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AMP-activated Protein Kinase Regulation and Biological Actions in the Heart

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Abstract

AMP-activated protein kinase (AMPK) is a stress-activated kinase that functions as a cellular fuel gauge and master metabolic regulator. Recent investigation has elucidated novel molecular mechanisms of AMPK regulation and important biological actions of the AMPK pathway that are highly relevant to cardiovascular disease. Activation of the intrinsic AMPK pathway plays an important role in the myocardial response to ischemia, pressure overload and heart failure. Pharmacologic activation of AMPK shows promise as a therapeutic strategy in the treatment of heart disease. The purpose of this review is to assess how recent discoveries have extended and in some cases challenged existing paradigms, providing new insights into the regulation of AMPK, its diverse biological actions and therapeutic potential in the heart.

Keywords

Protein kinases; cardiac metabolism; myocardial ischemia; cardioprotection; heart failure

AMP-activated protein kinase (AMPK) has gained attention over the last decade as a "cellular fuel gauge"[1] and "super metabolic regulator".[2] The AMPK pathway orchestrates the cellular response to a variety of stresses in the heart, regulating metabolism, organelle function and cell growth. The discovery of AMPK stemmed from early biochemical studies showing that acetyl-CoA carboxylase (ACC) and HMG-CoA reductase activities were regulated by phosphorylation in the liver. ACC synthesizes malonyl-CoA and is an important regulator of fatty acid synthesis, while HMG-CoA reductase is a key step in cholesterol biosynthesis. ACC phosphorylation was found to be modulated by the concentration of adenine nucleotides.[3] The discovery that the protein kinases for ACC and HMG-CoA co-purified and were regulated by AMP led to the first recognition of AMPK.[4] These early discoveries set the stage for the cloning and molecular characterization of AMPK and then early pioneering studies of AMPK in the heart.[5-7]

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Recent investigation has elucidated novel biological actions of AMPK that are highly relevant to cardiovascular disease. With the advent of new pharmacologic approaches to activate AMPK in a specific manner, the possibility of targeting this pathway for the treatment of human disease is emerging. The purpose of this review is to assess how recent investigation has extended and in some cases challenged existing paradigms, providing new insights into the regulation of AMPK, its biological actions and therapeutic potential in the heart.

Molecular structure and physiology of AMPK

The molecular structure and mechanisms regulating AMPK activation are important to understanding its physiologic function and to the development of innovative strategies to activate the kinase. AMPK is a heterotrimeric complex consisting of a catalytic alpha (α) subunit and regulatory gamma (γ) and beta (β) subunits (Figure 1). The α-subunit contains the AMPK serine-threonine kinase domain, which has a typical activating residue within the catalytic cleft (Thr^{172}). Phosphorylation of this amino acid by upstream kinases is essential for AMPK activity and its phosphorylation status is often used as an indicator of the activation state of the kinase.[8] The α-subunit also includes an auto-inhibitory domain (AID) (residues 313-335) and conformational changes induced by AMP binding to the γ-subunit, relieve auto-inhibition of the complex.[9, 10] An "α hook" region (residues 360-394) interacts with the γ-subunit nucleotide binding site when AMP is bound, leading to a change in the configuration of the complex, which promotes activation of the kinase domain.[11]

The β-subunits were initially thought to function as a bridge between the *α*-catalytic and the γ- regulatory subunits. However, β-subunits also contain a functional glycogen-binding domain (GBD) and glycogen appears to regulate the activity of the kinase.[12] The GBD binds best to glycogen with a single glucose *α*1-6 branch,[13] which inhibits AMPK activation by upstream kinases.[14]

The γ-subunits contain four potential nucleotide-binding sites termed CBS1-4,[15] due to their structural homology to binding domains in cystathionine beta-synthetase (CBS). They are also more simply referred to as sites 1-4.[16] Recent crystallographic studies show that only sites 1, 3, and 4 bind nucleotides in the mammalian enzyme. Site 1 is a high affinity site, which mediates the allosteric activation of the complex and binds only AMP and ATP.[17] Site 3 is a lower affinity site that binds AMP, ADP and ATP and influences the phosphorylation state of Thr^{172} by upstream kinases and phosphatases. Site 4 binds AMP tightly in a non-exchangeable manner.[17]

Molecular mechanisms of AMPK activation

The activity of AMPK is primarily determined by the cellular energy state, which is reflected in the ratio of AMP (and ADP) to ATP. Cellular stress leads to the breakdown of ATP to ADP and the subsequent production of AMP through the action of adenylate kinase (2 ADP → AMP + ATP). To a lesser extent AMP is also generated by the cleavage of pyrophosphate from ATP and via *de novo* purine biosynthesis. AMP content is also determined in part by its degradation by AMP-deaminase and 5′-nucleotidase and inhibition of 5′-nucleotidase is emerging as a novel strategy to activate AMPK.[18]

Cellular AMP is highly protein bound and the concentration of free AMP in the heart is normally in the low μM range.[19] ATP is present in much higher concentrations than AMP, but the predominant form of cellular ATP, Mg^{++}ATP, has a lower binding affinity for AMPK than AMP or ADP, enabling the latter nucleotides to competitively bind to the complex.[17]

According to the conventional paradigm, AMP is the primary activator of AMPK, but recent studies have challenged this concept. ADP is present in higher concentrations than AMP and under mild stress conditions appears to be a more important regulator of site 3, which induces a conformational change that modulates phosphorylation of the Thr^{172} site by upstream protein kinases and phosphatases.[11] Under more intensive stress, AMP levels rise and allosterically activate the complex through binding to site 1. This previously under-appreciated role of ADP led to brief discussions about renaming the kinase, “ADP-activated protein kinase”, but AMP is the primary allosteric regulator of the complex and the original terminology has managed to prevail.

AMPK phosphorylation and other post-translational modifications

Phosphorylation of the Thr^{172} site markedly increases the activity of AMPK and is determined by the balance of action of upstream kinases and protein phosphatases. In the heart, the liver kinase B1 (LKB1) is the major upstream AMPK kinase. Each of the AMPK subunits contains two or more isoforms and phosphorylation of the complexes containing the predominant α2 isoform is entirely dependent on LKB1 during ischemia.[20] In contrast, the kinase responsible for phosphorylating α1 containing AMPK complexes in the heart has yet to be identified.[20]

The calcium-calmodulin activated protein kinase kinase β (CaMKKβ) is a potential alternative upstream kinase that has an important role to activate AMPK in the brain and other non-cardiac cells.[21] However, CaMKKβ is expressed in much lower amounts in cardiomyocytes and its role in the heart is still not well understood. Transforming growth factor-β–activated protein kinase-1 (TAK1) is known to phosphorylate SNF1, the yeast homolog of the mammalian AMPK α-subunit.[22] Although TAK1 is present in the heart and is activated during ischemia, it appears to modulate LKB1 activity rather than directly phosphorylate AMPK.[23]

Protein phosphatases also have a critical role in regulating Thr^{172} phosphorylation. Both protein phosphatase 2A (PP2A) and PP2C dephosphorylate AMPK in cell free *in vitro* assays.[24] AMP binding to the γ subunit inhibits the action of PP2C to dephosphorylate Thr^{172} *in vitro*.[25] Alterations in protein phosphatase expression modulate AMPK activation in the heart, for example, increased PP2C expression decreases AMPK activity in the rodent cardiac lipotoxicity model.[26] PP2A also dephosphorylates AMPK, and elevated serum fatty acids stimulate PP2A activity and decrease AMPK phosphorylation in endothelial cells.[27] However, at the current time, there is limited understanding of the specific phosphatases, let alone which of their isoforms are physiologically responsible for dephosphorylating Thr^{172} and maintaining the low basal activity of AMPK in the normal heart.

AMPK activation is also modulated by phosphorylation at other sites. The α1 subunits are phosphorylated on Ser^{173}, Ser^{485} and Ser^{497}, while the β1 subunits are phosphorylated on Ser^{24}.[28] Phosphorylation of Ser^{173} by protein kinase A blunts the phosphorylation of Thr^{172} by upstream AMPK kinases.[28] Ser^{485} in α1 and the corresponding Ser^{491} in α2 subunits are phosphorylated by both Akt and protein kinase A, inhibiting activation at the Thr^{172} site.[29] Phosphorylation of Ser^{485} or Ser^{491} is responsible for the effect of high insulin concentrations or constitutively active Akt to blunt AMPK activation in the heart.[30]

In addition to phosphorylation, recent evidence indicates that AMPK undergoes post-translational acetylation of its α subunits. The acetylation state of AMPK is determined by the reciprocal actions of the acetylase p300 and the histone deacetylase 1 (HDAC1).[31] Deacetylation of AMPK promotes its interaction with upstream LKB1, which phosphorylates AMPK and stimulates its activation.[31] Interestingly, LKB1 itself is also regulated by acetylation, and the deacetylated form of LKB1 more readily leaves the nucleus and binds to its partner STRAD in the cytoplasm, forming the active LKB1 complex.[32] These findings indicate that acetylation is a potentially important determinant of the activity of the LKB1-AMPK pathway, although there is no information on the extent to which this mechanism is operative in the heart.

