AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism

Review

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The AMP-activated protein kinase (AMPK) is an evolutionarily conserved sensor of cellular energy status, and recent data demonstrate that it also plays a critical role in systemic energy balance. AMPK integrates nutritional and hormonal signals in peripheral tissues and the hypothalamus. It mediates effects of adipokines (leptin, adiponectin, and possibly resistin) in regulating food intake, body weight, and glucose and lipid homeostasis. AMPK is regulated by upstream kinases of which the tumor suppressor, LKB1, is the first to be identified. Complex signaling networks suggest that AMPK may prevent insulin resistance, in part by inhibiting pathways that antagonize insulin signaling. Through signaling, metabolic, and gene expression effects, AMPK enhances insulin sensitivity and fosters a metabolic milieu that may reduce the risk for obesity and type 2 diabetes.

Introduction

Over millennia, mammalian evolution selected for genes that allowed for survival during times of famine or drought. In modern society, this genetic constellation has become maladaptive owing to the fact that industrialization has made food supply more abundant and reduced the need to be physically active. Hence, the prevalence of obesity and its associated morbidities (including type 2 diabetes, hypertension, cardiovascular disease, and the metabolic syndrome) continues to climb. Understanding the fundamental biology of energy balance is essential to developing new approaches to "stay the tide" of these metabolic diseases. Historically, the AMP-activated protein kinase (AMPK) pathway was viewed primarily as a sensor and regulator of energy balance at the cellular level. Indeed, the fact that orthologs of AMPK are found in single celled eukaryotes such as the yeast *Saccharomyces cerevisiae* and the primitive protist *Giardia lamblia* (Carling, 2004; Hardie et al., 2003) is consistent with this being the function for which it originally evolved. However, during the last three years, it has become apparent that in mammals, AMPK is also involved in the fundamental regulation of energy balance at the whole body level by responding to hormonal and nutrient signals in the central nervous system and peripheral tissues that modulate food intake and energy expenditure.

Structure and regulation of AMPK

AMPK is the downstream component of a kinase cascade that acts as a sensor of cellular energy charge, being activated by rising AMP coupled with falling ATP. Because of the near equilibrium reaction catalyzed by adenylate kinase (2ADP ↔ ATP + AMP), the cellular AMP:ATP ratio varies approximately as the square of the ADP:ATP ratio, making the former a more sensitive index of energy status. AMPK exists as a heterotrimer comprising a catalytic α subunit and regulatory β and γ subunits. In mammals, each subunit is encoded by multiple genes (α1, α2, β1, β2, γ1, γ2, γ3) (Carling, 2004; Hardie et al., 2003), with splice variants and alternative promoters further increasing the complexity. In mammalian cells, AMP allosterically activates the complex, although this has not yet been convincingly demonstrated with orthologs from lower eukaryotes. In mammals, AMP also promotes activation of AMPK by stimulating phosphorylation of the kinase domain at a critical threonine residue in the activation loop (Thr-172) by an upstream kinase(s) whose identity is discussed later. The nucleotide also inhibits dephosphorylation of Thr-172 by protein phosphatases; the effects of AMP on both phosphorylation and dephosphorylation appear to be due to binding of the ligand to the *substrate*, AMPK, and not to the upstream kinase or phosphatase. The fact that AMP activates AMPK by three mechanisms, rather than one, means that the system is exquisitely sensitive to small changes in cellular AMP (reviewed in Hardie et al., 2003). All three effects of AMP on the AMPK system (allosteric activation, phosphorylation, and inhibition of dephosphorylation) are antagonized by high concentrations of ATP, so the system essentially monitors changes in the cellular AMP:ATP ratio.

What are the functions of the three subunits of the complex?

The α subunit contains an N-terminal kinase domain and a C-terminal domain involved in complex formation. The β subunits contain conserved C-terminal domains that are sufficient on their own to form a complex with α and γ (Hudson et al., 2003). The β subunits in all eukaryotes also contain a carbohydrate binding domain that is related to domains found in enzymes that metabolize the α1→6 branch points in α1→4 linked glucans, such as starch and glycogen (Hudson et al.,

2003; Polekhina et al., 2003). The function of this domain remains unclear, although it causes association of AMPK complexes with glycogen particles, where one physiological target, i.e., glycogen synthase, resides. In both rat and human muscle there is also evidence that a high cellular glycogen content represses activation of AMPK (Wojtaszewski et al., 2002, 2003). This raises the intriguing possibility that, as well as being able to sense the immediate availability of energy in the form of ATP, the AMPK system can also sense its more medium-term availability in the form of glycogen, although this phenomenon has not yet been reproduced in a cell-free system.

The γ subunits of AMPK contain four repeats of a sequence motif of about 60 residues first recognized by Bateman (Bateman, 1997) and originally termed a CBS domain. These occur in a number of proteins in archaea, bacteria, and eukaryotes, and since the functional unit is a dimer, Kemp (Kemp, 2004) has suggested the term "Bateman domain" to describe the structure formed by two tandem CBS motifs. Unusually, the γ subunits of AMPK contain four repeats, forming two tandem Bateman domains. Each of these domains binds one molecule of AMP or ATP in a mutually exclusive manner (Scott et al., 2004), consistent with the findings that high concentrations of ATP antagonize activation of AMPK by AMP. Constructs containing both Bateman domains bind two molecules of AMP or ATP with strong positive cooperativity, which may be a further mechanism to increase the sensitivity of the AMPK system to small changes in nucleotide levels.

Functional information from naturally occurring mutations in AMPK

Interestingly, six different point mutations in the gene encoding the γ2 subunit isoform, four affecting the N-terminal Bateman domain and two affecting the C-terminal domain, cause a hereditary heart disease in humans, i.e., Wolff-Parkinson-White syndrome (WPWS), which is usually associated with hypertrophic cardiomyopathy (HCM) (Scott et al., 2004). These mutations have similar effects on binding of AMP to the isolated Bateman domains and on activation of the heterotrimeric complex by AMP (Daniel and Carling, 2002; Scott et al., 2004), providing strong evidence that the Bateman domains provide the regulatory nucleotide binding sites in the AMPK complex. How these mutations cause WPWS and HCM is a complex issue that remains the subject of intense investigation. All the mutations interfere with AMP activation of AMPK. However, there is evidence, albeit indirect, that some mutations also increase the basal phosphorylation and activation of AMPK (Arad et al., 2002; Minokoshi et al., 2004). If correct, this would help explain why the mutations are dominant and appear to act as gain-of-function mutations, as does a similar mutation in γ3 in pigs (R225Q, equivalent to R302Q in γ2) (Milan et al., 2000). The equivalent mutation in γ1 (R70Q) has not yet been found to occur naturally but appears to be more active than the wild-type under basal conditions when expressed in cultured cells (Hamilton et al., 2001).

