AMPK in Health and Disease

GREGORY R. STEINBERG AND BRUCE E. KEMP

Protein Chemistry and Metabolism, St. Vincent’s Institute of Medical Research, University of Melbourne, Fitzroy, Victoria, Australia

I. Introduction and Historical Background 1026
   A. Discovery of the “adenylate charge” regulatory kinase 1026
   B. Metabolic stress sensing in yeast 1027
   C. Emergence of AMPK subfamily 1027

II. Structure and Regulation of AMPK 1027
   A. AMPK genes, transcripts, and variation 1027
   B. AMPK subunit composition: broad roles 1029
   C. Subunit structures 1029
   D. Nucleotide binding to the \gamma subunit 1031
   E. Mechanism of autoregulation 1032
   F. Myristoylation of the \beta subunit 1033
   G. AMPK interaction with glycogen 1034
   H. AMPK substrate recognition 1035

III. Regulation by Phosphorylation 1036
   A. Activating upstream kinases 1036
   B. Multisite subunit phosphorylation 1037

IV. AMPK Regulation of Carbohydrate Metabolism 1038
   A. Glucose uptake 1038
   B. Glycolysis 1040
   C. Glycogen metabolism 1040
   D. Hepatic glucose production 1041
   E. Hypothalamic glucose sensing 1042

V. AMPK Regulation of Lipid Metabolism 1042
   A. Fatty acid uptake 1042
   B. Fatty acid partitioning 1042
   C. Triacylglycerol turnover 1044
   D. Mitochondrial biogenesis 1045
   E. Cholesterol synthesis 1046

VI. AMPK Regulation of Protein Metabolism, Cell Polarity, Growth, and Apoptosis 1046
   A. Protein synthesis 1046
   B. Cell growth and apoptosis 1048
   C. Regulation of cell polarity and ion flux 1048

VII. Integrative Role of AMPK as a Regulator of Whole Body Energy Metabolism 1049
   A. Exercise 1049
   B. Regulation of appetite 1050
   C. Regulation by nutrients 1051
   D. Endocrine regulation 1052

VIII. AMPK Dysregulation in Disease 1054
   A. Aging and longevity 1054
   B. Obesity and the metabolic syndrome 1055
   C. Cardiovascular disease and reperfusion injury 1056
   D. Cancer 1056
   E. Dementia, neurogenesis, and stroke 1057

IX. Opportunities for Therapeutics 1057
   A. Metformin 1057
   B. Thiazolidinediones 1058
   C. Life-style interventions 1059
   D. Ciliary neurotrophic factor 1059
   E. Natural compounds 1059
   F. Small molecule activators 1060

X. Conclusions and Future Directions 1061
metabolic coupling of anabolic and catabolic pathways. The “adenylate charge hypothesis” for the activities are modulated allosterically by the ratio of [ATP]/[ADP]/[AMP]. The “adenylate charge hypothesis” for enzymes in metabolic pathways such as glycogen phosphorylase, glycogen synthase, pyruvate dehydrogenase, and pyruvate kinase (269). Both acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (HMGR) were inactivated by their respective associated kinases present in liver cytosol and could be reactivated by phosphatase treatment (43, 224).

It was recognized early on that both the HMGR and ACC kinase activities were stimulated by nucleotides. At first, HMGR kinase was thought to be regulated by cAMP (41), then ADP (61, 366), but it was subsequently shown to be potently (micromolar range) activated by AMP (145), then ADP (61, 366), but it was subsequently shown to be potently (micromolar range) activated by AMP (145) with the apparent activation by ADP due to AMP contamination. Yeh et al. (565) were first to report that the ACC kinase activity associated with acetyl-CoA carboxylase (79) and HMG-CoA reductase, which are respectively (41) the rate-limiting regulatory enzymes for fatty acid and cholesterol synthesis. At this time, enzyme-catalyzed protein phosphorylation was emerging as a regulatory mechanism for enzymes in metabolic pathways such as glycogen phosphorylase, glycogen synthase, pyruvate dehydrogenase, and pyruvate kinase (269). Both acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (HMGR) were inactivated by their respective associated kinases present in liver cytosol and could be reactivated by phosphatase treatment (43, 224).

I. INTRODUCTION AND HISTORICAL BACKGROUND

A. Discovery of the “Adenylate Charge”

AMP-activated protein kinase (AMPK) is an $\alpha\beta\gamma$ heterotrimer comprising an $\alpha$-catalytic subunit with $\beta\gamma$-regulatory subunits. Although mammalian AMPK was purified and sequenced in 1994 (76, 123, 341), it has a long history dating back to independent studies in the laboratories of Ki Han Kim at Purdue University and David Gibson at Indiana University, who reported a protein kinase activity associated with acetyl-CoA carboxylase (79) and HMG-CoA reductase, which are respectively (41) the rate-limiting regulatory enzymes for fatty acid and cholesterol synthesis. At this time, enzyme-catalyzed protein phosphorylation was emerging as a regulatory mechanism for enzymes in metabolic pathways such as glycogen phosphorylase, glycogen synthase, pyruvate dehydrogenase, and pyruvate kinase (269). Both acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (HMGR) were inactivated by their respective associated kinases present in liver cytosol and could be reactivated by phosphatase treatment (43, 224).

The name AMP-activated protein kinase was first proposed in 1988 when Munday et al. (351) demonstrated that the enzyme formerly known as ACC kinase-3 was the primary kinase responsible for the large reductions in ACC V_max. The name AMP-activated protein kinase was adopted, in 1989 when HMG kinase and ACC kinases were recognized to be one and the same by Carling et al. (77), who showed the respective kinases copurified through six steps from rat liver. Because AMPK is activated under conditions of low energy charge and typically inhibits anabolic reactions and promotes catabolism, it is arguably the best example of the “adenylate charge hypothesis” in action. For example, in response to a reduction in the energy charge (decrease in ATP and increase in AMP), AMPK switches off anabolic pathways such as fatty acid, triglyceride, and cholesterol synthesis as well as protein synthesis and transcription that consume ATP, and switches on catabolic pathways that generate ATP, such as fatty acid oxidation and glycolysis. While the name AMPK has been universally adopted and was entirely appropriate when AMPK was considered a metabolic stress-sensing protein kinase under the primary influence of cellular AMP concentrations, it is now known that Ca^{2+} pathways, mediated by calmodulin-dependent kinase kinase $\beta$ can activate AMPK independent of cellular AMP levels. Moreover, AMPK influences energy metabolism at the organism level by promoting food intake. For this reason, an alternate name (PKE energy kinase) has been proposed to embrace AMPK’s broader mechanisms of regulation and physiological functions (544). Nevertheless, the mammalian AMPK remains sensitive to AMP (allosteric activation) whatever the upstream kinase utilized; for this reason, AMPK remains appropriate. However, the yeast snf1 kinase (AMPK homolog), and possibly other orthologs yet to be tested, is not activated by AMP. Once the mechanism of control is understood in more detail across the AMPK subfamily, the need for a possible name change will become more apparent.
B. Metabolic Stress Sensing in Yeast

AMPK has homologs in all eukaryote organisms. When mammalian AMPK was purified and sequenced (339, 551), the catalytic subunit was found to correspond to the *Saccharomyces cerevisiae* protein kinase Snf1 identified in 1986 (85). Genetic studies had revealed that SNF1 mutants (sucrose nonfermenting) were unable to grow on sucrose because of a defect in activation of glucose-repressed genes when the carbon source was switched from glucose to sucrose. One of these glucose-repressed genes is *SUC2*, which encodes invertase, the enzyme secreted to hydrolyze sucrose into fructose and glucose. SNF1 mutants also do not grow on nonfermentable carbon sources, such as glycerol, ethanol, and lactate, which also require derepression of glucose-repressed genes (reviewed in Refs. 80, 192, 236). It has been estimated that Snf1 kinase controls the expression of over 400 genes out of several thousand, the transcription of which changes with glucose deprivation (reviewed in Ref. 297). In the presence of glucose, the Mig1 repressor binds the glucose-repressed gene promoters. But, in the absence of glucose, Snf1 kinase is activated and phosphorylates Mig1, altering its association with the transcriptional coactivators Cyc8 and Tup1 (383), as well as causing Mig1 to migrate to the cytoplasm and allow derepression of glucose suppressed genes (reviewed in Refs. 184, 192). Other substrates are involved as mutation of the Snf1 kinase site in Mig1 alone is not sufficient to block regulation. Recent studies from the laboratories of Thompson and Berger and co-workers (238a) have shown that Snf1 kinase phosphorylates histone H2b on Thr-39, and mutation of this site alone is sufficient to give a ΔSnf1 phenotype and block growth on nonfermentable carbon sources (238). Although knowledge of Snf1 protein kinase’s role in regulating transcription was well advanced by 1994, no biochemical studies had been done and no metabolic enzyme substrates were known. Following the discovery that AMPK was a homolog of Snf1 kinase, it was quickly found that ACC was a substrate for Snf1 kinase (339, 551), and yeast ACC activity was inhibited by glucose deprivation provided Snf1 kinase was present (551). These observations provided compelling evidence that the AMPK/ Snf1 kinases were key protagonists in a highly conserved metabolic stress-sensing pathway responsible for matching metabolic energy demand with supply. This conservation extends to plants, with the plant homolog RKIN1 able to complement SNF1 in yeast (76). With glucose deprivation, it was expected that AMP would increase and trigger Snf1 kinase activation. Indeed, Snf1p activity increases dramatically with glucose withdrawal, and this correlated with increases in AMP/ATP ratio (536); however, Snf1 kinase is not directly activated by AMP in vitro (339, 536). The signaling mechanism for activation of Snf1 kinase in response to glucose deprivation remains a conundrum.

C. Emergence of AMPK Subfamily

Multiple mammalian AMPK subunit isoforms encoded by distinct genes were identified with two α subunits (465), two β subunits (91, 497), and three γ subunits (95). The regulatory β and γ subunits associated with the mammalian α-catalytic subunit have yeast Snf1-interacting protein counterparts with the β subunit related to the *S. cerevisiae* GAL83-SIP1-SIP2 gene family and the γ subunit corresponding to Snf4 (158, 464, 549). The corresponding AMPK subunit genes are named PRKA followed by the subunit identifier A1, A2, B1, B2, G1, G2, or G3 (e.g., PRKAG3). They are distributed across five chromosomes [α1(5p12), α2(1q31), β1(12q24.1), β2(1q21.1), γ1(12q12-14), γ2(7q35-36), γ3(2q35)]. The AMPK α1 and α2 subunits are similar (~550 residues), both having conserved NH2-terminal catalytic domains and divergent COOH-terminal tails. The AMPK catalytic domains are found in the branches of the kinome belonging in the CAMK group (calcium/calmodulin-dependent protein kinases) between the MLCK and CaMK subfamilies (310). The 12 most closely related kinases are MELK, BRSK2, BRSK1, NUAK2, NUAK1, QIK, QSK, SIK, MARK4, MARK3, MARK1, and MARK2. A feature of these AMPK-related protein kinases is that they are activated by the AMPK upstream kinase LKB1 (299). The AMPK β subunits (~270 residues) vary in the first 65 residues but are otherwise highly conserved. In contrast, the γ subunits vary in length [γ1(331), γ2(569), and γ3(489)], but beyond their diverse NH2 termini share a conserved COOH-terminal ~300 residues containing four CBS repeat sequences (see below). Thus mammals differ from yeast in having multiple α and γ subunits but only two β subunits rather than the three present in *S. cerevisiae*. There is even greater diversity in plants and malaria, where βγ subunit fusions occur in addition to regular β and γ subunits (see Ref. 397). The plant βγ fusion subunits (β3) consist of the β-carbohydrate-binding module (CBM) sequence fused to an intact γ.