AMPK subcellular localization

Subcellular localization is an important determinant of cell signaling events and targeting of protein kinases to subcellular domains provides selectivity to specific substrates. Although AMPK has traditionally been considered to be a cytosolic enzyme, evidence is emerging that it may also be targeted to the nucleus and specific membrane domains.

AMPK has well-established functions in the nucleus where it phosphorylates transcription factors, histone proteins and histone deacetylase enzymes.[33] The α2, but not the α1 isoform, of the AMPK catalytic subunit has a nuclear localization signal,[34] although both α isoforms contain a nuclear export sequence.[35] Nuclear translocation of AMPK requires phosphorylation at the Thr^{172} site,[34] indicating that only activated AMPK goes to the nucleus. Exercise causes AMPK activation and nuclear translocation of AMPK complexes containing α2 subunits in skeletal muscle,[36] but there are no published data yet demonstrating cardiac AMPK translocation to the nucleus.

Localization of AMPK to specific membrane domains is potentially another important determinant of its action. Myristoylation of AMPK at the Gly^{2} site of its β-subunits serves to localize the AMPK complex to membranes.[37] Interestingly, myristoylation also increases the ability of AMP to allosterically activate AMPK and to promote phosphorylation of the Thr^{172} site by upstream kinases,[37] suggesting possible preferential activation of AMPK at membrane sites. Additional mechanisms may indirectly localize AMPK to membrane domains. For instance, AMPK binds to LKB1, which co-localizes with E-cadherin in adherens junctions in polarized epithelial cells.[38] The targeting of AMPK to specific membrane domains is difficult to study in cardiomyocytes, but could be an important determinant of its biological activity to regulate ion channels, membrane associated signaling proteins, cell polarity and cell junction formation in the heart.

AMPK signaling may also be localized to specific cytosolic domains through interacting with scaffold proteins. Scaffold proteins insulate kinase pathways from surrounding signaling cascades or alternatively promote the interaction of various signaling pathways. Interestingly, AMPK interacts with the TAK1–binding protein 1 (TAB1),[39] a scaffold protein better known for its role in mediating TAK-1 activation and p38 MAPK autophosphorylation. The interaction with TAB-1 appears to be functionally important in the heart, where impaired AMPK activity decreases p38 MAPK recruitment to TAB1 and blunts p38 activation during ischemia.[39]

AMPK expression and turnover

Although previous attention has focused primarily on the molecular mechanisms responsible for the acute activation of AMPK complexes, the expression and turnover of AMPK, upstream LKB1 and their component subunits are also regulated. In the heart, AMPK activity increases soon after birth, contributing to the metabolic switch from carbohydrate to fatty acid metabolism.[40] The total expression of AMPK does not appear to change significantly at birth in the mouse,[41] but there is a developmental shift in the expression pattern of specific AMPK subunit isoforms in the heart.[41, 42] There are 3 genes (PRKAG1, PRKAG2 and PRKAG3) encoding the γ subunit isoforms (γ1, γ2, and γ3).[43] Transcript levels of the γ1 isoform increase while γ3 content decreases during the development of the embryonic mouse heart.[42] The physiologic significance of these expression patterns is uncertain and prior studies have demonstrated that AMPK activity in the adult heart is primarily determined by complexes containing the ubiquitously expressed γ1 isoform.[43]

The γ2 subunit expression pattern is particularly important in the heart because mutations in the human PRKAG2 gene cause a cardiomyopathy, as subsequently discussed. The PRKAG2 gene encoding the γ2 isoform has 4 alternative transcripts: γ2a (long form), γ2b (short form), γ2c, and a recently discovered γ2-3B (the product of a variant transcript that starts in exon 3B).[42] Of the γ2 isoforms, γ2-3B and γ2b (short form) predominate in both human and mouse hearts and their expression increases during development.[42] Interestingly, the expression of γ2-3B is relatively restricted to heart, however, it is not known whether it has distinctive physiologic functions.

AMPK expression is also altered during pressure overload and after the development of heart failure. Aortic banding leads to upregulation of α2, β2, and γ2 isoforms in the rodent heart.[41, 44] In contrast, hearts explanted from patients with ischemic or non-ischemic cardiomyopathy, demonstrate increased expression of the α1, β1 and γ2c isoforms.[41] These differential isoform responses are of interest, although their functional significance is not understood; whether they represent species differences or distinct responses to various types of heart failure is also unknown.

The molecular mechanisms regulating AMPK subunit expression have not been defined. However, recent studies suggest that miRNAs may play a role in regulating the upstream LKB1 complex and thus would modulate AMPK pathway activation. Specifically, miR-451 regulates the expression of the LKB1 binding partner MO25 α, a protein that is required for LKB1 activity, in glioma cells.[45] Further study is needed to determine whether the extent to

which the expression of AMPK or LKB1 in the heart is regulated by miRNAs, transcriptional regulators or both.

Protein degradation is also emerging as an important determinant of AMPK subunit levels. Free AMPK subunits are relatively labile when not incorporated into the heterotrimeric AMPK complex. For instance, when inactivated or "kinase-dead" α catalytic subunits are expressed in the hearts of transgenic mice, they compete with native α subunits for incorporation into the AMPK heterotrimeric complex and the remaining free α subunits are degraded.[46] Recent studies show that AMPK subunits are degraded by ubiquitin-dependent proteosome activity, based on findings that cell death-inducing DFF45-like effector A (cidea) knockout mice have high levels of α, β and γ subunits in adipose tissue.[47] Whether a ubiquitin-dependent proteasome mechanism of AMPK degradation is operative in the heart is not yet known.

Stimuli activating AMPK

Heart AMPK activity is increased by a wide array of stimuli, including pathologic and physiologic stress, hormones and cytokines, and drugs (Figure 2). One of the best-studied pathologic stimuli for AMPK activation in the heart is ischemia.[48] AMPK is activated by both severe no flow ischemia[48] and partial ischemia[46] in isolated perfused rodent hearts, as well as during the regional ischemia that accompanies coronary ligation *in vivo*.[49, 50] AMPK activation is rapid and generally sustained during ischemia.[51]

Oxidative stress also induces AMPK activation. Hydrogen peroxide activates AMPK in isolated cardiomyocytes,[52] although the mechanisms involved remain uncertain. In endothelial cells, oxidative stress triggers peroxynitrite formation[53], which is reported to increase AMPK activation through a PKC zeta/LKB1- dependent mechanism.[54] In non-excitable cells, an additional mechanism mediating AMPK activation by oxidative stress involves the activation of calcium-release activated calcium channels with subsequent calcium-induced activation of CaMKK β and downstream AMPK.[55] In contrast, there is also evidence that oxidative stress might sometimes inactivate the AMPK pathway in the intact heart. In the spontaneously hypertensive rat model, LKB1 is inactivated via formation of lipid peroxidation 4-hydroxy-2-nonenal (HNE)-conjugates, leading to decreased activation of AMPK.[56] Thus, oxidative stress might have variable effects on the AMPK pathway, depending on the degree and duration of stress and the specific stimulus inducing oxidative stress.

Pressure overload stress increases AMPK subunit expression, but also the activation state of both α1 and α2 complexes in the heart.[44, 57, 58] The metabolic consequences of AMPK activation include increased rates of cardiac glucose transport[44] and glycolysis.[59] Increased expression of several metabolic genes during pressure overload is also AMPK-dependent.[60] However, as previously noted, not all forms of LV pressure overload activate the AMPK pathway *in vivo*[56] and it is also noteworthy that agonists, which induce hypertrophy in neonatal cardiomyocytes, do not increase AMPK activity.[61]

The primary physiological stress that activates AMPK is exercise, in both skeletal muscle[62] and the heart.[63, 64] The activation of heart AMPK during exercise is associated with

stimulation of downstream GLUT4 translocation and ACC phosphorylation,[63] mechanisms that promote the metabolism of glucose and fatty acids, respectively. The degree of activation of $\alpha 2$ containing complexes appears greater than that of $\alpha 1$ complexes,[63] consistent with the reported higher sensitivity of $\alpha 2$ complexes to changes in AMP concentration *in vitro*.[65] However, the precise mechanisms responsible for AMPK activation during exercise are not well-defined.[63] It is interesting that increased afterload does not appear to activate AMPK in the perfused rat heart,[66] raising the possibility that additional factors other than simply cardiac work are required for AMPK activation during exercise.