Activators of AMPK

Cellular stresses

The AMPK system is activated by a large variety of cellular stresses that deplete ATP (Carling, 2004; Hardie, 2004; Hardie et al., 2003) including metabolic poisons such as inhibitors of the tricarboxylic acid cycle (arsenite), the respiratory chain (antimycin A, azide), or the mitochondrial ATP synthase (oligomycin), as well as uncouplers of oxidative phosphorylation such as dinitrophenol. AMPK is also activated by pathological stresses such as glucose deprivation, ischemia, hypoxia, oxidative stress, and hyperosmotic stress. With the exception of the latter (Fryer et al., 2002), all of these are associated with increases in the cellular AMP:ATP ratio. The finding that AMPK is activated by glucose deprivation is particularly interesting because extensive genetic studies show that the budding yeast ortholog of AMPK, i.e., the SNF1 complex, is required for the survival response to glucose starvation (Hardie et al., 1998).

Exercise and contraction

The pathological or pharmacological stresses described above interfere with ATP production, but a metabolic stress that activates AMPK under more physiological conditions by increasing ATP *consumption* is exercise (Winder and Hardie, 1996) or contraction (Hutber et al., 1997) in skeletal muscle. The degree of AMPK activation depends on the intensity of exercise and is thought to be induced by changes in AMP/ATP and creatine/phosphocreatine ratios (reviewed in Aschenbach et al., 2004). However, exercise also activates AMPK in liver and adipose tissue, which may result, in part, from exercise-induced release of molecules such as interleukin 6 from muscle (Kelly et al., 2004) and also from systemic alterations in substrate availability. AMPK activation in response to exercise acutely inhibits ATP-consuming pathways and activates carbohydrate and fatty acid metabolism to restore ATP levels in muscle. It also plays a role in the adaptive response of muscle to endurance training by altering muscle fuel reserves and expression of exercise-responsive genes (Aschenbach et al., 2004). Increased physical activity reduces the risk of developing insulin resistance and Type 2 diabetes in susceptible human populations (Hu et al., 2001), and AMPK activation may account for at least some of the beneficial effects of exercise such as increased fatty acid oxidation (Merrill et al., 1997; Aschenbach et al., 2004), increased mitochondrial biogenesis (Zong et al., 2002), and possibly glucose uptake (Mu et al., 2001), although its role in the latter has become controversial (see below).

Antidiabetic drugs

Further interest in the potential role of the AMPK pathway in the treatment of Type 2 diabetes was generated by findings that AMPK is activated indirectly by metformin (Zhou et al., 2001) and thiazolidinediones (Fryer et al., 2002), widely used treatments for Type 2 diabetes. Both metformin (El-Mir et al., 2000) and thiazolidinediones (Brunmair et al., 2004) inhibit Complex I of the respiratory chain, and this may be how they activate AMPK. However, while thiazolidinediones (Fryer et al., 2002) cause increases in the cellular AMP:ATP ratio, this has not been demonstrated for metformin (Fryer et al., 2002; Hawley et al., 2002). Thiazolidinediones also increase plasma adiponectin and decrease plasma resistin levels, which could contribute to the indirect effects on AMPK activity (see below). Most of the actions of thiazolidinediones are thought to be explained by another target, the PPAR-γ, a nuclear receptor. While there is currently no other known target for metformin, more studies are needed to clarify how critical the activation of AMPK is for the therapeutic effects of these drugs.

Target proteins and pathways for the AMPK system—Role in systemic energy balance

In general, activation of AMPK switches on alternate catabolic pathways that generate ATP while switching off ATP-consum-

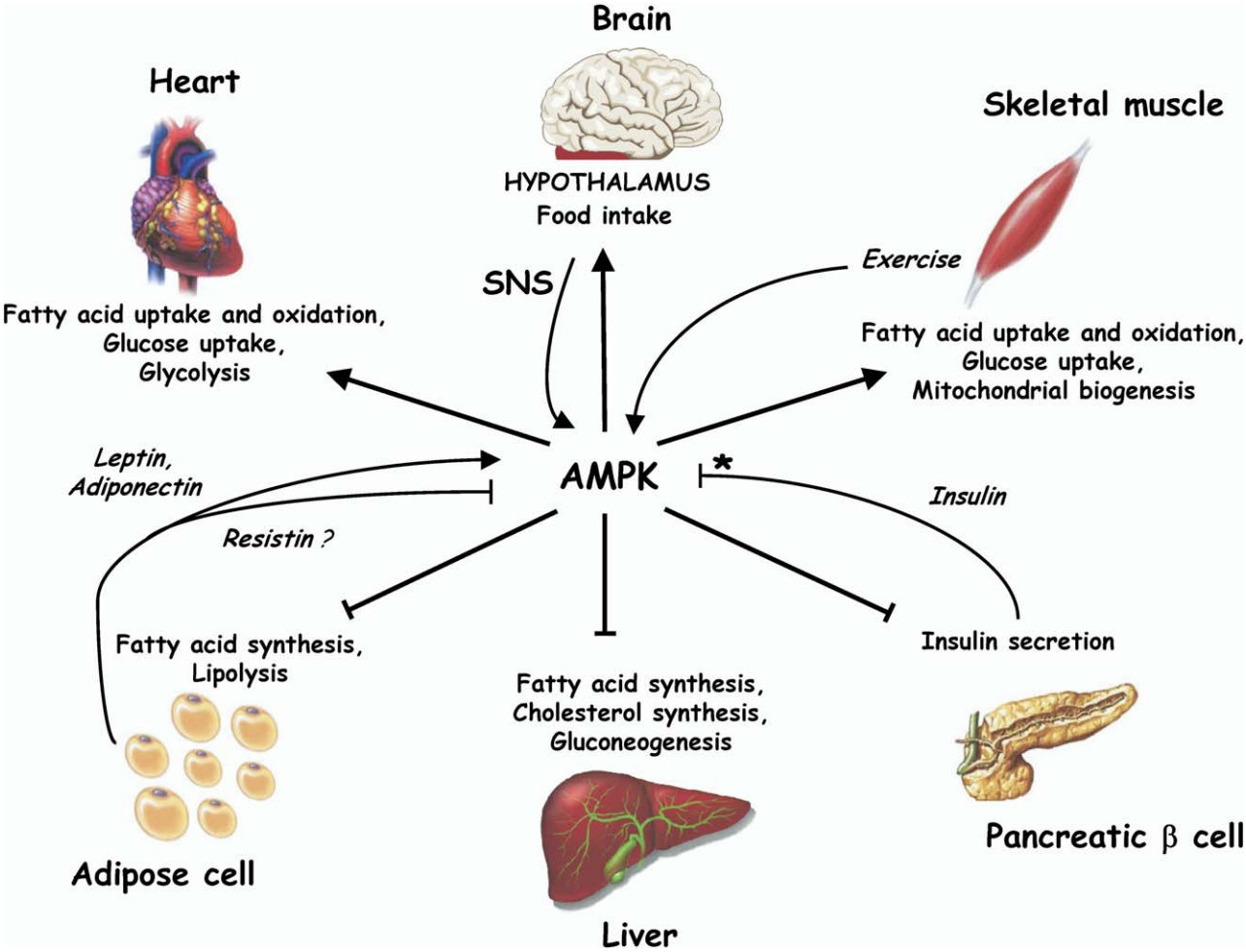

Figure 1. Role of AMP-activated protein kinase (AMPK) in the control of whole-body energy homeostasis