II. STRUCTURE AND REGULATION OF AMPK

A. AMPK Genes, Transcripts, and Variation

Since several of the AMPK subunits (α1, γ2, γ3) can occur in multiple forms generated by alternate initiation or splicing, we have adopted nomenclature where the longest transcript is denoted “1” and shorter transcripts are given higher values (e.g., γ3.1, γ3.2) (see Tables 1 and 2).

1. Human PRKAA1

The α1.3 isoform of 550 residues appears to be the most commonly expressed alternate transcript. There is
also an alternate 10 exon transcript version encoding a longer form of α, termed αL, that contains an in-frame “cassette-type” exon 3A of 45 bp between exons 3 and 4 that encodes a 15-residue insert between the D- and E-helices of the kinase large lobe (see below). The transcript is present in heart, liver, skeletal muscle, adipose tissue, and peripheral blood lymphocytes but does not appear in other mammals (B. J. W. van Denderen, unpublished data). There are four SNPs reported with only two in exons 1 (Met1Leu) and 9 (Val524Ala), respectively, giving rise to residue changes. Alternate initiation or the Met1Leu SNP could permit an upstream initiation at residue 9 Met, which provides for a 574 residue α1.1 isoform translated from 10 exons having the NH2-terminal sequence MRRLSSWRK-, compared with MATAEKQKH- for the 550 residue isoform. The alternate possible 574 (α1.1) and 559 (α1.2) residue isoforms have not been characterized biochemically.

2. Human PRKA2

There are six SNPs reported within the exons with two only in exons 3 (Ser84Ileu) and 7 (Val293Leu), respectively, giving rise to residue changes. In the case of PRKA2, intrinsic SNPs have been linked to cholesterol and low-density lipoprotein (LDL)-cholesterol levels in a cohort of 2,777 Caucasian females (459). There are two SNPs and one deletion [Met6Leu, Pro198Leu, and (65–67, SerProPhe)] reported for exons 1, 2, and 3, respectively. Rare mutations in γ2 giving rise to a cardiac glycogen storage phenotype are mentioned in section mD.

3. Human PRKAB1

There are four SNPs reported within the β1 exons but only two in exons 1 (Pro20Ala) and 3 (His135Leu), respectively, giving rise to residue changes.

4. Human PRKAB2

There are five SNPs reported within the β2 exons with only two in exons 4 (Val155Leu) and 5 (His211Tyr), respectively, giving rise to residue changes.

5. Human PRKAG1

There is one SNP reported within γ1 exon 5 (Thr89Ser). While this is a conservative substitution, it is nevertheless adjacent to Asp-90, which is the residue responsible for binding the AMP ribose, and therefore could conceivably have a subtle effect on AMPK regulation.

6. Human PRKAG2

There are two additional γ2 isoforms, with γ2.2 (C) transcribed from Met-44 in exon 2 (525 residues) and isoform γ2.3 (B) from Met-242 in exon 6 (328 residues). There are two SNPs and one deletion [Met6Leu, Pro198Leu, and (65–67, SerProPhe)] reported for exons 1, 4, and 3, respectively. Rare mutations in γ2 giving rise to a cardiac glycogen storage phenotype are mentioned in section mD.

7. Human PRKAG3

The β3 isoform 2 is initiated at Met-26 in exon 3 (γ3.2). There are a total of nine reported SNPs, with six predicted to alter the residue (Pro71Ala exon 3, Phe139Leu exon 4, Leu153Val exon 4, Gln260Arg exon 8, Arg340Trp exon 10, and Ala484Val exon 13). The SNPs at 260, 340, and 484 occur in the sequence containing the four repeat CBS sequences involved in nucleotide (AMP/ATP) binding, but their effects if any on AMPK regulation have not been reported. In a study of 761 obese subjects, Costford et al. (108) reported 87 variants in human PRKAG3 and its flanking regions. There were six common variants, including three intronic polymorphisms, one synonymous variant, and two nonsynonymous variants (Pro71Ala and Arg340Trp). There were 81 rare variants of which 30 were coding variants. Weyrick et al. (534) studied 1,061 subjects and found no association between PRKAG3 SNPs and prediabetic conditions (body fat distribution, insulin resistance, or insulin secretion) but did detect a significant associa-

<p>| Table 1. Human AMPK genes and transcripts |
|---------------------------------|-------|-------|------|----|</p>
<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Size, kb</th>
<th>Transcript, kb</th>
<th>Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1</td>
<td>PRKAA1</td>
<td>38.816</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>α2</td>
<td>PRKAA2</td>
<td>70.013</td>
<td>2.44</td>
<td>9</td>
</tr>
<tr>
<td>β1</td>
<td>PRKB1</td>
<td>13.668</td>
<td>2.388</td>
<td>7</td>
</tr>
<tr>
<td>β2</td>
<td>PRKB2</td>
<td>17.44</td>
<td>5.418</td>
<td>7</td>
</tr>
<tr>
<td>γ1</td>
<td>PRKG1</td>
<td>16.537</td>
<td>1.689</td>
<td>12</td>
</tr>
<tr>
<td>γ2</td>
<td>PRKG2</td>
<td>319.77</td>
<td>3.405</td>
<td>16</td>
</tr>
<tr>
<td>γ3</td>
<td>PRKG3</td>
<td>9.408</td>
<td>2.301</td>
<td>14</td>
</tr>
</tbody>
</table>

<p>| Table 2. Alternate transcripts |
|---------------------------------|-------|----------|</p>
<table>
<thead>
<tr>
<th>Alternate Transcripts</th>
<th>Size, residues</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1.1</td>
<td>574</td>
<td>Uncharacterized</td>
</tr>
<tr>
<td>α1.2</td>
<td>550</td>
<td>Uncharacterized</td>
</tr>
<tr>
<td>α1.3</td>
<td>550</td>
<td>Common</td>
</tr>
<tr>
<td>α2.1</td>
<td>552</td>
<td>Common</td>
</tr>
<tr>
<td>β1.1</td>
<td>270</td>
<td>Common</td>
</tr>
<tr>
<td>β2.1</td>
<td>272</td>
<td>Common</td>
</tr>
<tr>
<td>γ1.1</td>
<td>331</td>
<td>Common</td>
</tr>
<tr>
<td>γ2.1</td>
<td>560</td>
<td>Common</td>
</tr>
<tr>
<td>γ2.2 (C)</td>
<td>525</td>
<td>Uncharacterized</td>
</tr>
<tr>
<td>γ2.3 (B)</td>
<td>328</td>
<td>Uncharacterized</td>
</tr>
<tr>
<td>γ3.1</td>
<td>489</td>
<td>Common</td>
</tr>
<tr>
<td>γ3.2</td>
<td>444</td>
<td>Uncharacterized</td>
</tr>
</tbody>
</table>
tion between LDL-cholesterol and apolipoprotein B-100 levels and two SNPs, Pro71Ala (rs692243) in exon 3 and rs6436094 located in the 3’-untranslated region (UTR). Both studies on PRKAG3 SNPs indicate that many more SNPs will be discovered for the AMPK genes as more sequencing is done on larger populations.

B. AMPK Subunit Composition: Broad Roles

The AMPK subunits show differential tissue-specific expression and activation. Early on, Northern blots revealed that AMPK α1 was relatively evenly distributed across rat heart, liver, kidney, brain, spleen, lung, and skeletal muscle (465), whereas AMPK α2 subunit was highly abundant in rat skeletal muscle and, to a lesser extent, in heart and liver followed by brain and kidney and detectable in lung (465, 516). Quantitative real-time PCR (qRT-PCR) of mouse tissues has shown that AMPK α1 is evenly distributed across liver, kidney, lung, heart, red vastus, and brain, but with higher levels in adipose tissue and lower levels in white vastus, spleen, and pancreas (N. Dzamko, unpublished data). The mouse tissue qRT-PCR measures of AMPK α2 showed highest levels in the red vastus, followed by white vastus then heart kidney and liver, with very low levels seen in the lung, brain, and adipose tissue and negligible amounts in the pancreas and spleen (Dzamko, unpublished data).

The β1 subunit shows a widespread expression profile comparable to α1 based on Northern blots, qRT-PCR, and immunoblotting. High expression of β2 occurs in skeletal muscle and to a lesser extent heart (91, 497) (Dzamko, unpublished data).

The tissue distribution of the γ subunits has been studied reasonably comprehensively (95, 309). The γ1 isoform is ubiquitously expressed, whereas the γ3 isoform shows the most restricted expression, being confined to skeletal muscle (309). The γ2 isoform is also widely expressed but highest in the heart, followed by brain, placenta, and skeletal muscle (95). Yu et al. (569) found in mice the γ3 is most highly expressed in fast-twitch glycolytic muscle (type IIb) with no detectable expression in the slow-twitch oxidative soleus muscle that contains type I fibers. Type IIa fast-twitch fibers and red oxidative glycolytic-muscle have intermediate levels of γ3 expression. The expression profile of γ3 reported for the mouse is consistent with results from pigs carrying the RN- mutation (γ3 R200Q; Ref. 333), which have a glycogen storage disease of the muscle with glycogen accumulation in the white muscle (140). The longer isoform γ3.1 (termed γL; Ref. 569) is the predominant form in mouse muscle, whereas γ3.2 (γ3S) is thought to be the form expressed in human muscle (569).

C. Subunit Structures

The major structural elements of the AMPK subunit sequences are illustrated in Figure 1. The α subunit comprises an NH2-terminal kinase domain followed by an autoinhibitory sequence (AIS) and a subunit interacting domain (β-SID) that binds β (112, 227) (Fig. 1). The β subunit contains two characterized elements, a mid-molecule glycogen binding domain (GBD), now termed CBM (215, 395), and a COOH-terminal subunit binding sequence responsible for binding α and γ (αγ-SBS) (227) (Fig. 1). The γ subunit comprises four tandem repeat

Fig. 1. AMPK subunit functional features. Mammalian AMPK subunits showing regions of shared known structure are shown (colored cylinders). Numbers above α1 and β1 subunit cylinders denote NH2- and COOH-terminal residues of crystal structures. Light blue cylinder in S. cerevisiae sequence denotes insert from 335); -SBS, domain (Fig. 1). The β1 subunit comprises four tandem repeat.