Additional diverse pathologic stimuli are known to activate AMPK in non-cardiac cells, including hypoglycemia, osmotic stress, heat shock and UV irradiation (Figure 2). Whether these mechanisms are operative or important in the intact heart is less certain. Although nutrient deprivation classically activates AMPK, it is interesting that high concentrations of palmitate or oleate appear to activate AMPK in isolated perfused hearts.[67] These findings do not conform with the notion of AMPK as a “fuel gauge”, and have been construed as a feed-forward mechanism to facilitate fatty acid oxidation. One alternative explanation is that high fatty acid concentrations might induce oxidative stress leading to AMPK activation in the heart.

Hormones and cytokines in the activation of AMPK

The traditional paradigm of AMPK as a energy stress-activated kinase has expanded to include what might be considered an outer shell of regulation by endocrine, paracrine and autocrine mechanisms (Figure 2). A diverse array of hormones and cytokines modulate AMPK activity in non-cardiac cells, including adiponectin,[68] leptin,[69] resistin,[70] ghrelin,[71] interleukin-6[72] and ciliary neurotrophic factor.[73] The specific mechanisms mediating these effects on AMPK are largely unknown.

In the heart, adiponectin is perhaps the best-studied AMPK-activating hormone. AMPK contributes to adiponectin's cardioprotective effects during ischemia[74] and inhibitory effects on cardiac hypertrophy after aortic banding.[68] The canonical AdipoR1 and R2 receptors that typically mediate adiponectin action are expressed in the heart,[75] but interesting recent findings indicate that the glycoprotein T-cadherin might be the critical receptor for adiponectin binding and AMPK activation in cardiomyocytes.[76]

The anti-obesity hormone leptin also modulates AMPK activity in a variety of tissues, increasing fatty acid oxidation in skeletal muscle[69], while also interestingly inhibiting AMPK in the hypothalamus which decreases food intake.[77] Leptin also appears to demonstrate AMPK-dependent actions in the heart. Cardiac specific leptin receptor knock-out mice have impaired activation of AMPK after myocardial infarction, which is associated with impaired glycolytic metabolism, increased apoptosis and adverse LV remodeling.[78] In addition, ischemic post-conditioning and AMPK activation are impaired in hearts of leptin deficient ob/ob mice subjected to ischemia/reperfusion,[79] indicating a modulating effect of leptin on AMPK activation in the heart.

Pro-inflammatory cytokines also modulate AMPK activation. In the heart, short-term IL-6 infusions appear to reduce AMPK α subunit content and activation.[80] Mice fed a high fat

diet have elevated plasma levels of IL-6, which may contribute to the downregulation of AMPK seen in this model.[80] In contrast, interleukin-6 (IL-6) has a specific autocrine-paracrine effect in skeletal muscle to augment AMPK activation,[72] while intravenous infusion of IL-6 has little effect on muscle AMPK activity.[81]

Macrophage migration inhibitory factor (MIF) is a master-regulator of inflammatory cytokines, and curiously is highly expressed in cardiomyocytes. Hypoxia increases cardiac MIF expression, via a hypoxia-inducible factor-1 α dependent mechanism,[82] and ischemia triggers MIF secretion.[83] Endogenous cardiac MIF has an important autocrine-paracrine action to modulate AMPK activation during ischemia and hypoxia in the heart.[83] Extracellular MIF activates AMPK via its cell surface receptor CD74 with subsequent activation of the signal transducer CD44.[83] MIF knockout mice have impaired heart AMPK activation and are more susceptible to ischemic injury.[83] These observations have potential clinical relevance based on the observation that a common polymorphism in the *MIF* gene promoter leads to diminished MIF release and AMPK activation in response to hypoxia in human cells.[83] In addition, heart MIF expression is decreased with aging and appears to be responsible for diminished AMPK activation during ischemia in old mice.[84] These findings raise the possibility that older patients or those with the low expression *MIF* gene promoter polymorphism may be at increased risk for ischemic injury and might be more likely to benefit from therapeutics targeting AMPK activation.

Pharmacologic AMPK activators

A number of pharmacologic compounds have been identified that activate AMPK (Figure 2). The first one recognized was the nucleoside 5-aminoimidazole-4-carboxyamide-1-beta-D-ribofuranoside (AICAR), which is taken up by cells and phosphorylated to the AMP-mimetic “ZMP”. AICAR increases AMPK activity in several tissues including the heart.[85] However, AICAR is also an adenosine-regulating agent that was developed as “Acadesine” to protect the heart during cardiac surgery, [86] although it is unclear whether the doses administered were adequate to activate AMPK in the human heart. Although AICAR is used extensively for experimental studies, its complex pharmacology leads to variable degrees of AMPK activation and its off target effects render it less than ideal as a drug for clinical studies.

Metformin activates AMPK in various cell types, including cardiomyocytes.[87] Metformin inhibits complex 1 of the mitochondrial electron transport chain, which leads to an increase in the AMP to ATP ratio.[88] Recent data suggest that metformin might have an additional effect to inhibit AMP deaminase activity,[89] which would increase intracellular AMP concentrations and activate AMPK indirectly. Metformin also activates heart AMPK when administered to mice, and has a potent cardio-protective action in the post-ischemic mouse heart.[90] However, metformin is now recognized to have important AMPK-independent effects, such as its well-known action to suppress hepatic glucose production in type 2 diabetes.[91] A second important class of diabetes drugs, the thiazolidinediones, also activate AMPK in the heart.[26] However, it is not clear that they activate AMPK in the human heart at clinically prescribed doses and caution is warranted in terms of attributing their clinical cardiovascular effects to AMPK activation.

The lack of specificity of existing drugs has spurred an interest in the development of more direct and specific AMPK activators. Initial screens yielded compounds that interact directly to activate the AMPK complex. The thienopyridone compound A769662 interacts with β1 subunits and activates AMPK through allosteric mechanisms and by increasing phosphorylation of the Thr^{172} site.[92] In the heart, A769662 preconditions the heart through an AMPK-dependent mechanism,[50] as discussed below. Recently, the compound PT1 was shown to activate AMPK through interaction with the α1 subunit at the Glu^{96} and Lys^{156} sites that are adjacent to the auto-inhibitory domain.[93] PT1 promotes phosphorylation of Thr^{172} and activates AMPK in an AMP-independent fashion in L6 myotubes.[93] Although both of these novel compounds have relatively low potency, they serve as prototypes for the development of additional agents.

Two commonly used non-diabetes drugs have also been reported to activate AMPK. Statins activate AMPK in endothelial cells and AMPK appears to mediate their effect to promote nitric oxide production and angiogenesis.[94] The administration of atorvastatin to mice stimulates AMPK activation in both in the aorta and myocardium, although at doses that far exceed those prescribed clinically.[94] A recent study demonstrates that salicylate also activates AMPK in non-cardiac cells, through binding to the same β subunit site that interacts with A769662.[95] The salicylate concentration required to activate AMPK was in upper range of levels anticipated with high dose aspirin treatment. Although potentially relevant to high dose aspirin therapy in patients with inflammatory disease, these results have limited relevance to the traditionally low doses of aspirin prescribed to patients with cardiovascular disease.

AMPK activation by drugs, hormones and cytokines can involve subtle increases in the cellular ratio of AMP to ATP that are difficult to detect. AMP is present in low concentration and direct measures of the biologically active non-protein bound free AMP concentration are not feasible in intact cells. One innovative approach to determining whether drugs or hormones activate AMPK through alterations in the cellular AMP content, is by testing them in cell lines that express AMPK complexes containing mutant isoforms that are insensitive to AMP.[96] This approach shows AMP-dependence for many compounds, but A769662 and salicylate activate AMPK in an AMP-independent fashion.[96]

Additional strategies are under investigation to activate AMPK by decreasing AMP degradation. Knockdown of the AMP-degrading enzyme 5′-nucleotidase increases the AMP/ATP ratio, enhancing AMPK phosphorylation and the downstream activation of ACC and glucose transport in both myotubes and mouse skeletal muscle.[18] Although these results are of interest, the efficacy of inhibiting 5′-nucleotidase in the heart is not yet established and the potential sequelae of altering nucleotide metabolism warrant further consideration.