Activation of AMPK in many tissues switches off ATP-consuming processes while switching on catabolic processes that generate ATP. Some key metabolic effects are shown. The adipocyte-derived hormones leptin and adiponectin, as well as exercise, activate AMPK in skeletal muscle, stimulating fatty acid oxidation. Leptin's activation of AMPK in skeletal muscle involves the hypothalamic-sympathetic nervous system (SNS) axis. Adiponectin also activates AMPK in liver, increasing fatty acid oxidation and reducing gluconeogenesis, and in adipocytes, where the downstream biologic pathway has not been studied. Resistin inhibits AMPK in liver. AMPK inhibits insulin secretion from pancreatic β cells.

*Insulin inhibits AMPK activation in ischemic heart and hypothalamus, whereas it has no effect on AMPK in skeletal muscle or adipocytes. In hypothalamus, AMPK activity plays a role in regulation of food intake and body weight (see **Figure 3**).

ing processes, especially anabolic (biosynthetic) pathways. It achieves these effects both by rapid, direct phosphorylation of metabolic enzymes and via more long-term effects on gene and protein expression. Some of its overall effects on metabolism are summarized in Figure 1 and target proteins and pathways downstream of AMPK are listed in Table 1 and Supplemental Table 1 (which is more extensive with references) and reviewed in Carling (2004), Hardie (2004) and Hardie et al. (2003). In this article we will focus on recent findings regarding the role of AMPK in whole body energy balance.

Obesity results from an imbalance between energy intake and expenditure. It has long been known that increased adiposity is associated with insulin resistance and a markedly increased risk of developing type 2 diabetes, but only recently have data suggested that AMPK may play a role. AMPK affects energy intake, utilization, and storage through regulation of food intake and substrate flux and metabolism (Figure 1). It regulates many molecules and pathways in skeletal muscle, heart, adipose tissue, liver, pancreatic β cells, and the brain that control glucose and lipid uptake, storage, and utilization (see Table 1, Supplemental Table 1, and Figure 1) (Hardie et al., 2003). A major recent advance has been the discovery that AMPK elicits this regulation in part by responding to hormonal and nutrient signals.

One pathway that is central to the integrated effects of AMPK in peripheral tissues to reduce the risk for obesity and insulin resistance is the stimulation of fatty acid oxidation (Figure 2). AMPK phosphorylates acetyl CoA carboxylase (ACC), a rate-controlling step in the conversion of acetyl-CoA to malonyl CoA. This phosphorylation inhibits the activity of ACC, which results in decreased malonyl CoA levels. Malonyl CoA inhibits carnitine:palmitoyl-CoA transferase-1 (CPT1), a rate-limiting

Table 1. Examples of targets of AMPK and their biologic effects*

Organ/cell type		Immediate outcome	Biologic end result
Glucose homeostasis — Primary targets			
Muscle	glycogen synthase	enzyme activity ↓	glycogen synthesis ↓
Heart	6-phosphofructo-2-kinase	enzyme activity ↑	glycolysis ↑
C2C12 cells	insulin receptor substrate-1	PI-3-kinase binding ↑	insulin sensitivity ↑?
Liver	transcription factor ChREBP	DNA binding ↓	pyruvate kinase expression ↓
Lipid metabolism — Primary targets			
Liver	acetyl-CoA carboxylase-1/α	enzyme activity ↓	fatty acid synthesis ↓
Muscle	acetyl-CoA carboxylase-2/β	enzyme activity ↓	fatty acid oxidation ↑
Liver	HMG-CoA reductase	enzyme activity ↓	cholesterol synthesis ↓
Adipose tissue	hormone-sensitive lipase	activation by PKA ↓	lipolysis ↓
Mitochondrial biogenesis/function — No known primary targets. Secondary targets include:			
Muscle	transcription factor NRF1	DNA binding ↑	mitochondrial biogenesis ↑
Muscle	UCP3	expression↑	mitochondrial proton leak ↑
Muscle	co-activator PGC-1α	expression ↑	mitochondrial biogenesis ↑
Other (examples) — Primary targets			
Epithelia	CFTR	channel activity ↓	Cl-/fluid secretion ↓
Heart	endothelial NO synthase	enzyme activity ↑	NO production ↑
Cultured cells	TSC2	inhibition of TOR ↑	protein synthesis/cell growth ↓
Cultured cells	TOR	phosphorylation of S6 kinase ↓	protein synthesis/cell growth ↓?
Cultured cells	co-activator p300	coactivator-receptor binding ↓	gene expression ↓

"Primary targets" are direct targets for AMPK where the phosphorylation site(s) have been identified. "Secondary targets" are either regulated indirectly or the direct target remains unclear.

*See Supplemental Table 1 for multiple other secondary targets involved in glucose and lipid metabolism and other biologic processes. Supplemental Table I contains specific references for all targets.