Physiol Rev • VOL 89 • JULY 2009 • www.prv.org

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on November 6, 2017
sequences called CBS motifs, named after related sequences in cystathionine β synthase first recognized by Alex Bateman (35) (Fig. 1). The mammalian αβγ heterotrimer exists in solution as a single species (monomer) based on light scattering (556) and analytical ultracentrifugation analyses (226).

Substantial progress has been made in the past 2 years towards the complete structure of the AMPK αβγ heterotrimer. The crystal structures of the isolated catalytic domain of the mammalian α2 (PDB ID: 2H6D) and S. cerevisiae α subunit have been solved (PDB ID: 2H6D) (363, 420) together with the core αβγ interacting complex comprising a COOH-terminal fragment of β subunit, the complete γ1 subunit, and a C-terminal fragment of the α subunit (11, 501, 556). Broadly speaking, the structures of ~73% of the α subunit, 72% of the β subunit, and 100% of the γ1 structure are now known notwithstanding some regions of disorder in the α and β fragments (Figs. 1 and 2). The major gap in our knowledge is the juxtaposition of the α-subunit catalytic domain and its associated autoinhibitory sequence with the αβγ core complex. The first structure of the αβγ core complex was reported for the fission yeast Schizosaccharomyces pombe by Townley et al. (501). The β fragment forms a β-sheet structure that serves as a subunit-binding platform for both α and γ, as shown in Figure 2 [marked αγ-SBS green for both the α fragment (blue) and γ (red)]. Beyond the β subunit β-sheet platform, the backbone extends over the α-subunit interacting domain. This segment of the β sequence is devoid of its own hydrophobic core but instead makes hydrophobic contacts across the α-subunit interacting domain (501). On the other hand, the βγ interaction contains fewer hydrophobic interactions with the interface stabilized by hydrogen bonding (501). Consistent with this interpretation, alanine scanning mutagenesis of β (260–270) has shown that Tyr-267 side chain is important and, to a lesser extent, Thr-263 for stabilizing the βγ interaction, whereas the remaining residues can be replaced by Ala (226). The β Tyr-267 is juxtaposed to γ1 aromatic residue, Phe-51 in the mammalian structure (556). The α-subunit COOH-terminal fragment forms a discreet α-helical/β-strand domain that shares topology with components of the AMPK-related MARK kinases (499). The Townley structure has provided a clear picture explaining the earlier truncation mutagenesis results that showed that the COOH-terminal 25 residues of β (246–270) were essential for γ binding, whereas α binding required 85 residues β (186–270) (227) (Fig. 2).

The γ subunit forms an elliptical disk ~60 Å in diameter and 30 Å in depth made up of the four CBS motifs packed as pairs of Bateman domains (11, 501, 556). Each CBS motif contributes a pair of antiparallel strands that associate to form a Bateman domain with a pair of adenyl binding sites (see below) (Figs. 1 and 3).

**Fig. 2.** AMPK αβγ core structure. A: graphical representation of composite features from the mammalian AMPK heterotrimer and yeast orthologs: in blue, the α-subunit structures comprising a mammalian β-subunit interacting domain (SID) and regulatory sequence possibly unique to *S. cerevisiae* (RS); in green, the β-subunit structures [carbohydrate-binding molecule (CBM) from *S. cerevisiae* and αγ-subunit binding sequence (SBS) from *S. pombe*]; in red, mammalian γ-subunit structure. Three AMP molecules (yellow) evident in the mammalian AMPK structure and one ADP molecule (orange) seen in the *S. pombe* structure are shown in stick representation. B: 90° rotation of A.
The *S. cerevisiae* structure shows additional features to the mammalian and *S. pombe* structures, some of these are species specific while others are likely to be common to the architecture of mammalian AMPKs. In particular, the β subunit of the *S. cerevisiae* structure shows GBD nestled alongside γ, near the βγ binding junction (11). Previously, the GBD domain structures for β1 and β2 had been solved in the presence of β-cyclodextrin (396). On the basis of the β-cyclodextrin-GBD domain structure, it was possible to identify the sugar-binding site (see below). The position of the β-cyclodextrin has been modeled into the *S. cerevisiae* structure (11) to provide a guide to where glycogen is bound in the complex.

A larger fragment of the *S. cerevisiae* α subunit was crystallized: this now extends the known structure by ~30 residues (11). This makes it clear that the α-backbone path from the α-subunit interaction domain goes down over the γ subunit to a helix-loop-helix structure referred to as a regulatory sequence marked RS in the *S. cerevisiae* structure (blue in Fig. 2). There is uncertainty whether this feature will be found in the mammalian α-subunit structure, as *S. cerevisiae* and *S. pombe* both have insert sequences in this region, depending on how the alignments are done. Comparing the *S. cerevisiae* and mammalian α-subunit alignments, there are ~110 common residues between the known COOH-terminal α structure and the NH2-terminal kinase catalytic domain (Fig. 1). This segment of unknown structure contains the autoinhibitory sequence [α (313–335); see below].

### D. Nucleotide Binding to the γ Subunit

The structural studies have revealed that γ contains four adenylation binding sites comprising a helix-loop-strand motif with one contributed from each CBS unit (235, 501, 556). The mammalian γ structure was solved with 3 mol of AMP bound, two of which were interchangeable with ATP (see below) (556). In contrast, the *S. pombe* γ structures have been solved with either 1 mol of AMP bound (501) or 2 mol of ADP bound, respectively (235). To simplify the nomenclature for the binding sites, we have proposed that they be named 1–4 corresponding to their CBS unit location (249). The adenylation binding sites are surface pockets where the adenine moiety sits in a hydrophobic environment making hydrogen bonds to two strands via backbone groups (556). There are no aromatic residues in the AMP binding pockets, a feature of the

---

**Fig. 3.** The γ1-subunit nucleotide binding sites. **A:** graphical representation of the composite nucleotide binding sites for mammalian γ1 and *S. pombe*. Nucleotide binding sites 1-4 are shown with the corresponding old nomenclature used previously by Xiao et al. (556) and Jin et al. (235) shown in parentheses. AMP molecules occupy sites 1, 3, and 4, with an ADP occupying site 2 from the *S. pombe* structure (see Fig. 1). **B:** detailed view of AMP site 3 in mammalian γ1, showing hydrogen bonds between hydroxyl groups of the ribose moiety and Asp-244. **C:** hydrogen bond interactions between phosphate groups of 3 AMP molecules and basic residues lining the solvent accessible core of γ1.
allosteric AMP binding site in phosphorylase. The adenylyl group is also anchored through its ribose 2' and 3' hydroxyls to Asp residues in three of the four CBS units (CBS1, Asp90; CBS3, Asp245; CBS4, Asp317; see Fig. 1). These Asp residues are conserved across all species of AMP γ subunits. In CBS2, the corresponding position for the Asp in the structure is substituted with Arg-171, and in the mammalian structure this site is unoccupied (556). However, in the ADP-bound form of the S. pombe structure, both CBS2 and CBS4 are occupied with ADP showing that site 2 in CBS2 can bind nucleotide (235). In this case, the equivalent γ1 Arg-171 side chain is pointing away and the ADP ribose 2', 3' hydroxyls hydrogen bonds to Asp-250, Gln-251, and Ser-252 (S. pombe) contributed by the β-subunit 12-residue extended loop termed the β flap (235). In the mammalian structure, the corresponding β-subunit loop carrying Asp-224 (h β1) is disordered (556). Whether a nucleotide can bind to the mammalian CBS2 with the aid of the β1 Asp-224 in the sequence DPA252 remains to be determined.

A particularly striking feature of the γ's four AMP binding sites is that the phosphate groups point inwards in a solvent accessible core of the γ subunit that contains a constellation of basic residues. Thus, rather than having basic individual residues allocated to the phosphate groups from each AMP, there is a high degree of connectivity (see Fig. 3). The key basic residues are Arg-70, His-151, Arg-152, Lys-170, His-298, and Arg-299 (556). The four CBS units make varying contribution of basic residues with Arg-70 from CBS1 and none from CBS3. The CBS2 unit contributes His-151, His-152, and Lys-170 and CBS4 contributes His-298 and Arg-299 (Fig. 1). As mentioned, the initial S. pombe structure contained a single AMP bound to CBS4 (501). This site was also occupied in the mammalian structure but was found not to exchange and was present during purification of both the holoenzyme and the crystallized αβ core complex (556). The CBS1 and CBS3 sites in the mammalian structure bound AMP but would readily exchange ATP (556). These results are consistent with the reported early binding studies that found each Bateman domain (439) bound a single mole of AMP or ATP.

Since no biochemical studies have been reported for S. pombe, it is not clear whether nucleotide binding affects activity for either AMP or ADP. Mammalian AMPK was initially thought to respond to ADP (61, 366), but it was subsequently found that AMP was the genuine activator (145). What is clear is that mammalian AMPK is activated allosterically by AMP and inhibited by ATP, and the structure now identifies these as the exchangeable sites CBS1 and CBS3. However, we are left with several questions regarding the function of the nonexchangeable AMP at the CBS4 site as well as the question of whether the mammalian CBS2 site binds nucleotide at all.

The γ-subunit structures have provided an important road map for understanding the structure-function relationships of naturally occurring glycogen storage disease causing mutations. These mutations include the Hampshire pig Rendement Napole (RN−) mutation in γ3 (Arg302Gln) equivalent to Arg-70 in the γ1 structure as well as 10 human mutations in γ2 that give rise to Wolf-Parkinson-White cardiomyopathy (reviewed in Ref. 19). When mapped on the human γ1 structure, 8 of the 10 mutations occur in amino acids whose side chains are in proximity to the AMP binding sites (556). Notably, four mutations in γ2 Arg302Gln (γ1 Arg-70), His383Arg (γ1 His-151), Arg531Gly/Gln (γ1 Arg-299) correspond to the key phosphate interacting constellation of basic residues in the γ1 solvent accessible core structure. While no disease-causing mutations have been detected in γ1, a transgenic mouse with muscle specific expression of the γ1 Arg70Gln mutation resulted in constitutive activation of AMPK in the muscle and accumulation of glycogen (34). In addition, in a screen of obese and lean individuals, Costford et al. (108) have identified two families carrying an Arg225Trp mutation in γ3 who have double basal glycogen levels and reduced intramuscular triglycerides. This mutation occurs in CBS1 and corresponds to the human γ2 Arg302Gln and the pig γ3 Arg200Gln mutation mentioned above.