AMPK inhibitors

Pharmacologic inhibitors are useful reagents to probe the function of protein kinases in both cells and intact animals. Unfortunately, like many kinase inhibitors, the existing AMP inhibitors lack specificity. The most widely used inhibitor, compound C, inhibits AMPK *in vitro* with an IC_{50} of 0.1-0.2 μM.[97] However, even at a concentration of 1 μM, which is

lower than typically used for most cell based experiments, compound C inhibits multiple other protein kinases, such as extracellular signal-regulated kinase 8, Src kinase and MAP kinase-interacting serine/threonine-protein kinase 1.[97] This lack of specificity makes it highly desirable to use more specific genetic approaches to inactivate the AMPK pathway rather than to rely primarily on pharmacologic results.

Metabolic pathway regulation by AMPK

AMPK activation coordinates the regulation of several steps in substrate transport and metabolism, which are critical for energy generation and conservation under times of stress (Figure 3). The metabolic actions of AMPK were instrumental in focusing scientific interest in understanding the AMPK pathway in the heart.[5-7] The earliest work on AMPK focused on its role in modulating cardiac fatty acid oxidation.[5, 48] AMPK phosphorylation of ACC2 inhibits its activity and the synthesis of malonyl-CoA, a potent inhibitor of carnitine palmitoyltransferase-1 (CPT-1). The decrease in malonyl-CoA levels, relieves the inhibition of CPT-1, which transports fatty acyl-CoA groups into the mitochondrial matrix and is the rate-limiting step for heart fatty acid oxidation.[5]

AMPK is rapidly activated by ischemia, but it has no discernible effect to increase fatty acid oxidation during ischemia because of the absence of oxygen.[46] However, AMPK activation persists for variable periods of time during reperfusion and plays a role in re-activating fatty acid oxidation early in the post-ischemic heart.[5, 46] AMPK also stimulates the myocardial uptake of fatty acids by increasing the translocation of the fatty acid transporter CD36 from intracellular storage membranes to the sarcolemma.[98] In addition, AMPK activates lipoprotein lipase, which facilitates fatty acid uptake from triglyceride-containing lipoprotein particles in the heart.[99] Thus, activated AMPK has several physiological effects that act in concert to promote fatty acid oxidation.

AMPK also stimulates glucose transport and glycolysis, which are particularly important to the metabolic adaptation of the ischemic heart. Glucose uptake is mediated by glucose transporter (GLUT) 4, and to a lesser extent by GLUT1 in the heart,[100] and AMPK increases GLUT4 translocation to the sarcolemma.[85] Neither GLUT4, nor GLUT4 containing vesicles, are directly phosphorylated by AMPK. Rather, AMPK phosphorylates Rab GTPase-activating proteins (GAP) that regulate Rab10, which modulates docking and fusion of GLUT4 vesicles with the plasma membrane. The Akt substrate 160 protein is a GAP expressed in heart and regulates the GTP form of Rab10.[101] In addition, AMPK may also inhibit endocytosis of GLUT4 in cardiomyocytes, increasing the sarcolemma GLUT4 content and glucose transport.[102]

AMPK also acts downstream to glucose transport, by indirectly increasing the activity of phosphofructokinase (PFK)-1, the rate-limiting enzyme in glycolysis. Activated AMPK directly phosphorylates and stimulates PFK-2 to synthesize fructose 2,6-bisphosphate, which in turn allosterically activates PFK-1.[7] These increases in myocardial glucose transport and glycolysis are important components of the metabolic response to ischemia or hypoxia.[103]

AMPK regulation of transcription

In addition to the well-established acute metabolic effects of AMPK, AMPK also translocates into the nucleus where it has more prolonged effects on cellular metabolism by regulating gene transcription.[60] In the heart, AMPK regulates genes related to mitochondrial energy metabolism, including medium chain acyl-CoA dehydrogenase (MCAD), CPT-1, cytochrome C, and uncoupling protein (UCP)-3.[60] These transcriptional effects are mediated in part by activation of the estrogen-related receptor alpha (ERRα) transcription factor.[60]

AMPK also appears to regulate peroxisome proliferator activated receptor gamma co-activator (PGC)-1α, a critical modulator of cardiac gene expression and mitochondrial biogenesis. AMPK activation increases the expression of PGC-1α in hypoxic cardiomyocytes.[104] More detailed mechanisms linking AMPK to PGC-1α and mitochondrial biogenesis have been delineated in skeletal muscle. AMPK increases muscle NAD^+ concentrations and the activity of the NAD^+-dependent deacetylase sirtuin-1 (SIRT1) during fasting and exercise, and the subsequent deacetylation of PGC-1α promotes its activity.[105, 106] How AMPK increases cellular NAD^+ concentration is less certain, but may result in part from increased expression of the NAD^+ biosynthetic enzyme nicotinamide phosphoribosyltransferase (NAMPT).[107] AMPK also directly phosphorylates and activates PGC-1α at Thr^{177} and Ser^{538}.[108] In addition, chronic activation of AMPK also enhances the binding activity of nuclear regulatory factor-1 (NRF-1) and mitochondrial biogenesis in skeletal muscle.[109] The extent to which these mechanisms function in the heart is unclear, but would be of interest to better understand mitochondrial biogenesis during growth and cardiac hypertrophy.

AMPK also modulates gene expression through novel mechanisms in non-cardiac cells. AMPK phosphorylates the histone deacetylase (HDAC)5 transcriptional repressor at both the Ser^{259} and Ser^{498} sites, which promotes GLUT4 transcription in myotubes.[33] Activated AMPK acts as a direct histone kinase, phosphorylating histone 2B on Ser^{36} and regulating gene transcription[110] AMPK also modulates the stability of mRNA transcripts by regulating the RNA-binding protein HuR egress to the cytoplasm.[111] Further studies will be needed to determine whether these mechanisms are operative in cardiomyocytes.

AMPK regulation of protein synthesis

AMPK has important actions to inhibit cellular protein synthesis, in line with its role to conserve energy by inhibiting energy-consuming biosynthetic pathways (Figure 4). Under conditions of potentially lethal energy deprivation, there is little need to consume ATP to synthesize long half-life proteins. Amongst the best-characterized targets of AMPK is the eukaryotic elongation factor-2 (eEF2) kinase, which AMPK phosphorylates at Ser^{398}, leading to the phosphorylation and inhibition of downstream eEF2.[112] This action may be functionally important in the heart to conserve energy during ischemia[50] and to prevent excess hypertrophy during pressure overload.[58]

Activated AMPK also inhibits the mammalian target of rapamycin (mTOR) pathway. First, AMPK activates the tuberous sclerosis complex (TSC): phosphorylation of TSC2 at Thr^{1227} and Ser^{1345} leads to an increase in the TSC1-TSC2 complex “Ras homologue enriched in

brain" (Rheb)-GTPase activity and to the downstream inhibition of the mTOR complex 1 (mTORC1).[113] In addition, AMPK has a TSC2-independent action to inhibit mTORC1 activity by phosphorylating raptor at Ser^{722} and Ser^{792}, which induces 14-3-3 binding and dissociation of raptor from mTOR.[114] With the caveat that these molecular mechanisms have been elucidated in non-cardiac cells, AMPK appears to be a potent inhibitor of the mTOR pathway and this action could have relevance to modulating cardiac hypertrophy in the setting of LV pressure overload and heart failure.