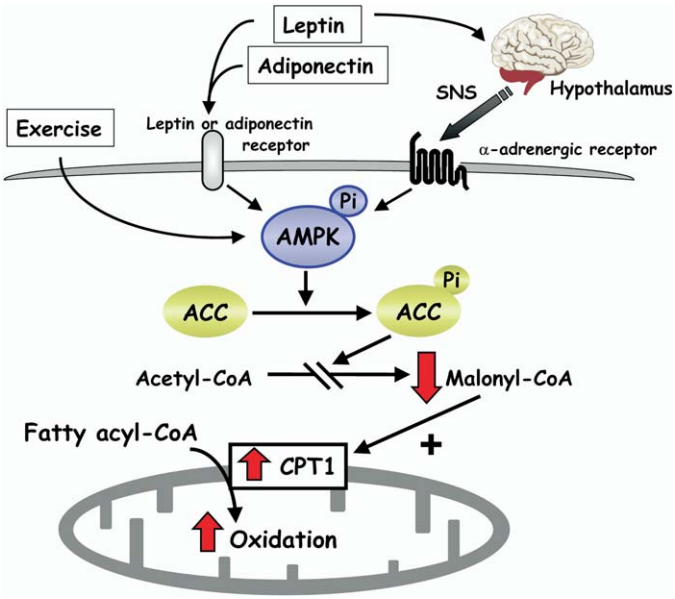

Figure 2. Model for the stimulatory effect of AMPK on fatty-acid oxidation in muscle

Exercise and adipokines (leptin and adiponectin) result in activation of AMPK, which phosphorylates and inhibits acetyl CoA carboxylase (ACC) activity. This reduces malonyl CoA synthesis, activating carnitine palmitoyl transferase (CPT1) and thereby increasing mitochondrial import and oxidation of long-chain acyl-CoA fatty acids in muscle. Leptin activates AMPK in muscle through two distinct mechanisms: one is a direct effect through the leptin receptor in muscle and the other is mediated by the hypothalamic-sympathetic nervous system (SNS) axis through α adrenergic receptors in muscle (Minokoshi et al., 2002).

step for the entry of long-chain fatty acyl-CoA into mitochondria for oxidation. A fall in malonyl CoA levels disinhibits CPT1, resulting in increased fatty acid oxidation. This pathway is thought to be central to the effects of AMPK to reduce lipid stores in muscle and liver and hence to its insulin-sensitizing effects.

AMPK mediates metabolic effects of adipokines

Another major advance in understanding metabolism over the last decade has been the recognition that adipose tissue is an endocrine organ that secretes a large number of hormones, cytokines, and lipids that have systemic effects on processes such as glucose and lipid homeostasis, body weight regulation, blood pressure, and immune function (Kershaw and Flier, 2004). These molecules have been termed adipokines and many of their metabolic actions are mediated by AMPK.

Leptin

Leptin is an adipocyte-secreted hormone that plays a pivotal role in regulation of food intake, energy expenditure, body weight, and neuroendocrine function (Friedman and Halaas, 1998). Leptin stimulates fatty acid oxidation (Minokoshi et al., 2002; Muoio et al., 1997) and glucose uptake (Haque et al., 1999; Kamohara et al., 1997) and prevents lipid accumulation in nonadipose tissues, which can otherwise lead to functional impairments (lipotoxicity) (Unger, 2002). While many of leptin's effects are mediated through the Jak-Stat pathway leading to changes in gene transcription, many key metabolic effects of leptin take place too rapidly to be ascribed to changes in gene transcription. Hence, investigators sought another signaling pathway mediating leptin's metabolic effects. Leptin was found to selectively stimulate phosphorylation and activation of the α2 catalytic subunit of AMPK (α2 AMPK) in skeletal muscle

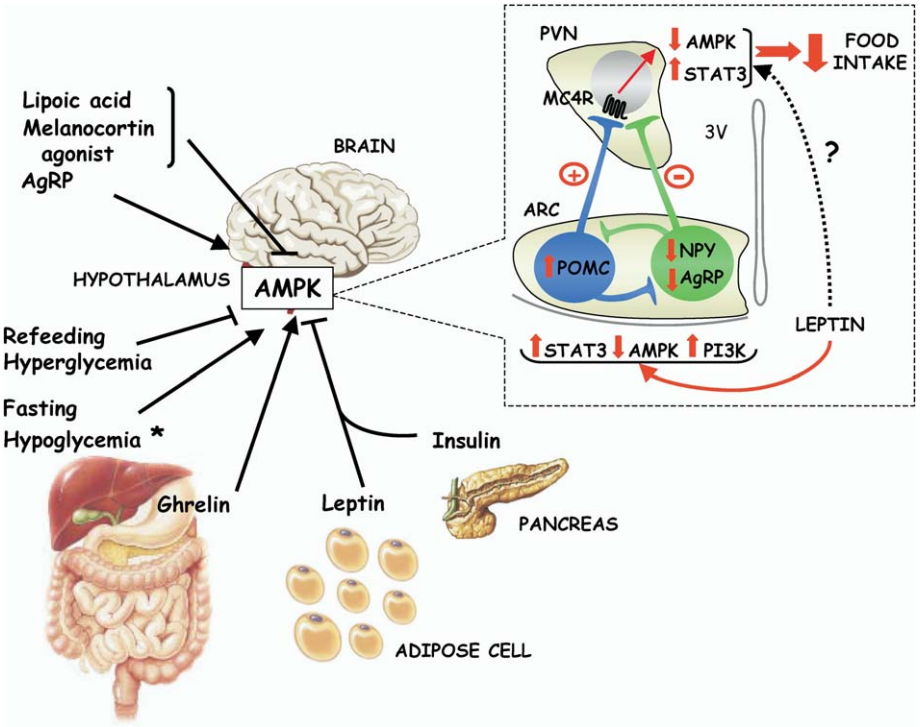

Figure 3. Regulation of AMPK activity in the hypothalamus by hormonal and nutrient signals

Peripheral hormones that control food intake modulate the activity of hypothalamic AMPK. The anorexigenic hormones insulin and leptin decrease AMPK activity in hypothalamic nuclei including arcuate nucleus (ARC) and paraventricular nucleus (PVN). Conversely, the stomach-derived orexigenic hormone, ghrelin, increases hypothalamic AMPK. Nutritional state also modulates hypothalamic AMPK; fasting or hypoglycemia increases hypothalamic AMPK while refeeding or hyperglycemia decreases AMPK activity. α-lipoic acid decreases hypothalamic AMPK activity and food intake. Manipulation of the melanocortin pathway in the hypothalamus alters AMPK activity. The orexigenic peptide Agouti-related protein (AgRP), an antagonist of melanocortin 4 receptor (MC4R), increases AMPK activity in the PVN while the anorexigenic melanocortin agonist (MT-II) decreases PVN AMPK activity.

*A "hypoglycemic-like state" was induced by injection of 2-deoxyglucose.

Inset: Leptin's anorexigenic action in the hypothalamus (shown in red) involves inhibition of AMPK and activation of STAT3 and PI3K activity in the ARC and the PVN. Decreased AMPK activity results in inhibition of Neuropeptide Y (NPY)/AgRP neurons, while increased Stat3, and possibly PI3K, activities both inhibit NPY/AgRP neurons and activate Proopiomelanocortin (POMC) neurons in the ARC, which project to the PVN. Leptin inhibition of AMPK in the PVN requires the MC4R, suggesting that leptin's action is mediated by projections of POMC neurons from the ARC, but a direct effect of leptin on AMPK in the PVN is possible. Abbreviations: 3V, third ventricle;NPY, neuropeptide Y; AgRP, agouti related peptide; PI3K, phosphoinositide 3-kinase.