E. Mechanism of Autoregulation

Many protein kinases are autoregulated by a variety of mechanisms (258). In the case of the CAMK branches of the kinome to which AMPK belongs, smMLCK, skMLCK, TTN, CaMK1, and CaMK2 have all been found to contain autoregulatory sequences beyond the COOH terminus of the catalytic domain which blocks catalytic activity (258). In the case of TTN (213) and CaMK1 (168), structural studies have shown that the autoregulatory sequence binds to the protein substrate groove and extends into the active site (257), although the extent of interaction is less for CaMK2 (418). While both CaMK1 and TTN are members of the CAMK group in the kinome, they occur in different branches/subfamilies. It is possible that the autoregulatory features may be more closely conserved within each major branch of the CAMK group. In the case of the calmodulin-activated protein kinases, the calmodulin binding sequence overlaps the autoregulatory sequence and provides a mechanism for relieving the autoinhibition when calmodulin binds (258). Typically truncation mutagenesis to remove the autoregulatory sequences permits the kinase to become constitutively active (i.e., independent of calmodulin). Like many kinases (492), AMPK has an absolute requirement for phosphorylation of its activation loop Thr-172 for activity (186). In addition to Thr-172 phosphorylation, it was found that the α1
(1-312) was active in the absence of γ or β, whereas a longer version α1 (1–392) was inactive, consistent with the presence of an autoregulatory sequence between 312 and 392 (112). Further detailed mapping of the autoinhibitory sequence has shown that it resides within a 23-residue sequence 313–335 (380). Pang et al. (380) have used homology modelling based on the crystal structure of the AMPK-related kinase, MARK2 (382), to position the autoinhibitory sequence of AMPK. The MARK kinases occur on the same main branch of the CAMK group as AMPK. The modeled AMPK autoinhibitory sequence is present as a 3-helix unit (residues 290-335) juxtaposed to the small lobe of the kinase. While there is low sequence identity between MARK2 and AMPK (232) in this region, the model is nevertheless attractive in that the positioning of the autoregulatory sequence could modulate the orientation of the kinase small lobe to block catalytic activity. The autoinhibitory sequence corresponds to the position of the ubiquitin-associated domains (UBA) found within other members of the AMPK-related protein kinase subfamily that includes MARK2 kinase. Furthermore, the MARK2 kinase UBA domain plays an important role in the regulation of phosphorylation by the upstream kinase LKB1 (232). In support of this model, mutation of the residues Leu328Gln and Val298Gly that are conserved across the MARK family result in a constitutively active AMPK (380). Formal proof of the model of AMPK autoregulation will depend on a crystal structure of the catalytic domain containing the autoregulatory sequence.

In addition to the autoregulatory sequences identified in the α subunit, Hardie and colleagues (441) have reported a pseudosubstrate sequence [human γ1; LEIDADYSSLRNKIH1531R152LP(V155)IDPE1590] within the γ-subunit CBS2 sequence that closely resembles the consensus substrate recognition sequence for AMPK in ACC (GIJOAJDGLAFHRMRRSSMS70GLHL) in terms of the juxtaposition of basic and hydrophobic residues (underlined) with Val-55 occupying the site corresponding to the phosphorylatable Ser-79 in ACC1 (440, 441). This model requires the pseudosubstrate sequence in CBS2 to deform and associate with the catalytic domain substrate-binding site. It is envisaged that the binding of AMP to the γ subunit leads to association with His-151, Arg-152 and dissociation of the pseudosubstrate sequence from the active site substrate-binding groove. Since the orientation of the catalytic domain to CBS2 is not yet known, there is insufficient structural information to test whether this model is plausible. Nevertheless, when the γ2 pseudosubstrate peptide Val155Ser mutation was made, this residue was autophosphorylated (441), implying that the autoregulatory sequence was accessible to the active site provided the phosphorylation was an intramolecular, rather than an intermolecular, event. Further evidence in support of the model was obtained in yeast where mutation of the corresponding residues of the p-3 Arg-146 and p-12 Leu-137 in yeast γ (SNF4) gave a phenotype of constitutive AMPK activity (Snf1p) (441). Overall, the biochemical data are strongly supportive of the γ pseudosubstrate model, but the major uncertainty remains the structural likelihood of the γ CBS2 domain adopting a different structure to accommodate binding the α-subunit active site.

The β subunit also appears to contribute to the suppression of activity since either NH2-terminal truncation of the first 65 residues up to the CBD (215) or the Gly2Ala mutation, which blocks myristoylation (525), causes an increase in activity. There is no structural information available for the NH2 terminus of the β subunit to provide clues to the possible mechanism underlying its inhibitory effects. The β1 NH2-terminal sequence contains adjacent Ser-24 and Ser-25 that are autophosphorylated, indicating that this sequence is accessible to the active site and phosphorylation on Ser-24/25 may be involved in sustaining AMPK activation (340). The available evidence supports the concept that AMPK is autoregulated at multiple levels involving all three subunits acting in concert. What is missing at this point is structural data to evaluate the proposed mechanisms.

F. Myristoylation of the β Subunit

Myristoylation is a commonly used posttranslational modification adding myristic acid (14 carbon lipophilic group) typically to glycine-2 following processing on NH2-terminal Met, but can also occur on Gly residues exposed by endoproteinase activity (406). The myristoyl group acts as a lipid anchor, tethering the host protein to cellular membranes and is widely used to secure signaling proteins to membranes. The sequences of the mammalian β1 and β2 subunits (MGTTSSEN) share NH2-terminal consensus sequences (MGNXXS/T) for myristoylation (Web resource: http://mendel.imp.ac.at/myristate/) (8). With the use of ESI-mass spectrometry of the purified rat liver AMPK (α1β1γ1), it was confirmed that the β1 subunit was myristoylated and phosphorylated (Mt, 30,552, 30,635, and 30,722 of the myristoylated mono-, di-, and tri-phosphorylated forms, respectively) (340). When AMPK is expressed in insect cells, there is complete processing of the β NH2-terminal Met by Met-aminopeptidase, but in complete myristoylation (226). Supplementing the insect cell culture media with myristic acid does not overcome the problem.

Myristoylation of proteins is typically associated with facilitating membrane binding, as is, for example, the case with MARCKS protein ADP-ribosylation factor 1. However, in other instances, such as for calcineurin-B and the PKA catalytic subunit, myristoylation may not affect...
membrane association (340). Proteins may undergo a “myristoyl switch” where ligands (e.g., Ca\(^{2+}\)–calmodulin, GTP) or phosphorylation may trigger the myristoyl group to flip inside the protein and dissociate from the membrane (406). Removal of the β-subunit myristoylation site (Gly2Ala mutant) results in a fourfold activation of the enzyme and relocalization of the β subunit from a particular extranuclear distribution to a more homogeneous cell distribution (525). One interpretation of the increase in AMPK activity in the absence of the myristoyl group is that it may be tethering the NH\(_2\)-terminal 65 residues, which otherwise has an autoinhibitory effect. In the PKA catalytic subunit structure, the myristoyl group is positioned in a hydrophobic pocket at the tail of the large lobe anchoring the PKA NH\(_2\)-terminal A-helix. It is not known whether something similar occurs with the AMPK α-subunit catalytic tail binding the β-myristoyl group. There is also no information on whether AMPK could undergo a “myristoyl switch” between membrane and soluble forms in response to phosphorylation or ligand binding signals.

E. AMPK Interaction With Glycogen

Initial bioinformatics sequence/secondary structure searches revealed a midmolecule sequence in the β subunits related to the starch binding domains found in plants (isoamylase domains) (215, 395). Most eukaryote β subunits contain a carbohydrate-binding domain with the exception of the plant AKINB3 subunits (164). The AMPK β glycogen-binding domain has recently been reclassified as a member of the CBM-containing family, CBM48 (http://afmb.cnrs-mrs.fr/CAZY). The mammalian AMPK domain limits were established experimentally by partial proteolysis (395), and a stable rat β1(68-163) fragment was expressed and crystallized in the presence of β-cyclodextrin (an α 1-4 linked heptaglucan), as had previously been performed for other starch binding domain structural studies (396). The corresponding human β2 CBM structure has also been solved in the absence of cyclodextrin (PDB ID: 2F15). The isolated β(68-163) domain was found to bind glycogen, which was blocked with β-cyclodextrin \(K_{\text{d}} \approx 1.3\) mM. Cell-based studies with fluorescently tagged AMPK have also confirmed that AMPK associates with glycogen and glycogen synthase (215). However, it has recently been claimed that AMPK is not found associated with isolated glycogen α-particles (110–290 nm) from rat liver (386). It is clear that more detailed histochemical studies are required to establish under what conditions and in which tissues AMPK is associated with glycogen.

The β1 CBM structure revealed that cyclodextrin bound in a sugar-binding pocket cradled between a pair of aromatic residues, Trp-100, Trp-133 that accommodates three sugars from the cyclodextrin (Figs. 1 and 4). When other nearby contact residues including Asn-150 and Lys-126 are mutated, there is a loss of glycogen binding (396). Another feature of the structure is the extension of the side chain of Leu-146 through the core of the bound cyclodextrin. Solution studies using NMR have also been reported, showing that the CBM binds maltohexose and maltohepulose with \(K_p\) values <1 mM while shorter oligosaccharides bind substantially less strongly (256). The NMR results also showed chemical shifts in all the key sugar binding residues in the presence of maltoheptulose with the exception of Trp-100. Because the CBM binds most tightly to carbohydrates containing α 1-4 glycosidic linkages with a single glucose α 1-6 branch, it has been proposed that AMPK may bind to partially degraded glycogen (184, 256).

The physiological significance of AMPK’s association with glycogen has been investigated in several studies. High muscle glycogen levels correlated with reduced activation of AMPK in response to exercise (127, 415, 475, 531, 546) or 5-aminimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) (545), leading to the idea that AMPK may be inhibited by glycogen. Intuitively, it seems reasonable that AMPK in mammals would be switched off as energy stores such as glycogen become replete. However, there has been conflicting data on whether glycogen is inhibitory. We found that AMPK activity is not altered by binding to glycogen, as measured by adding purified rat liver AMPK to glycogen (395). However, this does not exclude the possibility that glycogen can affect the AMPK activation by an upstream kinase or inactivation by a phosphatase. In the case of patients with an inherited
defect in glycogen phosphorylase (McArdle disease) and associated excess skeletal muscle glycogen, AMPK is still activated by exercise (365). In a recent comprehensive study, McBride et al. (317) have reinvestigated the effects of glycogen on AMPK. They show addition of glycogen to rat liver AMPK is inhibitory, and when recombinant AMPK is expressed in CCL13 cells, this inhibition is dependent on the presence of CBM. In agreement with Koay et al. (256), they provide evidence that inhibition is mediated by the α1→6 linkages and require the key sugar binding Trp residues in the CBM. Isomaltose, but not maltose, inhibited rat liver AMPK with a half-maximal effect at 16 mM (317). A series of synthetic branched oligosaccharides with a single α 1-6 linkages were found to be more potent inhibitors of AMPK activity as well as inhibiting AMPK phosphorylation by CaMKK but not dephosphorylation by protein phosphatase 2C (317). The inhibitory effect of the oligosaccharides is lost when the CBM Trp residues are mutated, indicating that the CBM is essential. These results have led Hardie and colleagues (317) to propose that AMPK acts as a cellular glycogen sensor through its CBM containing β subunit.

