 1167-1174 (2001).

## AMPK $\alpha$ s

ISOFORMS	$\beta$ 1	$\beta$ 2
OTHER NAMES	—	—
MOLECULAR WEIGHT/ STRUCTURAL DATA	30.1 kDa 269 aa Myristoylation	30.3 kDa 272 aa
SPECIES	Human	Human
DOMAIN ORGANIZATION	Glycogen binding, complex formation	Glycogen binding complex formation
PHOSPHORYLATION SITES	Ser <sup>24</sup> /Ser <sup>25</sup> , Ser <sup>96</sup> , Ser <sup>101</sup> , Ser <sup>108</sup> , Ser <sup>182</sup>	Not known
TISSUE DISTRIBUTION	Ubiquitous	Skeletal/cardiac muscle, others
SUBCELLULAR LOCALIZATION	Extranuclear, nuclear	Cytoplasm
BINDING PARTNERS/ ASSOCIATED PROTEINS	$\alpha$ and $\gamma$ subunits, glycogen	$\alpha$ and $\gamma$ subunits, glycogen
UPSTREAM ACTIVATORS	Not known	Not known
DOWNSTREAM ACTIVATION	Not known	Not known
ACTIVATORS <sup>a</sup>	Not known	Not known
INHIBITORS	Not known	Not known
SELECTIVE ACTIVATORS	Not known	Not known
PHYSIOLOGICAL FUNCTION	Glycogen-binding	Glycogen-binding
DISEASE RELEVANCE	Not known	Not known

## FOOTNOTES

# AMPK $\gamma$ s

ISOFORMS	$\gamma$ 1	$\gamma$ 2	$\gamma$ 3
OTHER NAMES	—	—	—
MOLECULAR WEIGHT/ STRUCTURAL DATA	37.6 kDa 331 aa	63.1 kDa 569 aa Myristoylation	51.5 kDa 464 aa
SPECIES	Human	Human	Human
DOMAIN ORGANIZATION	AMP/ATP-binding (two sites)	AMP/ATP-binding (two sites)	AMP/ATP-binding (two sites)
PHOSPHORYLATION SITES	Not known	Not known	Not known
TISSUE DISTRIBUTION	Ubiquitous	Skeletal/cardiac muscle, others	Skeletal muscle
SUBCELLULAR LOCALIZATION	Cytoplasm	Cytoplasm	Cytoplasm
BINDING PARTNERS/ ASSOCIATED PROTEINS	$\alpha$ and $\beta$ subunits	$\alpha$ and $\beta$ subunits	$\alpha$ and $\beta$ subunits
UPSTREAM ACTIVATORS	Not known	Not known	Not known
DOWNSTREAM ACTIVATION	Not known	Not known	Not known
ACTIVATORS	Not known	Not known	Not known
INHIBITORS	Not known	Not known	Not known
SELECTIVE ACTIVATORS	Not known	Not known	Not known
PHYSIOLOGICAL FUNCTION	AMP/ATP-binding	AMP/ATP-binding	AMP/ATP-binding
DISEASE RELEVANCE	Not known	Mutations: cardiac glycogen increased, cardiac arrhythmias	Mutations (pig): skeletal muscle glycogen increased

## FOOTNOTES