(Minokoshi et al., 2002). There is a dual effect, i.e., early and transient activation of AMPK by leptin directly at the level of muscle and a more sustained activation lasting at least 6 hr after a single injection of leptin (Figure 2). The latter is mediated through the hypothalamic-sympathetic nervous system (SNS) axis and α adrenergic receptors in muscle (Minokoshi et al., 2002). The direct effect may be due to increased AMP levels in muscle, whereas no change in muscle AMP concentrations was detected with the indirect effect. Hence, it could result from activation of upstream AMPK kinases (see below) or another novel mechanism. In parallel with AMPK activation, leptin suppresses ACC2 activity, thereby stimulating fatty acid oxidation in muscle (Figure 2). AMPK activation appears to be critical for leptin's effects on fatty acid oxidation in muscle (Minokoshi et al., 2002). AMPK also inhibits lipogenesis in the liver. To the extent that obesity and type 2 diabetes are associated with "ectopic" deposition of triglycerides in nonadipose tissues and resistance to leptin action (Unger, 2002), AMPK activators may bypass leptin resistance and reduce insulin resistance and the risk for type 2 diabetes. In addition to mediating leptin's action in skeletal muscle, AMPK is also a key regulator of leptin action in the hypothalamus (see below).

Adiponectin

Adiponectin is an adipocyte-secreted molecule that circulates in macromolecular complexes, increases fatty acid oxidation and glucose uptake, inhibits gluconeogenesis, and prevents atherosclerosis by direct effects on the vasculature (Goldstein and Scalia, 2004). Adiponectin stimulates AMPK phosphorylation and activity in muscle and liver in vivo and in vitro (Yamauchi et al., 2002). In muscle, the activation of AMPK is necessary for adiponectin's effects on fatty acid oxidation and glucose transport and is associated with increased AMP levels. In liver, AMPK activation is necessary for the effects on inhibition of PEPCK and glucose 6 phosphatase expression and hepatic glucose production (Yamauchi et al., 2002). Furthermore, blocking the effect of adiponectin to activate AMPK in liver using a dominant-negative AMPK abrogates the glucose lowering effect of adiponectin (Yamauchi et al., 2002). Adiponectin also activates AMPK in isolated adipocytes, although the biologic effect is unknown (Wu et al., 2003). Thus, the AMPK pathway is critical for the metabolic- and insulin-sensitizing actions of adiponectin as well as leptin.

Resistin

Resistin is a hormone secreted by adipocytes in rodents and possibly in humans that impairs insulin action on hepatic glucose production (Muse et al., 2004). Resistin decreases AMPK phosphorylation (Banerjee et al., 2004; Muse et al., 2004) in liver. Whether this is critical for the effects of resistin on hepatic glucose output remains to be determined.

Signaling through α and β adrenergic receptors activates AMPK

The β adrenergic agonist, isoproterenol, stimulates AMPK activity in isolated adipocytes (Moule and Denton, 1998), while α adrenergic receptors, as well as other G protein-coupled receptors linked to inositol trisphosphate release via Gq, activate AMPK in cultured cells (Kishi et al., 2000) and skeletal muscle (Minokoshi et al., 2002). As discussed above, α adrenergic receptors in muscle mediate leptin's effects through the hypo-

Table 2. Genetic manipulation of AMPK subunits in mouse models

Model	Brief description of phenotype	Reference
Total α1 KO	No detectable metabolic phenotype	Jorgensen, 2004
Total α2 KO	Insulin-resistance, increased catecholamine secretion in urine, no effect on muscle glucose uptake in response to contraction, increased fat mass following high-fat diet	Jorgensen, 2004 Viollet, 2003 Villena, 2004
Muscle-specific overexpression of α2 dominant-negative (muscle creatine kinase promoter)	Decreased glucose uptake in skeletal muscle in response to contraction, decreased glucose uptake in heart during ischemia, decreased fatty acid oxidation following reperfusion, increased cardiac dysfunction and apoptosis	Mu, 2001 Russell, 2004
Cardiac-specific overexpression of α2 dominant-negative (α myosin heavy chain promoter)	Decreased glucose uptake in heart, increased depletion of ATP, increased left ventricular dysfunction during ischemia	Xing, 2003
Cardiac-specific overexpression of γ2 wild-type (α myosin heavy chain promoter)	No detectable phenotype observed	Arad, 2003
Cardiac-specific overexpression of γ2 N488I mutant (α myosin heavy chain promoter)	Marked cardiac hypertrophy, glycogen accumulation, ventricular preexcitation (similar to Wolff-Parkinson-White syndrome)	Arad, 2003
Total γ3 KO	Reduced glycogen synthesis following recovery from exercise	Barnes, 2004
Skeletal muscle-specific overexpression of γ3 wild-type (myosin light chain 1 promoter)	No detectable phenotype observed	Barnes, 2004
Skeletal muscle-specific overexpression of γ3 R225Q mutant (myosin light chain 1 promoter)	Elevated level of glycogen in muscle, increased synthesis of glycogen following recovery from exercise, reduced fat accumulation, and insulin-resistance in muscle following a high-fat diet.	Barnes, 2004

KO = gene knockout mice

thalamus and sympathetic nervous system on AMPK activation and fatty acid oxidation in muscle (Figure 2).

AMPK regulates food intake by integrating hormonal and nutrient signals in the hypothalamus

Perhaps one of the most exciting recent advances is the discovery that AMPK is a master regulator of food intake. Its activity in the hypothalamus is modulated by normal physiology, i.e., fasting increases AMPK activity in the hypothalamus, while refeeding suppresses it (Figure 3) (Minokoshi et al., 2004). These effects could result from changes in glucose and insulin levels, since hyperglycemia and hyperinsulinemia suppress hypothalamic AMPK activity (Minokoshi et al., 2004). Administration of 2-deoxyglucose, which depletes cellular ATP, activates hypothalamic AMPK activity (Kim et al., 2004b). Leptin inhibits AMPK activity specifically in the arcuate and paraventricular nuclei, and this inhibition is essential for leptin's anorexigenic and weight loss effects (Figure 3). The anorexigenic effect of leptin is mediated in part through melanocortin 4 (MC4) receptor signaling. An agonist for this receptor that inhibits food intake also inhibits AMPK activity in the paraventricular hypothalamus. Mice lacking the MC4 receptor are hyperphagic and obese and fail to inhibit AMPK activity in response to either refeeding or leptin (Minokoshi et al., 2004), suggesting that MC4 receptors are necessary for the physiologic and hormonal regulation of AMPK in the paraventricular hypothalamus. ICV administration of α-lipoic acid, a short chain fatty acid and a cofactor of mitochondrial respiratory enzymes, also suppresses hypothalamic AMPK activity and is associated with decreased food intake and weight loss (Kim et al., 2004b), consistent with a critical role for hypothalamic AMPK in the central regulation of energy balance.