AMPK regulation of protein degradation and autophagy

AMPK also modulates protein degradation and the turnover of intracellular organelles. AMPK activates the ubiquitin proteasome system and protein degradation in both striated muscle cells[115] and the heart.[116] In the heart, AMPK increases the expression of the ubiquitin ligases atrogin-1 and the muscle RING finger protein 1 (MuRF1), the latter through activating MEF2.[116] Whether MuRF1 upregulation requires PGC-1α activation and how AMPK activates MEF2 in cardiomyocytes is uncertain. It would also appear that there is a complex interplay between AMPK and MEF2, and that activated AMPK counters the effect of MEF2 to promote cardiac hypertrophy.[117]

In keeping with its function to provide energy during nutritional deprivation, AMPK also promotes autophagy, providing important nutrients from the breakdown of macromolecules and organelles.[118] In the ischemic heart, AMPK activation induces autophagy in cardiomyocytes, contributing to their survival.[119] In a diabetic mouse heart failure model, autophagy is impaired and metformin treatment enhances autophagic activity leading to the preservation of cardiac function through an AMPK-dependent mechanism.[120]

The molecular mechanisms responsible for AMPK's stimulation of autophagy are now starting to be unraveled in non-cardiac cells (Figure 5). AMPK inactivates mTORC1, which normally suppresses autophagy when the nutrient supply is adequate. Intriguing recent evidence also indicates that AMPK directly phosphorylates the Unc-51-like kinase (ULK)-1, the mammalian homolog of the yeast kinase autophagy-related 1 (Atg1), which has a critical role in the induction of autophagy.[121, 122] AMPK is reported to phosphorylate ULK1 on various sites, including Ser^{467}, Ser^{555}, Thr^{574}, Ser^{637}, Ser^{317} and Ser^{777}.[121, 122] Interestingly, loss of function of either AMPK or ULK1 results in defective mitophagy.[121] AMPK appears to bind in a complex that includes ULK-1 and mAtg101, and this interaction is nutritionally–dependent and is inhibited by mTOR phosphorylation of ULK-1 on Ser^{757}.[122] Thus, there is a complicated interplay between AMPK, mTORC1 and ULK-1 in regulating autophagy and the specific molecular mechanisms through which AMPK regulates autophagy during ischemia, hypertrophy and heart failure require additional investigation.

AMPK action during myocardial ischemia-reperfusion

AMPK is activated during ischemia[5] and most evidence indicates that intrinsic AMPK activation protects the heart against injury during ischemia-reperfusion.[46, 90, 123, 124] Genetic inactivation of heart AMPK by the transgenic expression of a K45R $\alpha 2$ subunit impairs ischemic glucose uptake and ATP homeostasis, and results in poor recovery of LV

contractile function, increased cardiac necrosis and greater apoptosis after ischemia-reperfusion.[46] Similarly, hearts from mice expressing the KD D157A α2 subunit have reduced glucose uptake and greater ATP depletion during ischemia[123] and increased necrosis after coronary occlusion-reperfusion *in vivo*.[90] Hearts from global AMPK α2 knockout mice have reduced glycolysis, greater ATP depletion, more rapid development of contracture during ischemia, as well as impaired metabolism and contractile function after ischemia.[125, 126] However, not all studies have documented a protective function of the AMPK pathway during ischemia.[127]

AMPK inactivation models provide insight into the intrinsic role of the AMPK pathway in the response to ischemia. However, from the clinical perspective, the more important issue is whether pharmacologic AMPK activation represents a potential therapeutic strategy to reduce ischemic injury and evidence to date supports this possibility. Metformin activates AMPK and protects against regional ischemia when administered *in vivo* in both diabetic and normal mice, before or after ischemia.[90] The compound A769662, which directly activates AMPK, protects against ischemic injury during global ischemia *in vitro* and during regional ischemia *in vivo*.[50] A769662 treatment also enhances ischemic AMPK activation and reduces infarct size in diabetic rat hearts.[128] Although pharmacologic agents often have off-target effects, the benefit of metformin and A769662 appears to be specifically mediated by AMPK activation, based on the observation that neither drug has a cardio-protective effect in AMPK-inactivated mouse models.[50, 90]

AMPK activation has pleiotropic effects in the heart and which mechanisms are essential to the cardio-protective actions of AMPK are still poorly understood. AMPK activators both activate AMPK prior to ischemia and enhance the degree of ischemic AMPK activation,[50] and it is difficult to tease apart these actions experimentally. In addition to preserving energy stores,[50] modulating the induction of autophagy and other molecular mechanisms might be important. A well-known action of AMPK is to phosphorylate endothelial nitric oxide synthase on Ser^{1177}, which appears to be critical for the cardio-protective effect of metformin,[90] but is less important during A769662 treatment.[50] Recent evidence suggests that the cardio-protective effect of A769662 might also be mediated in part through inhibition of mitochondrial permeability transition pore opening.[128]

AMPK is activated by additional cardio-protective interventions, providing indirect support for the hypothesis that it defends against ischemic injury. For example, adiponectin activates AMPK and prevents ischemia-reperfusion injury and apoptosis in mice,[74] although adiponectin has additional mechanisms of action, including stimulation of cyclooxygenase-2[74] and ceramidase. [129] The latter enzyme generates the anti-apoptotic sphingolipid sphingosine-1-phosphate and its activity appears to be independent of the LKB1-AMPK pathway in cardiomyocytes.[129] Interestingly, short-term caloric restriction [124] and erythropoietin [130] both increase AMPK activation and have cardio-protective effects that are inhibited by the AMPK inhibitor compound C, although caution is warranted because of the known non-specific effects of this pharmacologic inhibitor[97].

There is some concern that persistent AMPK activation during early reperfusion promotes fatty acid oxidation, which can be detrimental in the post-ischemic heart.[131] Excessive fatty

acid oxidation impairs glucose oxidation via a Randle cycle mechanism, leading to persistent lactate production and intracellular acidosis that triggers sodium-proton exchange and calcium overload via sodium-calcium exchange.[131] High plasma fatty acid concentrations occur during the stress of myocardial ischemia and potentially might augment this phenomenon.[131] However, *in vivo* experiments in the AMPK-inactivated mouse models suggest that any potential adverse effects are counter-balanced by protective effects of AMPK activation to reduce myocardial necrosis during ischemia-reperfusion.[50, 90]

AMPK action in cardiac hypertrophy

Cardiac hypertrophy has an adaptive function to reduce wall tension in the setting of the LV pressure overload that accompanies hypertension or obstructive valvular heart disease. However, pressure overload can lead to pathologic LV remodeling, characterized by hypertrophy exceeding vascular perfusion capacity, interstitial fibrosis, diastolic dysfunction or overt systolic contractile failure. Thus, strategies that limit LV remodeling are potentially important and AMPK activation appears to modulate the myocardial response to pressure overload.

The effects of pro-hypertrophic stimuli are blunted by treatment with pharmacologic agents that activate AMPK. For instance, metformin, AICAR and resveratrol each activate AMPK and diminish hypertrophy induced by either phenylephrine or constitutively-active Akt in neonatal rat cardiomyocytes.[61] Adiponectin also decreases agonist-induced cardiomyocyte hypertrophy *in vitro* in an AMPK-dependent fashion, as does constitutively active AMPK.[68] In contrast, the diabetic heart has increased expression of the hormone resistin, which inhibits AMPK activation, worsening hypertrophy in neonatal rat cardiomyocytes.[70] Taken together with the known effects of activated AMPK to inhibit protein synthesis, these data support the paradigm of AMPK functioning as an anti-hypertrophic pathway in the cardiomyocyte.

In the intact heart, intrinsic activation of AMPK appears to have an important role in the functional response to pressure overload. Mice with global $\alpha 2$ AMPK deletion demonstrate greater hypertrophy, contractile dysfunction, fibrosis, heart failure and mortality after aortic banding.[58] Greater activation of the mTOR/S6-kinase signaling pathway, which is normally inhibited by AMPK, likely contributes to the excess hypertrophy in this model[58]. Exaggerated cardiac hypertrophy and contractile failure also occur in adiponectin knockout mice, which have reduced AMPK phosphorylation after aortic banding.[68] Recent data indicate that AMPK deficiency also exacerbates obesity-induced cardiac hypertrophy and contractile dysfunction.[132]

Although AMPK restrains the development of pathologic hypertrophy during aortic banding, intrinsic AMPK activation does not appear to regulate normal heart growth. It is striking that none of the mouse models with impaired heart AMPK activation develop cardiac hypertrophy.[46, 123] The greater influence of AMPK activation during pressure overload hypertrophy might reflect the fact that AMPK activation is more sustained after banding, as compared to the intermittent AMPK activation associated with exercise during normal growth. AMPK also blocks the hypertrophic response to aortic banding, by blunting

the increase in cardiac angiotensin II and endothelin concentrations and inhibiting the nuclear factor-κB and the calcineurin/nuclear factor of activated T cells pathways.[57, 133]

From a translational perspective, there appears to be an opportunity for therapeutic intervention, since AMPK activity is not maximally increased during pressure overload and can be further augmented by pharmacologic means. Treatment with the AMPK activator AICAR blunts LV hypertrophy induced by aortic banding in rats, although AICAR also decreased blood pressure confounding interpretation of these results.[57] Resveratrol has similar beneficial effects on AMPK and hypertrophy in this model, but has pleiotropic effects that might contribute to its action.[134] Adiponectin over-expression also activates AMPK in the heart and diminishes hypertrophy after banding in mice.[68] Thus, these initial observations support the possibility that AMPK activators might have a role in modulating the hypertrophic and functional consequences of pressure overload in the heart and warrant further study.