F. AMPK Substrate Recognition

AMPK’s substrate recognition is determined by the enzyme’s location, as well as residues present in the local phosphorylation site sequence of its target substrates. Our knowledge of AMPK’s substrate specificity has come from studies using synthetic peptides by Hardie and others, as well as site-directed mutagenesis (96, 119, 332, 374, 440, 533). The general consensus motif for AMPK includes hydrophobic residues in the P+4 and P-5 position relative to the Ser/Thr phosphorylation site at P0. The P-5 position is anchored in a hydrophobic pocket positioned between the kinase large lobe helices αF and αG around Leu-212. A basic residue, typically Arg, occurs in the P-1 to P-4 positions. Snf1 exhibits a strong preference for the P-3 basic residue (119, 533). There is also a positive influence of a second Arg at the P-6 position that interacts with the kinase acidic residues Asp-215 and Asp-217 slightly COOH-terminal to the αF helix (440). The simple consensus motif can be written φX(B, X)XX(Ser/Thr)XXXφ, where φ is a hydrophobic residue, B is basic, and X is any residue (96, 119, 332, 440, 533). It is important to recognize the consensus sequence requirements are a guide only and that sites consistent with this arrangement may not be phosphorylated in proteins for other overriding structural reasons while some naturally occurring AMPK phosphorylation sites may not have all the consensus determinants. A good example is the AMPK phosphorylation site in endothelial nitric oxide synthase (eNOS) at Ser-1177 (RIRTQSL[1177]FSLQLQ), which does not have the φ residues at -5 and +4 (93). We found the synthetic peptide corresponding to the PKA phosphorylation site (Ser-230) in the yeast transcription factor ADR1 (LTRRAS[230]FSAQ) is an excellent substrate for AMPK without conforming to the φ at P+4 consensus requirement (332). Subsequent specificity studies employing mutagenesis of the recombinant ACoAC1 (60–94) fragment containing the Ser-79 phosphorylation site found that Gln and Asn were well tolerated at the P+4 position (440). This led Hardie and colleagues (440) to revise the consensus to emphasize that hydrophobic residue at P-5/P-4 and a basic residue at P-4/P-3 may be the more dominant determinants. In our view, the original consensus sequence proposal for AMPK (119) has served the field well with the recognition that there are multiple exceptions.

We have found that AMPK substrate recognition was exquisitively sensitive to oxidation using the SAMS peptide rACoAC (73–87) A7B[86–87] (HMRSAMSF7GLHLVK) with Met oxidation at P-5 having the greatest impact (332). Thus far, the possibility that oxidation of Met could alter AMPK signaling pathways has not been investigated in natural or pathological states. Several web-based programs can be used to analyze AMPK specificity. These include Scansite (http://scansite.mit.edu/) (367), which uses a specificity matrix of preferred residues within the local phosphorylation site sequence to assign a probability for kinase sites. If one scans the mammalian data base using the Scansite AMPK peptide based specificity motif, the top hit is Ser-743 in the Tousled-like kinase sequence PHMRRSNS[435]SGNLHMS (consensus motifs underlined). However, we found recombinant Tousled-like kinase was not a substrate for AMPK (L. Macaulay, L. A. Castelli, and B. E. Kemp, unpublished data). Kobe and colleagues (59, 435) have developed an alternative approach called Predikin which uses a prediction model of the kinase substrate recognition groove to estimate the compatibility of any putative substrate sequence being phosphorylated (http://predikin.biosci.uq.edu.au). Predikin has been used to predict all the possible Snf1 kinase substrates in the yeast genome (60).

For other protein kinases such as the classic PKA signaling system, there are multiple scaffold proteins called AKAPs that serve to create signaling complexes with substrates and signal terminators such as phosphatases and phosphodiesterases. For a recent review, see Scott et al. (40).

While the AMPK β subunit can localize the AMPK to glycogen and membranes via its CBM and NH2-terminal myristoyl group, respectively, no studies have documented signaling complexes analogous to the PKA AKAPs. We can anticipate this will change and AMPK signaling complexes will be identified, especially where multiple isoforms of the αβγ subunits are present in the same cell.

Physiol Rev • VOL 89 • JULY 2009 • www.physrev.org

Downloaded from http://physrev.physiology.org/ by 10.220.33.2 on November 6, 2017
III. REGULATION BY PHOSPHORYLATION

A. Activating Upstream Kinases

Like many protein kinases, AMPK has an obligate requirement for phosphorylation by an upstream kinase on Thr-172 in the activation loop of the α-subunit catalytic domain (186). Three mammalian upstream kinases have been identified for AMPK and are discussed below.

1. LKB1

The identification of AMPK upstream kinases proved as difficult as that of AMPK, taking two decades. Mutation screens in yeast for sucrose nonfermenting (Snf1) phenotypes did not reveal an upstream kinase. With the advent of genome-wide studies and the systematic analysis of protein complexes by mass spectrometry, vital clues emerged concerning kinases that associated with members of the Snf1 kinase complex (Snf1, Snf4, Sip1, Sip2) (161, 188). In particular, Pak1 (now termed Sak1) was associated with snf1, and Tos3 was associated with Snf4. Nath et al. (361) showed that Pak1 phosphorylated and activated Snf1 kinase. A similar strategy was also employed independently by Hong et al. (205), who showed that all three related kinases Tos3, Pak1, and Elm1 were functional upstream kinases for Snf1 kinase. It required the triple deletion mutant Δtos3, Δpak1, and Δelm1 to exhibit the Δsnf1 phenotype of failure to grow on raffinose (205). The presence of three complementry upstream Snf1 kinase kinases explained why a single upstream kinase had not been revealed by mutagenesis. Hong et al. (205) also showed that the catalytic domain sequence of mammalian LKB1 was the most closely related kinase to the corresponding sequences in the three yeast kinases.

Furthermore, recombinant LKB1 phosphorylated and activated mammalian AMPK. Elm1 kinase was also shown independently to activate Snf1 kinase through use of a library of glutathione-S-transferase fusions containing all 119 yeast protein kinases (483). Again, it was found that the triple mutant Δtos3, Δpak1, and Δelm1 had the Δsnf1 phenotype (483). The possibility that LKB1 was the mammalian homolog to these three yeast Snf1 kinase kinases was investigated, and rat liver AMPK kinase (AMPKK) activity was shown to consist of a complex of LKB1 and its two accessory subunits STRADα, β, and MO25α/β (185).

LKB1 was also deduced as an upstream kinase for AMPK by comparison of its peptide substrate specificity requirements with the sequence around Thr-172 in the AMPK α-subunit activation loop (445). LKB1 has a requirement for Thr at the phosphorylation acceptor site and Arg at −1 and Leu at −2 features found in the EFLRT<sup>172</sup>SCG sequence. With the use of LKB1-deficient MEFs, it was found that there was negligible activation of AMPK following treatment with AICAR or H<sub>2</sub>O<sub>2</sub>, but the response could be restored by introducing wild-type (WT) but not kinase-dead LKB1 alleles (445). Similar results were also obtained with HeLa cells, which are LKB1 deficient on account of gene methylation (185, 445).

2. Calmodulin-dependent kinase kinase

Hawley et al. (189) were the first to report CaMKK phosphorylated and activated AMPK, discovering when they investigated the AMP dependence of partially purified fractions of liver AMPKK. However, CaMKK was initially dismissed as a genuine AMPKK on the basis that the partially purified liver kinase kinase was not calmodulin dependent (189). The discovery that AMPK could be activated by Ca<sup>2+</sup> ionophores in LKB1-deficient cells led three independent groups to demonstrate CaMKK was indeed an upstream kinase for AMPK. Using Hela cells and LKB1-deficient MEFs, Hurley et al. (217) showed that mannitol and ionomycin treatment activated AMPK Thr-172 phosphorylation and that the response was inhibited by STO-609, a relatively specific inhibitor for CaMKK. AMPK signaling in these cells could also be suppressed with siRNA for both CaMKKα and -β (217). Hawley et al. (188) found that up to 3 μM concentrations on the ionophore A23187 activated AMPK in HeLa cells without significant changes in ATP/ADP ratios, and this could be substantially blocked with 2.5 μM STO-609. Importantly, these authors investigated the selectivity of STO-609 and showed that other protein kinases, including AMPK itself, were inhibited at 10 μM STO-609. CaMKK is highly expressed in brain, and K<sup>+</sup> depolarization of brain slices activates AMPK threefold, independent of any change in cellular AMP/ATP ratio, indicating that CaMKK activation of AMPK may be a major pathway in the brain (188).

Woods et al. (550) also showed that activation of AMPK by ionomycin in HeLa cells was dependent on CaMKKβ. These authors also investigated the relative contribution of LKB1 and CaMKK in activating AMPK using NIH3T3 fibroblasts (which contain both CaMKKα and -β) and LKB1-deficient MEFs via a variety of treatments in conjunction with STO-609. Treatment of the 3T3NIH cells with STO-609 caused an ~20 and 50% inhibition of AMPK activation by ionomycin and AICAR, respectively. In contrast, in LKB1-deficient MEFs, there was a dramatic reduction in the AICAR stimulation of AMPK, but the residual was still inhibited by STO-609. For ionomycin-dependent activation of AMPK in the absence of LKB1, the total activation was maintained and ~90% in inhibited by STO-609; this is consistent with CaMKK being responsible (550). Notwithstanding the question of selectivity of STO-609, the results indicate that in NIH3T3 cells containing both LKB1 and CaMKK there is a contribution from CaMKK with AICAR stimulation, which is otherwise primarily mediated by LKB1. Woods et al. (550) also pro-
vided evidence to show that CaMKKβ had a much stronger preference for AMPK as a substrate relative to CaMKKα, whereas CaMKK-I was phosphorylated comparably by both kinase kinases (550). Using the yeast triple-deficient strain Δtos3, Δpak1 and Δelm1, these authors showed that CaMKKβ could rescue the Δsnf1 phenotype and allow growth on either glycerol-ethanol or raffinose (550). This provides strong genetic evidence for the functional similarity of CaMKK with Tos3, Sak1, and Elm1, even though yeast are devoid of CaMKKβ.

Since CaMKK was established as an AMPK upstream kinase using ionophores to promote Ca²⁺ signaling, several studies have shown this pathway is operational under normal physiological conditions. CaMKK mediates both thrombin and bradykinin-dependent AMPK activation and stimulation of fatty acid oxidation in endothelial cells (346, 463). Similarly, CaMKKβ also mediates thyroid hormone T₃, stimulation of fatty acid oxidation in C2C12 myoblasts (559), and the effect of antigen receptor stimulation in T lymphocytes (486).