On the other hand, orexigenic peptides such as the gut-derived hormone ghrelin (Andersson et al., 2004) and the neuropeptide agouti-related peptide (Minokoshi et al., 2004) stimulate hypothalamic AMPK activity, indicating that this system functions bidirectionally to regulate both appetite stimulation and inhibition (Figure 3). The possibility that changes in AMPK activity can account for the food intake effects is strengthened by the fact that direct activation of hypothalamic AMPK increases food intake and body weight (Andersson et al., 2004; Minokoshi et al., 2004), while inhibition of hypothalamic AMPK decreases food intake and body weight (Kim et al., 2004a; Minokoshi et al., 2004). These changes can be explained, at least in part, by alterations in neuropeptide expression in hypothalamic neurons (Figure 3 inset) (Minokoshi et al., 2004). Since constitutive activation of hypothalamic AMPK causes leptin resistance (Minokoshi et al., 2004), it will be important to determine whether AMPK modulation is impaired in leptin-resistant states such as obesity. If so, altering AMPK signaling or downstream pathways in the arcuate or paraventricular hypothalamus may circumvent leptin resistance.

The mechanism by which changes in hypothalamic AMPK activity alters food intake is unknown. The reduction in hypothalamic AMPK activity in response to leptin was associated with decreased ACC phosphorylation in one study (Andersson et al., 2004). Reduced ACC phosphorylation would result in increased ACC activity leading to increased malonyl CoA levels which would inhibit CPT1 and hence mitochondrial fatty acid oxidation (Figure 2). While some studies in which fatty acid synthase inhibitors were injected ICV in mice suggest that increases in hypothalamic malonyl CoA levels could act as anorexigenic signals (Hu et al., 2003; Loftus et al., 2000), this is controversial. Although the role of malonyl CoA is unclear, direct inhibition of CPT1 in the hypothalamus also has anorexigenic effects (Obici et al., 2003), further implicating this pathway in the regulation of food intake. In future studies it will be important to determine whether changes in hypothalamic AMPK activity also modulate energy expenditure and whether these changes are necessary for the effects of the hypothalamic-sympathetic nervous system axis on AMPK activity in peripheral tissues (Minokoshi et al. 2002).

Transgenic animal models

The AMPK transgenic animal models generated to date have yielded surprising findings (see Table 2). Deletion of both cata-

lytic subunits i.e., α1 and α2, results in a nonviable phenotype, with the embryos dying at ~10.5 days post conception (B. Viollet, personal communication). Interestingly, this is about the same stage in development that embryos that are null for the upstream kinase LKB1 die. Deletion of α1 alone has no detectable metabolic phenotype (Jorgensen et al., 2004), whereas deletion of α2 results in mild insulin-resistance and an insulin secretory defect (Jorgensen et al., 2004; Viollet et al., 2003). The glucose intolerance in the α2 knockout (KO) mice is not due to an inherent defect in skeletal muscle glucose uptake, but probably results from both impaired insulin action and inhibition of insulin secretion owing to increased catecholamine levels in the fed state (Viollet et al., 2003). The molecular basis for this defect is not understood, but it may be due to altered sympathetic nervous activity, consistent with a regulatory role of α2 within the CNS. α2 KO mice fed a high-fat diet exhibit increased body weight and fat mass due to increased size of preexisting adipocytes, compared to wild-type animals (Villena et al., 2004).

In addition to α1 and α2 AMPK KO models, many groups have used a catalytically inactive mutant of α2 that acts as a dominant-negative form of the kinase to inhibit AMPK in vivo. Although there are inevitable caveats with genetic models, some important findings have emerged. Surprisingly, inactivation of AMPK in skeletal muscle does not have a significant effect on glucose uptake in response to contraction, although the effect of AMPK activators, AICA riboside, and hypoxia are completely abolished (Jorgensen et al., 2004; Mu et al., 2001). More recently, the increase in GLUT4 expression in response to exercise and denervation has been reported not to require AMPK (Holmes et al., 2004). In heart, inactivation of AMPK by overexpression of dominant-negative α2 significantly reduced ischaemia-induced glucose uptake, exacerbating ATP depletion and increasing the degree of injury due to apoptosis (Russell et al., 2004; Xing et al., 2003). In addition, inactivation of AMPK prevented the increase in fatty acid oxidation following reperfusion of ischemic hearts (Russell et al., 2004).

To date, there have been no genetic models described for modulation of the β subunit isoforms of AMPK. Naturally occurring mutations in human γ2 or pig γ3 lead to metabolic abnormalities in heart and skeletal muscle, respectively (see above), and this has led to the generation of several animal models involving the γ isoforms (Table 2). Cardiac-specific overexpression of human γ2 harboring a missense mutation (asparagine 488 to isoleucine, N488I) in mice results in a phenotype remarkably similar to that seen in patients with γ2 mutations (Arad et al., 2003). In contrast, overexpression of wild-type γ2 has no significant effect, indicating that the phenotype is directly attributable to the mutation (Arad et al., 2003). Skeletal muscle-specific overexpression of γ3 harboring a missense mutation of arginine 225 to glutamine (R225Q) causes a marked increase in glycogen, similar to the naturally occurring phenotype in pigs (Barnes et al., 2004), whereas overexpression of wild-type γ3 had no detectable effect. In mice in which the γ3 gene was deleted, glycogen resynthesis following exercise was reduced, whereas it was increased in the γ3 mutant transgenic animals (Barnes et al., 2004). Interestingly, the γ3 mutant transgenic mice, but not the γ3 KO mice, were protected from excess fat accumulation and insulin resistance in skeletal muscle when fed a high-fat diet, implying that γ3 may play a role in substrate utilization in skeletal muscle (Barnes et al., 2004). At present, the effect of mutations in γ2 and γ3 on AMPK activity in vivo as well as the molecular basis for altered heart and skeletal muscle metabolism is unclear. Whether the mutations increase basal AMPK activity (Arad et al., 2002; Minokoshi et al., 2004) is controversial (see above), but if they do, the high glycogen content caused by these mutations might result from a persistently elevated glucose uptake. Thus, further studies in these transgenic models will be important for understanding the role of specific AMPK subunit isoforms and naturally occurring mutations in metabolism.