AMPK in heart failure

Heart failure is associated with numerous alterations in cardiac substrate and energy metabolism, leading to an interest in understanding the role of AMPK in mediating these changes and the potential benefit of pharmacologically activating AMPK in failing hearts. In the rapid pacing-induced canine heart failure model, both metformin and AICAR treatment significantly diminish contractile dysfunction, apoptosis and fibrosis.[135] Both treatments augment AMPK activation in the paced hearts, suggesting that heart failure does not maximally activate the AMPK pathway and that further pharmacologic activation might be beneficial.[135] These treatments also improve systemic insulin resistance and increase plasma nitric oxide levels and it is possible that these non-cardiac effects might contribute to their beneficial effects.[135] Metformin and AICAR also have additional effects on the heart, the former as a mitochondrial complex 1 inhibitor and the latter as an adenosine regulator. Some caution is warranted because the dose of metformin in experimental studies exceeds the doses used clinically.[135] However, recent observational studies suggest that metformin improves the survival of patients with heart failure and type 2 diabetes,[136] supporting the possibility that pharmacologic activation of AMPK might be beneficial in heart failure.

AMPK also appears to play a role in preventing heart failure in the mouse coronary occlusion model. Metformin administered at the time of reperfusion augmented AMPK activation, eNOS phosphorylation and PGC-1α expression and reduced LV remodeling 4 weeks after myocardial infarction. [137] AMPK activation had a critical role in mediating metformin's action, based on the observation that metformin had no effect in mice with genetically inactivated AMPK in the heart.[137]

As evidence accumulates that AMPK activation might be beneficial in preventing heart failure, a number of issues require careful consideration. First, there needs to be a better understanding of whether AMPK activation has benefit in the treatment of established LV dysfunction and heart failure, recognizing that these effects might vary depending on the model. Second, the specific role of AMPK activation needs to be confirmed in order to be certain that observed effects are indeed attributable to AMPK activation; more specific

AMPK activators and the use of AMPK-inactivated genetic models will be important in this regard. Finally, the long term therapeutic efficacy and safety of AMPK activators will need to be assessed.

AMPK γ subunit mutations

Mutations in the gene encoding the γ2 subunit (PRKAG2) on chromosome 7q3 are linked to familial hypertrophic cardiomyopathy associated with the Wolf-Parkinson-White (WPW) syndrome.[138-140] Unlike the more common forms of hypertrophic cardiomyopathy that are associated with mutations in sarcomeric proteins, the hallmark of the PRKAG2 syndrome is the accumulation of glycogen in cardiomyocytes without myofibrillar disarray.[140] PRKAG2 mutations cause cardiac glycogen overload similar to Pompe's or Danon's diseases, but do not cause impaired glycogenolysis.[141]

PRKAG2 mutations fall within or adjacent to the nucleotide binding domains (Figure 5).[138-140] The degree of hypertrophy and associated conduction system disease vary depending on the mutation and the individual. The syndrome of hypertrophic cardiomyopathy and WPW has been linked to mutations at H383R in CBS2,[138] T400N in CBS2,[140] N488I in CBS3,[140] R302Q in CBS1[139, 140] and a leucine insertion between CBS1 and CBS2.[138] In addition, the R531G mutation in CBS4 has been observed in children with WPW and conduction system disease without cardiac hypertrophy.[142]

The molecular mechanisms responsible for the cardiac phenotype are still under investigation. Complexes containing γ2-mutations generally have diminished activation by AMP in cell free systems, with reduced AMP dependence (R302Q, H338R) or sensitivity (N488I, R531Q, R531G) *in vitro*.[143, 144] They also appear to have reduced ATP binding which diminishes the inhibitory effects of ATP on AMPK activation.[143, 144] Although mouse hearts expressing the N488I[143], R531G[145] and R302Q[146] mutations all demonstrate cardiac glycogen overload, LV hypertrophy and WPW, they are reported to have variable AMPK activity. The N488I hearts have constitutively high AMPK activity,[143] while AMPK activity in R531G hearts is normal at 1 week and then decreases following the glycogen accumulation,[145] and surprisingly R302Q hearts have low AMPK activity.[146] The reason for these disparities in AMPK activity remains uncertain.

The N488I mouse model is the best studied to date and has constitutively high AMPK activity, which appears to be responsible for its phenotype.[147] The N488I phenotype is largely reversed when crossed with mice expressing inactivated AMPK in the heart.[147] The increase in activity of the N488I complex is due to a high level of Thr[172] phosphorylation, but what leads to this phosphorylation is not fully understood. Nonetheless, the high AMPK activity promotes glycogen synthesis, leading to cardiac glycogen overload that is the hallmark of the PRKAG2 syndrome.[148]

The finding that glycogen synthesis is increased in the PRKAG2 model appears paradoxical from the perspective that activated AMPK phosphorylates and inhibits glycogen synthase in non-cardiac cells.[149] However, AMPK stimulation of heart glucose uptake leads to an increase in glucose-6-phosphate concentrations, which both increase substrate availability and allosterically activate glycogen synthase.[148] Because AMPK also increases fatty acid

oxidation, which inhibits glucose oxidation, glucose-6-phosphate is preferentially shunted into glycogen synthesis in the N488I hearts. These hearts also have increased expression of UDP-glucose pyrophosphorylase, which synthesizes UDP-glucose, the direct substrate for glycogen synthesis,[148] although this enzyme is not rate limiting for glycogen synthesis. Thus, increased AMPK activity and glucose uptake in the absence of increased energy demand promotes glycogen accumulation in the N488I model.

The development of the WPW syndrome in patients with PRKAG2 mutations is an example of how altered metabolism affects cardiac electrophysiological development. WPW is characterized by ventricular pre-excitation, via one or more muscle bridges traversing the annulus fibrosis, which “bypass” the AV node and rapidly depolarize the ventricles. These bridges normally undergo apoptosis prior to birth, but in the N488I model, glycogen-filled myocytes are presumably resistant to apoptosis and persist as physiologic bypass tracks that disrupt the annulus fibrosis.[143] Consistent with the developmental basis of WPW, post-natal induction of the PRKAG2 mutation in mice fails to induce ventricular pre-excitation, although it does cause LV cardiac glycogen overload.[150]

Because the PRKAG2 mutations appear to be activating in some cases, this research has raised concerns that AMPK activators might cause cardiac glycogen overload with prolonged use in the clinical setting. However, there are potential biological differences between the cardiac effects of AMPK activating therapy and chronically activating PRKAG2 mutations. The degree of AMPK activation might differ, and the γ mutations might also alter the subcellular distribution of AMPK or its interaction with specific substrates. It is also noteworthy that existing diabetes drugs that activate AMPK do not cause cardiac glycogen overload, although the actual degree to which they activate AMPK in the human heart is not known. Nonetheless, if excess cardiac glycogen accumulation emerges as a clinical problem, then AMPK activators would have to be restricted to use on a short-term basis, or alternative pharmacologic strategies that activate AMPK outside of the heart would have to be considered.

Conclusions

In the last decade, there has been substantial progress in understanding the biological actions of AMPK and a growing appreciation of its importance in the cardiovascular system, but substantial gaps in knowledge remain to be addressed. In discussing many of the recent novel molecular mechanisms responsible for AMPK activation and action, it is readily apparent that most have been discovered in non-cardiac cells. Some of these mechanisms might be ubiquitous, while others might not prove to be operative in the heart.

Activation of AMPK is a highly regulated process that goes beyond changes in AMP and ATP, and role of local autocrine-paracrine factors, upstream kinases including CaMKKβ, and specific protein phosphatases in the heart are all areas that require further investigation. There is also a fundamental lack of understanding of the cell biology of AMPK complexes in the heart with respect to their subcellular localization and action. Although the complex membrane structure and myofibrillar proteins pose technical challenges to studying compartmentalized signaling mechanisms, this is a key area that warrants innovative

approaches to unravel. The heterogeneity of cell types in the heart presents an additional challenge to understanding the function of the AMPK pathway in the intact heart, since AMPK might be differentially regulated in endothelial cells, fibroblasts and smooth muscle cells.