3. Tak1 kinase

The transforming growth factor (TGF)-β-activated kinase-1 was recently identified as an upstream kinase for AMPK based on a genetic screen for mammalian kinases that could rescue the triple deficient strain Δtos3, Δpak1, and Δelm1 (342). Mice carrying a cardiac specific dominant negative mutation for Tak1 were reported to have a Wolff-Parkinson-White syndrome reminiscent of the human AMPK γ₂ mutations (557). Tak1-deficient MEFs had reduced AMPK activation to oligomycin, metformin, and AICAR, leading the authors to propose a pathway where Tak1 kinase is upstream of LKB1 and AMPK (557), whereas the yeast study suggested Tak1 could directly phosphorylate AMPK (342). Since the AMPK γ₂ mutations giving rise to Wolff-Parkinson-White syndrome are gain-of-function mutations with increased constitutive AMPK activity, it is not clear how the dominant negative Tak1 gives the same phenotype if Tak1 is an upstream AMPK kinase.

B. Multisite Subunit Phosphorylation

1. α- and β-Subunit phosphorylation

While the α subunit Thr-172 is the major AMPK activating site phosphorylated by the upstream kinases (LKB1, CaMKKβ, and Tak1), both α and β subunits have multiple phosphorylation sites; however, the functional role of these is relatively poorly understood. Initial studies by Mitchelhill et al. (340) revealed that native rat liver α1 was phosphorylated at Ser-485 and β1 at Ser-24/25, Ser-108, and Ser-182. Further autophosphorylation sites have been identified using recombinant protein (552) [α2Thr-258, α2Ser-491(α1Ser-485) and β1Ser-96, Ser-101, Ser-108]. With the use of immunoprecipitated AMPK from rat liver and on-line capillary liquid chromatography ESI-MS/MS, only α2Thr-258 and α2Ser-491 were confirmed (552).

A) SECOND REGULATORY SITES. We have shown that α1Ser-485 (α2Ser-491) is involved in the negative regulation of AMPK by PKA-mediated cAMP signaling (218). Other studies in the heart also indicate that insulin activation of Akt increases the phosphorylation of AMPK α1/α2 at Ser485/491 (209, 456) and inhibits AMPK signaling (37, 265). Ser-485 is in the α-subunit COOH-terminal β-binding structure positioned close to γ. It is not yet clear how this phosphorylation signal is transmitted to α or how this inhibits AMPK Thr-172 phosphorylation and enzyme activity.

B) SITES OF UNKNOWN FUNCTION. We have identified a number of additional phosphorylation sites (α1 Thr-373, Thr-379, Thr-481, Ser-485, Ser-499) by MS/MS sequencing from tryptic digests of recombinant human α1 AMPK phosphorylated in insect cells (Kemp et al., unpublished data). A recent large-scale mouse liver phosphoproteomics study (517) reported these sites and others (α1; Thr-373, Thr-379, Ser-485, Ser-499, Ser-514, Ser-515, Thr-517), but site identification was equivocal (summarized in Fig. 5) and will need corroboration. The phosphoproteomics analysis missed all known β phosphopeptides and α (Thr-172). Even though the data are incomplete, it is striking that there may be many phosphorylation sites in the

![Figure 5](https://www.prv.org/ampkinhealthanddisease/figure5.png)

**Fig. 5.** The distribution of phosphorylation sites on AMPKα1. The structures of the α-subunit catalytic domain and the COOH-terminal binding domain are shown together with the recently reported phosphorylation sites (see text for details).
COOH terminus of the α subunit (400–550). Understanding the role of these sites will depend on identifying physiological responses where their state of phosphorylation changes and interpreting this in relation to the emerging structural information on AMPK and intersecting signaling pathways. For example, Ser-514 and Ser-515 are positioned at the αβγ junction and would seem poised to affect signal coupling between the subunits.

IV. AMPK REGULATION OF CARBOHYDRATE METABOLISM

Glycogen and starch provide rapidly accessible stores of glucose for metabolism in eukaryotes. While we will concentrate on AMPK’s role in mammalian carbohydrate metabolism, there is rapidly growing evidence that the corresponding yeast and plant kinases play equally important roles. In yeast, glycogen and trehalose serve as carbohydrate reserves that accumulate late in the logarithmic growth phase in response to nutritional stress (524). Snf1 is required for both the accumulation of glycogen as well as its maintenance by autophagy. Studies in plants have revealed that SnRK1 is also important for starch biosynthesis through transcriptional upregulation of sucrose synthase and ADP-glucose pyrophosphorylase (397). Furthermore, in transgenic plants overexpressing SnRK1, there is starch accumulation (328) analogous to mammals where increased AMPK activity either by naturally occurring mutations or transgenic models leads to muscle glycogen accumulation (see below).

A. Glucose Uptake

The uptake of glucose across the plasma membrane is dependent on the glucose gradient as well as the expression of transmembrane proteins known as GLUTs, which display Michaelis-Menten saturation kinetics. In many tissues including skeletal muscle, GLUT1 and GLUT4 predominate, with GLUT4 translocation to the plasma membrane and t tubules considered rate-limiting for the uptake of glucose in response to stimuli such as muscle contraction and insulin; however, it should be noted that under some situations, glucose phosphorylation by hexokinase also appears to be important (154, 155). Importantly, during exercise and/or skeletal muscle contraction, glucose uptake is increased, an effect which is independent of the proximal part of the insulin signaling pathway (for review, see Ref. 241). Therefore, the finding that AICAR stimulated glucose uptake via a phosphatidylinositol 3-kinase (PI3K)-independent pathway generated significant interest towards the therapeutic utility of AMPK to bypass insulin resistance (46, 190, 331). In the heart, both AICAR and ischemia also activate AMPK, resulting in increased GLUT4 translocation and increased glucose uptake (421).

The loss of AMPK α2 but not AMPK α1 results in abolished AICAR-stimulated glucose uptake (244), an effect also observed in AMPK γ3 knockout mice (32) and mice lacking AMPK β2 (Dzamko et al., unpublished data), suggesting a key role for the AMPK α2, β2, γ3 heterotrimer in the AMPK-dependent regulation of glucose uptake in muscle. The expression of a constitutively active form of AMPKα1 is also sufficient to increase glucose uptake in muscle cells (151). The stimulation of glucose uptake by AICAR is mediated at least in part by increasing GLUT4 translocation to the plasma membrane (274, 421) but not to t tubules (287). These effects may be dependent on nitric oxide synthase (NOS) (152, 448), although these data are equivocal (476).

1. Rab GTPase-activating proteins: TBC1D1 and TBC1D4

The signaling events, by which activation of AMPK leads to the translocation of GLUT4, is an area of continuing excitement. Recent studies have revealed a critical role for the Rab GTPases TBC1D1 and TBC1D4 (AS160), a topic which has been discussed in detail in several excellent reviews (82, 426, 572). AS160 contains two phosphotyrosine-binding (PTB) domains at the NH2 terminus and a Rab-GAP (GTPase-activating protein) domain at the COOH terminus, which is proposed to promote hydrolysis of GTP to GDP by Rab protein(s) on the GLUT4 storage vesicle (GSV) (246, 577). Subsequent studies demonstrated that AS160 was phosphorylated at five Akt consensus sites (Ser-318, Ser-570, Ser-588, Thr-642, and Thr-751) in response to insulin treatment in 3T3-L1 adipocytes (432), an effect also observed in skeletal muscle (67). Surprisingly, AS160 phosphorylation as assessed using the PAS [the phospho (Ser/Thr)-Akt substrate] antibody was also detected following muscle contraction or treatment with AICAR, conditions which do not increase Akt phosphorylation, and the phosphorylation of AS160 correlated well with the increases in glucose uptake under these conditions. In cell-free assays, AMPK is shown to directly phosphorylate AS160 predominately at Ser-588, and to a lesser degree at Ser-341 and Thr-642, an effect which directly enhances binding to 14-3-3 (163). Taken together, these data suggest that the phosphorylation of AS160 by AMPK directly regulates binding to 14-3-3, which in turn controls GLUT4 vesicle recycling. In support of these findings, several groups using transgenic mice with diminished AMPK signaling have demonstrated that AS160 phosphorylation is markedly reduced in response to AICAR but not muscle contraction (266, 267, 502), consistent with changes in glucose uptake mediated by these stimuli. However, recent studies which immuno-depleted AS160 from muscle lysates have suggested
that in the experiments described above examining AS160 phosphorylation utilizing the PAS antibody were most likely measuring a close relative to AS160, TBC1D1 (491). In contrast to AS160, the phosphorylation of which by both insulin and AMPK increases 14-3-3 binding/inhibition recent studies (89) in L6 myotubes, demonstrate that TBC1D1 is phosphorylated on Ser-237 and binds to 14-3-3 proteins in response to AMPK activation. Importantly, while insulin promotes phosphorylation of Thr-596, it does not result in 14-3-3 binding (89) or the inhibition of TBC1D1 activity (87). TBC1D1 expression is high in glycolytic skeletal muscle compared with both soleus and adipose tissue where AS160 appears to predominate. Taken together, these data suggest there is a complementary system in which the phosphorylation of AS160 and TBC1D1 by Akt and AMPK is required for maximal GLUT4 translocation. The hierarchical control/dependence of this pathway is differentially regulated depending on the tissues examined. Interestingly, TBC1D1 has been identified as a candidate for a severe obesity gene, suggesting that defects in TBC1D1 signaling may contribute to obesity-related insulin resistance (284).

2. Insulin receptor substrate 1

It is well documented that a single bout of exercise is capable of improving skeletal muscle insulin sensitivity in patients with type 2 diabetes, long after AMPK signaling has returned to resting levels (128, 410). One possible explanation for the enhanced insulin sensitivity postexercise relates to the finding that AMPK phosphorylates insulin receptor substrate 1 (IRS-1) at Ser-789 in vitro and that AICAR treatment in C2C12 muscle cells increases IRS-1 Ser-789 phosphorylation and insulin-stimulated IRS-1 associated PI3K activity (231) (Fig. 6). However, future studies are required to deter-

---

**Fig. 6. Glucose uptake in skeletal muscle.** Schematic illustration of the interaction between AMPK, insulin signaling, and GLUT4 trafficking to the plasma membrane. Both AMPK and Akt phosphorylate different sites of Rab GTPase activating protein (GAP) domains of TBC1D1 and AS160. The phosphorylation of AS160 by Akt and AMPK results in 14-3-3 binding and increases GTP-bound Rab and GLUT4 exocytosis to the plasma membrane. In contrast, while TBC1D1 is phosphorylated by both AMPK and Akt, only AMPK increases 14-3-3 binding and inactivation of TBC1D1, suggesting that it is the predominant RabGAP regulating AMPK-stimulated GLUT4 exocytosis. The activation of AMPK also results in the phosphorylation of the transcriptional coactivator PGC1α, while also phosphorylating HDAC5, causing it to be sequestered out of the nucleus resulting in enhanced GLUT4 promoter activity and mitochondrial biogenesis. Green arrow, stimulation/activation; red oval, inhibition/deactivation. Dotted lines indicate proposed action, but direct phosphorylation site has not been identified.
mine whether this interaction occurs following exercise or in response to hormonal stimuli in fully differentiated skeletal muscle.