Upstream kinases in the AMPK cascade

Although it has been known for over 25 years that AMPK is the downstream component of a protein kinase cascade (Ingebritsen et al., 1978) the identity of the upstream kinase (AMPKK) remained elusive until very recently. In the intervening period, however, the major regulatory phosphorylation site was identified as Thr172 within the (α) catalytic subunit of AMPK (Hawley et al., 1996). This enabled a number of studies to demonstrate that phosphorylation of Thr172 is essential for activation of AMPK and that the degree of phosphorylation of Thr172 correlates with AMPK activity. Thus, to understand the regulation of AMPK in vivo, identification of the upstream kinase responsible for phosphorylation of Thr172 is critical.

A vital breakthrough in the search for upstream kinases came from studies identifying three closely related yeast kinases that phosphorylate and activate SNF1 in vitro (Hong et al., 2003; Sutherland et al., 2003). These kinases, Elm1, Pak1, and Tos3, appear to have overlapping and redundant functions, since deletion of all three kinases is required to abolish SNF1 activity in vivo and confer a phenotype similar to a *snf1* mutant phenotype (Hong et al., 2003; Sutherland et al., 2003). The most closely related protein kinases to Elm1, Pak1, and Tos3 in the human genomic database are members of the calmodulin-dependent protein kinase kinase (CAMKK) family, closely followed by the tumor suppressor LKB1. Previous studies showed that AMPKK activity was not dependent on calmodulin or calcium (Hawley et al., 1995), thus leaving LKB1 as a strong contender for the elusive AMPKK.

At first, LKB1 appeared an unlikely candidate for AMPKK. Mutations in the LKB1 gene cause a rare hereditary form of cancer, termed Peutz-Jeghers syndrome (PJS) (Jenne et al., 1998), a role not obviously related to the known functions of AMPK. PJS is characterized by the development of multiple hamartomatous polyps in the gastrointestinal tract combined with pigmentation of the mucous membranes. Nearly 100 different LKB1 mutations have been identified in patients with PJS, and the majority of these are predicted to inactivate the kinase, although this has not been tested (Boudeau et al., 2003b). Interestingly, LKB1, like AMPK, is a heterotrimer, requiring two other proteins, mouse protein 25 (MO25) and STE20-related adaptor protein (STRAD), for catalytic activity. STRAD is a pseudokinase while MO25 stabilizes the interaction of LKB1 and STRAD (Boudeau et al., 2003a). In addition to activating LKB1, binding of MO25 and STRAD appears to localize LKB1 in the cytoplasm (Boudeau et al., 2003a).

Convincing evidence that LKB1 is a physiologically relevant AMPK kinase came from the demonstration that (1) the AMPKK activity purified from rat liver corresponds to LKB1 (Hawley et al., 2003; Woods et al., 2003) and (2) in cells lacking LKB1, AMPK activity is dramatically reduced compared to cells ex-

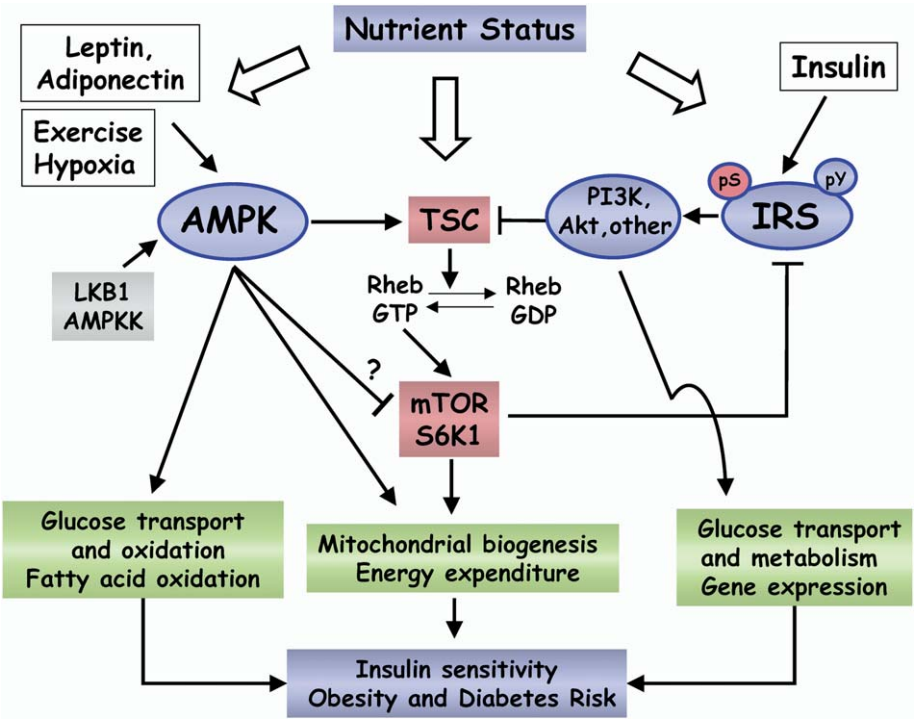

Figure 4. Proposed model for dual regulation of TSC-mTOR-S6Kinase pathway by AMPK and insulin-PI3K signaling: Potential role in insulin sensitivity and the risk for obesity and type 2 diabetes

The activity of the TSC-mTOR-S6Kinase pathway is the product of the integration of signals activating the PI3K/Akt signaling (e.g., insulin) and activating the AMPK pathway (e.g., exercise, leptin, adiponectin, or hypoxia). TSC controls the activity of the mTOR-S6Kinase signaling pathway by serving as a GTPase activating protein for the small protein Ras-like GTPase Rheb (Ras homolog enriched in brain). Once activated, TSC increases Rheb-GDP levels. Since mTOR is activated by increases in Rheb-GTP levels, this decreases mTOR activity. TSC activity is regulated inversely by AMPK and PI3K/Akt pathways. Activation of the PI3K/Akt pathway leads to inhibition of TSC activity and thus, activation of the mTOR pathway. In contrast, activation of AMPK activates TSC, thereby inhibiting the mTOR pathway. However, mTOR activity may also be directly inhibited by AMPK. Regulation of the mTOR-S6K1 pathway by AMPK may affect insulin sensitivity by modulating the phosphorylation and amount of IRS. Indeed, AMPK, by inactivating mTOR, decreases S6K1 activity, which results in decreased serine phosphorylation (pS) of IRS and increased IRS-dependent signaling. On the other hand, chronic activation of the mTOR-S6K pathway, e.g., by nutrient excess, results in reduced IRS tyrosine phosphorylation (pY) and increased serine phosphorylation (pS). These changes may reduce downstream insulin signaling and the metabolic and transcriptional effects of insulin. Alterations in the downstream biologic effects modulate the risk for obesity and type 2 diabetes. (Modified from Patti and Kahn, 2004).

pressing LKB1, and AMPK is not activated by stimuli which normally activate it (Hawley et al., 2003; Shaw et al., 2004; Woods et al., 2003). Perplexingly, the activity of LKB1 itself is not regulated directly by stimuli that activate AMPK, either in cells (Lizcano et al., 2004; Woods et al., 2003) or in skeletal muscle where there is no effect of contraction (Sakamoto et al., 2004), nor is it activated directly by AMP (Hawley et al., 2003; Woods et al., 2003). LKB1, therefore, appears to be "constitutively active," and the regulation of AMPK phosphorylation is presumably mediated via effects on AMPK itself, rather than on LKB1. It is possible that LKB1 is regulated indirectly, for instance, by altered association with MO25 and/or STRAD and/or changes in its subcellular localization.