Although the acute metabolic actions of AMPK in the heart are fairly well characterized, the regulation of gene expression in the heart by AMPK is in its infancy and further research in this area is needed. Similarly, the degree to which AMPK regulates mitochondrial function, autophagy/mitophagy and electrophysiological properties of the heart, requires additional investigation, particularly in relevant models of myocardial ischemia, pressure overload and heart failure.

The function of specific AMPK subunit isoforms has not yet been delineated in the heart. In particular, whether the individual γ2 variants confer distinct physiologic actions to AMPK complexes is an open issue. Defining the regulation and action of the γ2 variants might provide additional insight into the pathogenesis of the human PRKAG2 syndrome.

The potential translation of basic research on AMPK to the clinical arena is a goal of many ongoing research programs. Since the first discoveries that activated AMPK stimulates glucose transport in skeletal and heart muscles,[6, 151, 152] there has been a great deal of interest in the development of highly potent and specific AMPK activators for the treatment of type 2 diabetes. Since diabetic patients often have concomitant cardiovascular disease, understanding the potential cardiovascular benefits and any safety issues associated with AMPK activators would be important.

With regards to the potential clinical applications of AMPK activators in cardiovascular disease, there is still much work that needs to be done. For application to ischemia reperfusion, additional research is clearly needed to better assess the efficacy of AMPK activators administered at the time of reperfusion. Protective effects at the time of reperfusion would enhance the use of AMPK activators as potential adjunct therapy to coronary revascularization in patients with acute myocardial infarction. If long-term therapy with AMPK activators proves feasible, then it would be appropriate to consider their application to the treatment of heart failure. Additional studies would be needed to determine whether AMPK activators prevent the transition to heart failure during LV pressure overload and most importantly whether they might reverse LV dysfunction and adverse remodeling in the setting of established heart failure. Establishing efficacy and safety in large animal models would be essential before AMPK activators would be appropriate for introduction into clinical studies. Nonetheless, existing results to date offer encouragement to further pursue research on AMPK as a novel target for the treatment of cardiovascular disease.

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Non-standard abbreviations

ACC	acetyl-CoA carboxylase
AICAR	5-aminoimidazole-4-carboxyamide-1-beta-D-ribofuranoside
AMPK	AMP-activated protein kinase
AID	auto-inhibitory domain
Atg1	autophagy-related 1
CPT-1	carnitine palmitoyltransferase-1
CaMKKβ	calcium-calmodulin activated protein kinase kinase β
Cidea	cell death-inducing DFF45-like effector A
CBS	cystathionine beta-synthetase
ERRα	estrogen-related receptor alpha
eEF2	eukaryotic elongation factor-2
FOX	forkhead box
GLUT	glucose transporter
GBD	glycogen-binding domain
GAP	GTPase-activating proteins
HDAC	histone deacetylase
HNE	4-hydroxy-2-nonenal
IL-6	interleukin-6
LKB1	liver kinase B1
MIF	macrophage migration inhibitory factor
MCAD	medium chain acyl-CoA dehydrogenase
mTOR	mammalian target of rapamycin
mTORC1	mTOR complex 1
MuRF1	muscle RING finger protein 1
MEF	myocyte enhancer factor
NAMPT	nicotinamide phosphoribosyltransferase

NRF-1	nuclear regulatory factor-1
PGC	peroxisome proliferator activated receptor gamma co-activator
PFK	phosphofructokinase
PP2A	protein phosphatase 2A
SIRT1	sirtuin-1
TAB1	TAK1–binding protein 1
TAK1	transforming growth factor-β–activated protein kinase-1
TSC	tuberous sclerosis complex
ULK	Unc-51-like kinase
UCP	uncoupling protein
WPW	Wolf-Parkinson-White

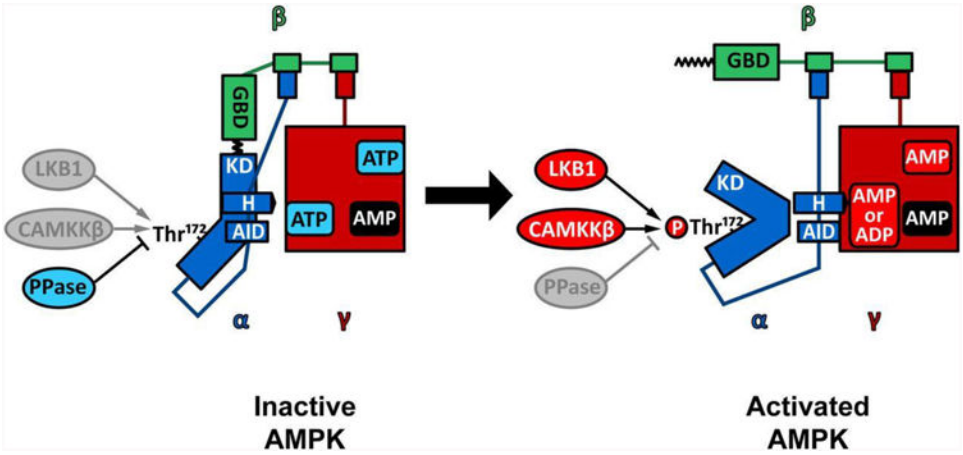

Figure 1. Molecular structure of the AMPK complex

The AMPK complex is composed of a catalytic subunit (α, blue) and two regulatory subunits (β, green and γ, red). The α subunit contains a serine-threonine kinase domain (KD), which is highly activated by phosphorylation of the Thr^{172} residue in the catalytic cleft by upstream kinases (LKB1, CAMKKβ). AMPK is maintained in an unphosphorylated inactive state by the interaction of kinase domain with an autoinhibitory domain (AID) and with the myristoylated N-terminus of the regulatory β subunit. AMP interaction with the nucleotide binding sites in the γ-subunit induces a conformational change in the heterotrimeric complex via an α hook domain, that relieves the autoinhibition by the AID and promotes phosphorylation of the Thr^{172} residue. The catalytic cleft of activated AMPK is in a closed conformation, which protects phosphorylated Thr^{172} from being dephosphorylated by protein phosphatases (PPase).

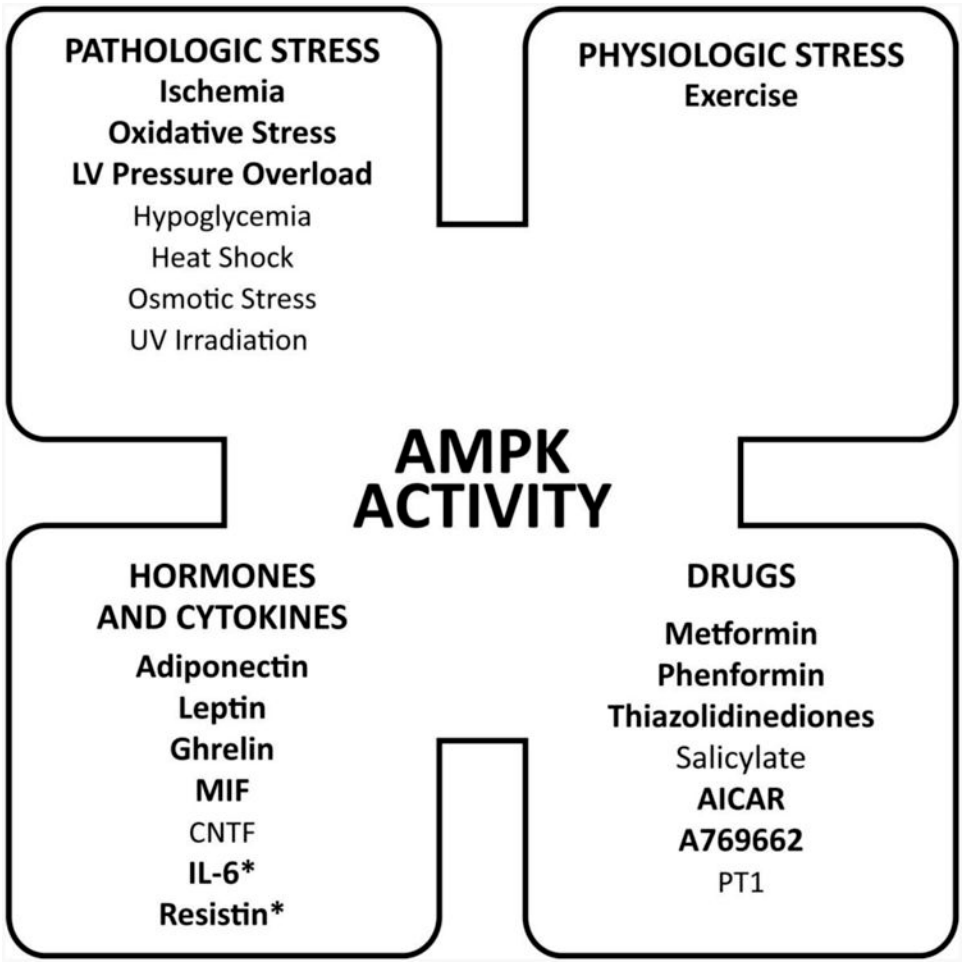

Figure 2. Regulators of AMPK activity

AMPK activity is modulated by several physiologic, pathologic and pharmacologic factors. Factors that are known to regulate heart AMPK are highlighted in bold, while inhibitors of heart AMPK activity are designated by asterisks and interventions that have been shown only in non-cardiac cells are in italics.