A second potential mechanism by which AMPK may improve insulin sensitivity is through the suppression of inhibitory serine phosphorylation of IRS1. The serine phosphorylation of IRS by JNK, IKK, and S6 kinase is an important factor contributing to the development of insulin resistance in obesity (210). Recent studies have shown that the activation of AMPK by adiponectin results in improvements in insulin sensitivity in C2C12 myotubes (520). This improved insulin sensitivity is attributed to the downregulation of mTOR/S6 kinase signaling (to be discussed in detail below) and is eliminated following the overexpression of wild-type Rheb (Ras homology-enriched in brain) or a TSC2 mutant lacking the AMPK phosphorylation site (TSC2 Ser1345Ala). It will be interesting to examine whether a similar phenomenon mediates improvements in insulin sensitivity postexercise in obese insulin-resistant skeletal muscle.

3. Transcriptional regulation of GLUT4 and hexokinase

Increased insulin-stimulated glucose uptake following exercise may also be mediated through transcriptional upregulation of rate-limiting enzymes for the uptake of glucose, such as GLUT4 and hexokinase. In skeletal muscle, the chronic activation of AMPK by AICAR has been shown to increase GLUT4 (581) and hexokinase II (HKII) (480) transcription similar to exercise training (202, 242, 373, 541). Several years ago, studies by Salt et al. (429) reported that AMPKα2 was enriched in the nucleus in cultured INS-1 and CCL13 cells but did not observe any change in the cytoplasmic/nucleus ratio of α2 in response to glucose deprivation (INS-1) or arsenite (CCL13) treatment that would be expected to activate AMPK in these cells. More recently, AMPKα2 has been shown to localize to the nucleus of human skeletal muscle following exercise (323, 324, 475). The mechanism by which AMPK increases GLUT4 expression (26, 391) involves the transcription factors myocyte enhancer factor (MEF) 2A and MEF 2D (295, 296). As illustrated in Figure 6, the activity of these transcription factors may be controlled through AMPK phosphorylation of both peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) (209) and histone deacetylase (HDAC) 5 (325). Taken together, these data support the concept that AMPK is a positive regulator of GLUT4 and HKII gene transcription; however, it should also be noted that studies using mice with mutated AMPK signaling suggest that AMPK activation during exercise is by no means obligatory (203, 245).

B. Glycolysis

1. Phosphofructokinase 2

The cardiac isoform of 6-phosphofructokinase-2 (PFK2) controls the synthesis and degradation of fructose 2,6-bisphosphate, which in turn is a potent stimulator of PFK1, a key enzyme in glycolysis (216). PFK2 activity is regulated through phosphorylation within the regulatory domain at the COOH terminus that contains phosphorylation sites at Ser-466 and Ser-483 (126). In the heart, AMPK can increase the rate of glycolysis through phosphorylation of PFK2 at Ser-466 (313). There are four genes for PFK-2 (PFK2FB1-4) in mammals with alternate splice variants generating additional isoforms (411); in addition to the cardiac isoform PKK2FB2, the ubiquitously expressed PKK2FB3 isom is also phosphorylated (Ser-461) and activated by AMPK and is responsible for stimulating glycolysis in leukocytes (314).

C. Glycogen Metabolism

Glycogen represents the most easily accessible large-scale source of energy and is an important substrate in muscle during exercise and in the liver during fasting. The absolute level of glycogen is a function of both its production (glycogenesis) and its breakdown (glycogenolysis), which are mediated by the enzymes glycogen synthase (GS) and glycogen phosphorylase (GP), respectively. The role of AMPK in the regulation of glycogen metabolism in muscle has been an area of great interest for a number of laboratories and dates back to initial studies by Carling and Hardie (78) demonstrating that AMPK phosphorylates GS at Ser-7, a known inhibitory site of the enzyme. Experiments several years later by Young et al. (568) added further evidence for a role of AMPK in glycogen metabolism by showing that activation of AMPK by AICAR in rat muscle cell preparations caused an increase in GP activity. However, subsequent studies demonstrated that this was an artifact due to allosteric activation of GP by the AICAR derivative 5-aminoimidazole-4-carboxamide-1β-D-ribofuranosyl-5′-monophosphate (ZMP) (302). Given that AMPK causes an activation of GP and the suppression of GS, it was anticipated that AMPK would reduce muscle glycogen levels; however, in vivo the chronic activation of AMPK using AICAR actually increased muscle glycogen content and GS activities (20, 202). However, subsequent studies demonstrated that the AICAR effect on glycogen content and GS activities was due to an increase in glucose uptake and cytosolic glucose and subsequently glucose-6-phosphate (G-6-P), which allosterically activates GS independent of phosphorylation (20). These findings are supported by studies in AMPK γ3 mutant Hampshire pigs (333) and mice (32, 301), which have increased AMPK signaling and muscle glycogen contents, while the
inverse is observed in AMPK α2 null mice (240). Therefore, while the phosphorylation of GS at Ser-7 by AMPK does inhibit GS activity under basal conditions, stimuli that increase the concentration of intracellular G-6-P (such as AICAR-stimulated glucose uptake) are capable of overcoming the inhibitory effects of Ser-7 phosphorylation by AMPK.

D. Hepatic Glucose Production

The regulation of hepatic glucose production is critical for maintaining glucose homeostasis and is vital for survival. As a result, this system has adapted a number of diverse and redundant regulatory cues, which allow for the storage of glucose as glycogen and triacylglycerols following a meal and to conversely increase glucose output during a fast. Since AMPK is sensitive to changes in hormones and nutrients, it is not surprising that it plays a critical role in the regulation of this pathway by acting to regulate a number of pathways in the liver and to sense circulating glucose levels in the brain. The activation of AMPK was first shown to inhibit hepatic glucose production by Bergeron et al. (44) using AICAR during a clamp in rodents. However, studies by Wasserman and colleagues (72) demonstrated the opposite effects in dogs even in the presence of high levels of physiological insulin, an effect believed to be mediated through indirect stimulation of glycogenolysis by ZMP. The most convincing evidence supporting a role for AMPK in inhibiting glucose production comes from AMPKα2 knockout mice which display fasting hyperglycemia, glucose intolerance, and increased hepatic glucose output (16, 519). Similarly, LKB1 floxed mice injected with adenovirus Cre are also hyperglycemic and exhibit increased mRNA expression of gluconeogenic enzymes (446). In addition, studies in isolated hepatocytes treated with metformin (582) and adiponectin (16, 561) support an important role for AMPK in suppressing hepatic glucose output effects mediated by reduced gluconeogenic gene expression as will be discussed below.

Hepatic glucose production is controlled through phosphorylation of transcriptional coactivators and transcription factors, resulting in the inhibition or activation of their cognate promoters, that in turn control the expression of enzymes critical for regulating lipid and carbohydrate metabolism (Fig. 7). Initial experiments demonstrated that the activation of AMPK negatively regulates the transcription of the gluconeogenic enzymes L-type pyruvate kinase (L-PK) (117, 278), phosphoenolpyruvate carboxykinase (PEPCK) (300), and glucose-6-phosphatase (G-6-Pase) (548) in response to elevated glucose. L-PK, along with GLUT-2 and other genes involved in glucose and lipid metabolism, is regulated by the transcription factor hepatic nuclear factor 4α (HNF-4α) (337). It has recently been demonstrated that HNF-4α can be regulated by AMPK and that this regulation may be important for the regulation of L-PK (279). AMPK has been shown in vitro to phosphorylate HNF-4α on Ser-304, reducing the ability of the transcription factor to form the homodimers required for stability and DNA binding (206). This experiment provides a link between AMPK activation and the regulation of L-PK, but future studies are required to determine whether this relationship is also observed in vivo in genetic models of AMPK deficiency.

1. CREB-regulated transcription coactivator 2

Recently, CREB-regulated transcription coactivator 2 [CRTC2; formally known as transducer of regulated CREB activity 2 (TORC2)] has emerged as a critical regulator of gluconeogenesis (264). Under fasting conditions, glucagon triggers the transcription of gluconeogenic genes via the cAMP-responsive factor CREB (CRE binding protein) and subsequent recruitment of the coactiva-
tor CBP and CRTC2 to the nucleus (Fig. 7). This recruitment leads to the expression of the coactivator PGC-1α, which in turn drives the transcription of PEPCK and G-6-Pase. The phosphorylation of CRTC2 by AMPK and AMPK-related kinases, salt-inducible-kinase1 (SIK1) and -2 promotes CRTC2 binding to 14-3-3 proteins in the cytoplasm and prevents the translocation of CRTC2 to the nucleus, thereby reducing CREB-dependent mRNA expression of PEPCK and G-6-Pase (264). The mutation of CRTC2 Ser171Ala blunts SIK1- and AICAR-mediated suppression of G-6-Pase, PEPCK, and PGC-1α expression in primary hepatocytes, suggesting that CRTC2 might be a critical downstream target of both AMPK and the AMPK-related kinase SIK1 in the regulation of hepatic gluconeogenesis. Supporting this finding is the observation that the hyperglycemia in liver-specific LKB1 null mice (446) is much more severe than that seen in mice with liver deletion of both AMPKα1 and -α2 (16).

E. Hypothalamic Glucose Sensing

In recent years, the role of central pathways in regulating hepatic glucose fluxes has gained considerable support (303). Specifically, glucose-sensing neurons within the ventral medial hypothalamus have been shown to play a critical role in counterregulatory responses to hypoglycemia, with several reports demonstrating that activation of AMPK in the hypothalamus with AICAR (319, 320) or 2-deoxyglucose (10) increases hepatic glucose production. Glucose sensing also occurs in the portal vein with the activation of AMPK being important for controlling glucose uptake in skeletal muscle, although the signals regulating this response are not understood (69).

V. AMPK REGULATION OF LIPID METABOLISM

During fasting, postabsorptive conditioned lipids are the predominant substrate for the maintenance of whole body energy metabolism (251). The ability to efficiently store fuel in the form of energy-dense lipids and their mobilization during times of low carbohydrate availability was an essential development in evolution, allowing organisms to survive periods of famine and prolonged fasting. This coordinated release of free fatty acids from adipose tissue combined with the ability to actively fine tune the gradient between fat and carbohydrate metabolism in metabolically active tissues in response to a number of dynamic physiological stimuli requires an integrated metabolic system of control. This synchronized regulation of metabolism occurs acutely, as well as through transcriptional control by AMPK. A list of known AMPK substrates and their role in regulating lipid metabolism is shown in Figure 8.