The finding that LKB1 is an AMPK kinase yielded a double breakthrough as it not only provided identification of AMPKK, but it also provided a downstream target for LKB1, which previously had no identified physiological target. Recently, it was shown that LKB1 can activate 11 AMPK-related kinases by phosphorylation of a threonine residue equivalent to Thr172 in AMPK (Lizcano et al., 2004). This suggests that LKB1 may act as a master upstream kinase and clearly raises the possibility that some of the functions of LKB1 are mediated by the AMPK-related kinases, rather than AMPK itself. Currently, however, the physiological role of the AMPK-related kinases and of LKB1 in regulating these kinases is not clear. In yeast, there are three upstream AMPK/SNF1 kinase cascades, and, naively, one might anticipate the same number or more in the more complex mammalian system. This may be the case, since AMPK has some activity and is weakly phosphorylated on Thr172 in LKB1$^{-/-}$ cells (Hawley et al., 2003). At present, the identity of the kinase(s) that phosphorylates AMPK in the absence of LKB1 remains unknown.

Integration of AMPK and insulin signaling pathways and modulation of the risk for obesity and diabetes

Recent studies suggest that the mTor-S6 kinase 1 (S6K1) pathway may play an important role in regulation of insulin sensitivity and adiposity (Figure 4). S6K1$^{-/-}$ mice are protected against age- and high fat diet-induced obesity and insulin resistance due to increased energy expenditure associated with enhanced tissue mitochondrial content (Um et al., 2004). S6K1 phosphorylates IRS proteins on serine residues, which decreases tyrosine phosphorylation and insulin signaling (Harrington et al., 2004). In addition, S6K1 activation decreases IRS protein levels through alterations in transcription and protein degradation (Harrington et al., 2004; Shah et al., 2004). Since AMPK inhibits phosphorylation of S6K1 on Thr389, and, thus, its activation (Kimura et al., 2003), this could provide a molecular mechanism by which AMPK increases insulin sensitivity in addition to its effects on lipid metabolism.

S6K1 is regulated by an upstream cascade involving the tumor suppressor products Tuberous Sclerosis Complex (TSC) proteins 1 and 2 that control the activity of the upstream kinase mTOR (Hay and Sonenberg, 2004) (Figure 4). TSC serves as a GTPase activating protein for the small GTPase Rheb (Ras homolog enriched in brain). Once activated, TSC increases Rheb-GDP levels, which decreases mTOR activity and S6K1 activation (Figure 4). TSC activity is regulated by both AMPK and PI3K/Akt pathways (Hay and Sonenberg, 2004). Activation of the PI3K/Akt pathway inhibits TSC activity, and, thus, activates the mTOR pathway. In contrast, phosphorylation of TSC

by AMPK activates TSC, leading to inhibition of the mTOR pathway. AMPK may also inhibit mTOR activity by direct phosphorylation (Cheng et al., 2004). Thus, we propose that AMPK may affect insulin sensitivity by modulating the mTOR-S6K pathway, which in turn alters phosphorylation and protein levels of IRS (Harrington et al., 2004). Inactivation of TSC leading to activation of mTor-S6K signaling results in a negative feedback program that attenuates insulin signaling through IRS and PI3K and, hence, causes insulin resistance (Harrington et al., 2004; Shah et al., 2004). AMPK may function on both TSC and mTor directly to prevent impaired insulin signaling and obviate the metabolic changes that increase the risk for obesity and diabetes (Figure 4).

Summary and future directions

Major advances now reveal that the evolutionarily conserved cellular fuel gauge, AMPK, acts as a master regulator of metabolism not only at the cellular level but also at the whole body level. As the complexity has broadened, concepts that once had almost the status of dogma have now been challenged, such as the idea that AMPK plays a key role in contraction-stimulated glucose transport. The nature of the effects of AMPK on glycogen synthesis is also increasingly complex due to multiple regulatory factors. Other key challenges include (1) identifying the molecules that lie upstream and downstream of AMPK in the hypothalamic regulation of food intake (Figure 3), (2) determining the mechanisms by which the naturally occurring mutations in the γ subunits produce alterations in cardiac or skeletal muscle function (Table 2), and (3) determining the physiological significance of the potential effects of AMPK on the insulin signaling cascade (Figure 4).

The identification of LKB1 as an upstream kinase in the AMPK cascade is a long-awaited advance in the field. Now it will be important to determine the full extent of the role of LKB1 in regulating AMPK. Similarly, we need to determine what functions of LKB1 are mediated by AMPK and whether these are involved in cell proliferation effects such as those seen in PJS. An attractive hypothesis would link nutrient regulation of metabolism with cell proliferation, and the connection between LKB1 and AMPK is an obvious link. Identification of other potential upstream AMPK kinases will also be critical, as these could provide alternative pathways for regulation of AMPK, perhaps through different signaling mechanisms, e.g., different stimuli, or by tissue-specific expression of the different upstream kinases.

Recent discoveries demonstrating that the AMPK cascade plays a central role in the regulation of body weight, systemic glucose homeostasis, lipid metabolism, mitochondrial biogenesis, and possibly even insulin signaling make it an attractive therapeutic target. Indeed, an AMPK activator has therapeutic effects in obese and diabetic rodents and in humans, with some side effects (reviewed in Musi and Goodyear, 2002). The challenge is high, since AMPK's effects are widespread. Furthermore, a therapeutic agent would ideally activate AMPK in peripheral tissues (to increase fatty acid oxidation and glucose uptake and reduce gluconeogenesis) while inhibiting it in the hypothalamus (to reduce food intake and body weight). Research in this field will undoubtedly continue to elucidate the intricate biology of energy balance and provide critical information to understand the pathogenesis of obesity and type 2 diabetes.

Supplemental Data

Supplemental Data includes one table and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/1/1/15/DC1/.

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