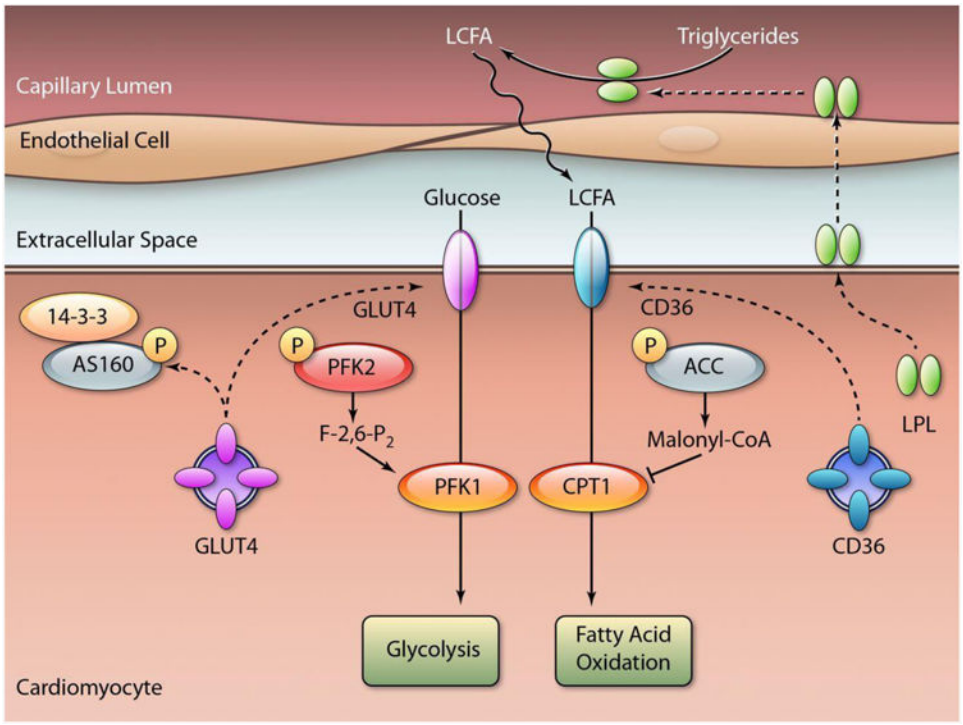

Figure 3. AMPK regulation of heart glucose and fatty acid metabolism

AMPK regulates substrate transporters and the concentrations of allosteric regulators of glycolysis and fatty acid oxidation. Molecules that are phosphorylated by AMPK are designated by the symbol “P”. AMPK phosphorylates the Rab GTPase AS160, which induces GLUT4 glucose transporter translocation to the sarcolemma. AMPK phosphorylates phospho-fructokinase 2 (PFK2), leading to the synthesis of fructose-2,6-bisphosphate, an allosteric activator of PFK1 and glycolysis. AMPK promotes lipoprotein lipase (LPL) translocation from the myocyte sarcolemma to the luminal surface of capillary endothelial cells, where it catalyzes the release of long-chain fatty acids (LCFA) from triglyceride-containing lipoproteins for heart substrate metabolism. Activated AMPK also stimulates CD36 translocation to the sarcolemma increasing cardiomyocyte free fatty acid uptake. AMPK also phosphorylates and inactivates acetyl-coenzyme A carboxylase (ACC) decreasing the concentration of the fatty acid oxidation inhibitor malonyl-CoA. Proteins activated by AMPK are highlighted in red and proteins inactivated by AMPK in grey. (Illustration credit: Ben Smith).

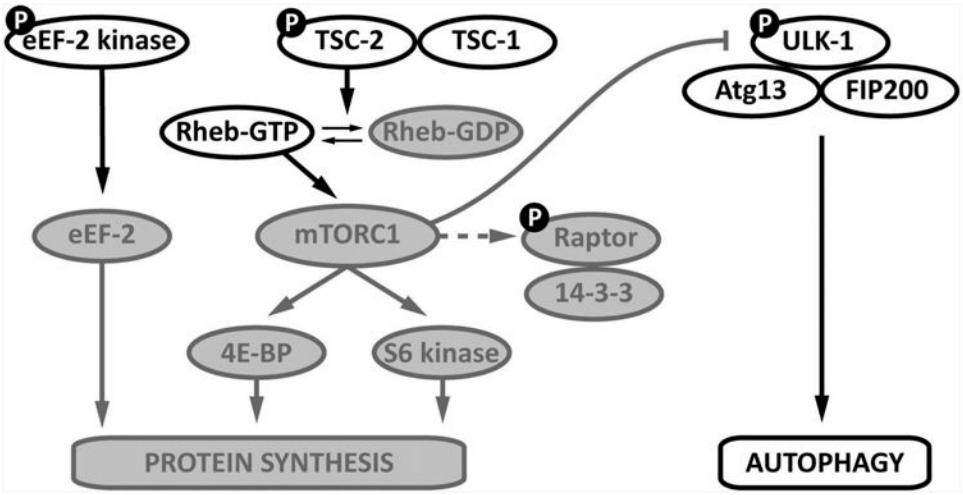

Figure 4. Activated AMPK inhibits protein synthesis and increases authophagy

Proteins phosphorylated by AMPK and designated with the symbol "P". AMPK phosphorylates eukaryotic elongation factor (eEF)-2 kinase, which inhibits eEF-2 activity. AMPK also phosphorylates tuberous sclerosis complex (TSC)-2, which increases the GTP-ase activity of the TSC1-TSC2 complex. AMPK also phosphorylates raptor, which removes it from the mammalian target of rapamycin complex (mTORC) 1 complex. Coordinated TSC2 and raptor phosphorylation result in inactivation of mTOR signaling, which inhibits the function of mTOR in the activation of protein synthesis and inhibition of autophagy. AMPK also phosphorylates Unc-51-like kinase 1 (Ulk1) in the ULK1-Atg13-FIP200 complex to directly promote autophagy. Direct phosphorylation of eEF2 has been shown in the heart, while the other pathways were demonstrated in non-cardiac cells.

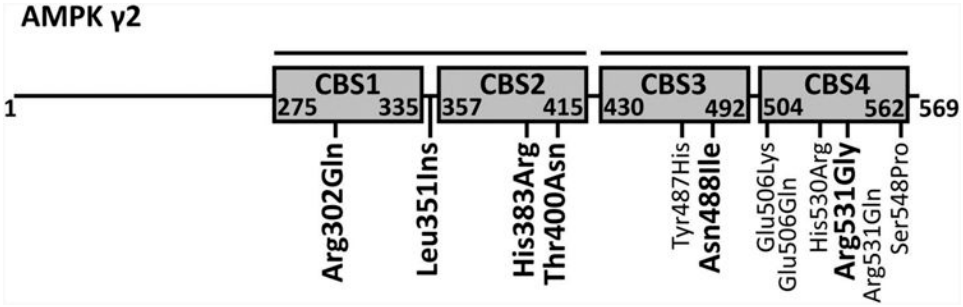

Figure 5. Mutations in the human γ2 subunit of AMPK

Schematic diagram of the PRKAG2 gene product γ2 isoform highlights the nucleotide binding domains (Bateman domains) and lists mutations with clinical expression curated in the OMIM database (http://www.omim.org/). All mutations cause cardiac hypertrophy and those that cause Wolff-Parkinson-White syndrome are highlighted in bold.