A. Fatty Acid Uptake

The first step in the regulation of fatty acid (FA) metabolism involves their movement across the plasma membrane. In obesity, skeletal muscle displays an increased propensity towards the uptake of fatty acids, suggesting this may be a principal defect contributing to the accumulation of intramuscular lipids (56). While fatty acids due to their hydrophobic nature may passively diffuse across plasma membranes via a flip-flop evidence from genetic models of fatty acid translocase (FAT/CD36), fatty acid binding protein (FABP) deficiency has indicated that these key proteins also play an essential role in mediating the process (for review, see Ref. 53). The uptake of fatty acids is dependent on the metabolic rate of the tissue, as both contraction (55) and exercise (447) increase fatty acid uptake suggesting that AMPK may be important. Supporting this idea are studies demonstrating that the pharmacological activation of AMPK with AICAR increases both skeletal muscle (448, 473) and heart (305, 447) fatty acid uptake. AMPK may also be important in the regulation of FAT/CD36 transcription, with endurance training and chronic electrical stimulation increasing protein expression levels (54), while fasting-induced increases in FAT/CD36 are suppressed in AMPK α3 null mice (301). However, it should be cautioned that in muscle specific AMPK DN mice, total fatty acid uptake is not altered in response to muscle contraction (137), a finding also supported by Turcotte et al. (506) who have shown that an ERK inhibitor inhibits contraction-stimulated fatty acid uptake. If AMPK is involved to some degree in fatty acid uptake, future studies will need to identify whether AMPK phosphorylates FAT/CD36 directly or whether translocation is mediated indirectly as is the case for the regulation of glucose uptake via TBC1D/AS160.

B. Fatty Acid Partitioning

Once fatty acids are taken up across the plasma membrane and activated to fatty acyl-CoA, they can either be directed towards oxidation or storage depending on the metabolic demands of the tissue at the time. In addition, in lipogenic tissues such as adipose and liver, glucose can also be stored as lipid. AMPK plays a critical role in determining the fate of glucose and fatty acids through regulation of key substrates, as discussed below, and summarized in Figure 8.

1. Acetyl-CoA carboxylase

Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl CoA to malonyl CoA, a metabolic intermediate, that is either incorporated into fatty acids during their synthesis by fatty acid synthase or acts allosterically...
to inhibit carnitine palmitoyltransferase 1 (CPT1), which controls transport of activated fatty acids into the mitochondria for oxidation (48, 350). ACC exists in two isoforms (3): ACC1 (also known as ACCα) and ACC2 (also known as ACCβ), with the major difference between the isoforms being an NH₂-terminal extension of 146 amino acids in ACC2, localizing the enzyme to mitochondria (2). This localization is thought to be important for ACC2 regulation of fatty acid oxidation, since malonyl-CoA will be produced in close proximity to CPT1, which resides on the outer mitochondrial membrane. Liver, brown adipose tissue, and the brain contain both ACC1 and ACC2 isoforms, while skeletal muscle and cardiac muscle contain predominantly ACC2, whilst white adipose tissue expresses predominantly the ACC1 isoform (3, 229).

Wakil and colleagues (4 – 6, 311) have provided evidence supporting a critical role of the two ACC isoforms in the regulation of fatty acid metabolism using ACC1 and ACC2 null mice. These studies have shown that whole body ACC2 null mice have greatly reduced levels of skeletal muscle malonyl-CoA but normal levels of malonyl-CoA in adipose tissue and liver. This results in much higher rates of fatty acid oxidation in skeletal muscle (4, 6) and surprisingly (given the low levels of ACC2 expressed) also adipose tissue (370). Importantly, these elevated rates of fatty acid oxidation result in a lean phenotype that protects mice against high-fat diet-induced muscle and liver insulin resistance despite hyperphagia (4, 6). An important note from these studies is that this pronounced phenotype occurs despite normal ACC1 expression, which would be expected to compensate for the reduced malonyl-CoA especially in tissues such as liver and adipose tissue, thus highlighting the potential importance of the intracellular localization of malonyl-CoA for the regulation of fatty acid oxidation. In the liver-specific ACC1 null mice, there is a 70% reduction in malonyl-CoA levels but no change in fatty acid oxidation (311). However, in contrast to these findings suggesting two autonomous pools of malonyl-CoA in the regulation of fatty acid oxidation, studies by Savage et al. (436) using antisense oligonucleotide have found additive effects of ACC1 and ACC2 inhibition on liver fatty acid oxidation, indicating that in this model there is overlap and that malonyl-CoA derived from ACC1 can suppress fatty acid oxidation in liver tissue.

The whole body deletion of ACC1 is embryonic lethal, demonstrating the absolute requirement for the enzyme in fatty acid synthesis during development (5).
terms of cross-talk between malonyl-CoA produced by ACC1 and ACC2, the ACC1 liver-specific null mice have reduced accumulation of liver triacylglycerol (TAG) when fed a short-term high-sucrose/low-fat diet (311). Similarly, studies by Savage et al. (436) using anti-sense oligonucleotides have also found that the suppression of ACC1 inhibits lipogenesis, whereas a reduction in ACC2 has no effect on lipogenesis, supporting the theory of independent pools of malonyl-CoA for the regulation of lipogenesis. However, a recent report in ACC1 liver-specific null mice questions this conclusion because their ACC1 liver-specific null mice had increased expression levels of ACC2 and normal fatty acid synthesis and importantly were not protected against fatty liver disease in response to a high-sucrose diet (183). Overall, there is not a clear consensus on the degree of malonyl-CoA overlap between synthesis and oxidative pathways between ACC isoforms.

Future studies, in mice with targeted tissue-specific knock ins, of dead ACC2 or ACC1 that preserve the respective enzyme architectures may be necessary to clarify this important question.

The short-term regulation of ACC is achieved by reversible phosphorylation and to a lesser extent, allosteric regulation by citrate (350). Early experiments using liver purified ACC and AMPK concluded that AMPK phosphorylated ACC at three sites: Ser-79, Ser-1200 (351), and subsequently Ser-1215 (124). A limited proteolysis approach suggested that Ser-79 was the major site responsible for the inhibition of ACC activity (124), and this was later confirmed using site-directed mutagenesis (176). Experiments using AICAR confirmed that Ser-79 is the physiologically relevant phosphorylation site for inhibition of ACC1 by AMPK (107), but due to the NH₂-terminal extension, the equivalent site in ACC2 is Ser-221 (1). While initial studies conducted in vitro showed that Ser-79 phosphorylation may be inhibited by PKA phosphorylation of Ser-77 (352), this was not observed in hepatocytes treated with glucagon, suggesting the Ser-77 phosphorylation is not physiologically significant (453), a finding also confirmed in rodent skeletal muscle (542). Phosphorylation of ACC1 (Ser-79) and ACC2 (Ser221) by AMPK is commonly used as an in vivo measure of AMPK signaling in response to a variety of stimuli ranging from hormones to exercise, but so far, genetic evidence supporting their physiological importance at the whole animal level has not been available. In this regard, we have recently generated mice carrying Ser/Ala knock ins for these sites that may provide clues to their physiological importance in regulation of fatty acid metabolism.

2. Malonyl-CoA decarboxylase

Malonyl-CoA levels are also regulated by decarboxylation by the enzyme malonyl-CoA decarboxylase (MCD) (134). The overexpression of MCD in liver increases hepatic FA oxidation and rescues whole body insulin sensitivity in obese rats (12). While MCD activity is increased in response to AICAR and contraction in skeletal muscle (385, 423), this regulation appears to be indirect as AMPK does not phosphorylate MCD in vitro (177). Increased rates of fatty acid oxidation in the diabetic rat heart are associated with elevated MCD activity (425), an effect that is reversed in the presence of MCD inhibitors (135). However, recent studies by Reszko et al. (407) have suggested that the primary determinant of malonyl-CoA production under physiological conditions and in the absence of inhibitors to MCD is the rate of acetyl-CoA carboxylation.

3. Fatty acid synthase

Fatty acid synthase (FAS) is a multifunctional enzyme found in lipogenic tissues such as adipose and liver, which catalyzes the synthesis of long-chain fatty acids, primarily palmitate, using acetyl-CoA and malonyl-CoA as substrates (Fig. 8). A decade ago, Foretz et al. (147) demonstrated that AMPK inhibited the glucose-stimulated transcription of FAS. FAS expression is regulated through the transcription factor sterol regulatory element binding protein 1c (SREBP1c), which is a critical transcription factor regulating many lipogenic genes (FAS, GPAT, SCD1, etc.). In the liver, overexpression of a CA-AMPK (146) or treatment with metformin (582) reduces SREBP1c gene expression, but the exact mechanism by which AMPK regulates this transcription factor is still unknown. Recent studies have suggested that FAS may also be regulated posttranslationally by AMPK, as treatment with AICAR or peroxynitritites which activate AMPK inhibits FAS activity within minutes in 3T3-L1 cells (13). These effects were inhibited by the overexpression of an AMPK dominant negative or following treatment with Compound C (13). Further studies utilizing 32P labeling confirmed that both AICAR and metformin dramatically increased the incorporation of label into FAS, an effect associated with increased Thr phosphorylation at an unidentified site (13). As obesity-associated hepatic steatosis is associated with increased SREBP1c and FAS, identifying the exact mechanism by which AMPK inhibits their activity may help reveal novel treatments for this prevalent condition.

C. Triacylglycerol Turnover

The regulation of triglyceride (TG) turnover is a balance between biosynthetic pathways responsible for TG synthesis and hydrolysis (Fig. 8). In recent years, the control of this pathway has become an area of great interest because even though total TG content in muscle and liver does not appear to adversely affect insulin sensitivity, lipid intermediates in the form of DG, ceramides,
and long-chain acyl-CoA activate serine threonine kinases and protein phosphatases that directly inhibit insulin signaling (530). One hypothesis is that in obesity and insulin resistance there is a mismatch between rates of esterification and hydrolysis, which results in futile cycling and the build up of reactive lipid intermediates, which in turn may impede insulin sensitivity.

1. **Glycerol-3-phosphate acyl-transferase**

The synthesis of TG is regulated in part by the supply of the substrates glycerol-3-phosphate (from carbohydrate metabolism) and fatty acyl-coenzyme A supplied through intracellular hydrolysis and/or transport from exogenous sources. The first committed step in phospholipid and TG synthesis is the formation of lysophosphatic acid from glycerol-3-phosphate. GPAT is the rate-limiting enzyme in this reaction and is reported to exist as both mitochondrial (mt) and microsomal isoforms based on N-ethylmaleimide (NEM) inhibition. TG formation is regulated by the mitochondrial 828 residue isoform (accession number Q8N1G6) that is an integral membrane protein (221). The mtGPAT gene consists of 19 exons on chromosome 10q25.2. Null mice have reductions in body weight, total adipose tissue weight, and lower hepatic TG content (181), while overexpression of GPAT reduces fatty acid oxidation and increases TG esterification (296). The activation of AMPK by AICAR reduces hepatic GPAT activity and TG esterification (354). Similarly, the activation of AMPK in liver and adipose tissue by endurance exercise also reduced mtGPAT activity (385) effects, which were not observed in skeletal muscle, likely due to its relatively low expression compared with lipogenic tissues such as liver and adipose. In agreement, we have also detected no significant reduction of mtGPAT activity following endurance exercise in human skeletal muscle (529). Despite the significant correlative data, perhaps the best evidence supporting a role of AMPK in regulating mtGPAT activity comes from Muoio et al. (354) who reported that incubating mtGPAT with recombinant AMPK inhibits the enzymes activity. In this study, it remains possible that an associated protein may be inhibiting mtGPAT activity as direct GPAT phosphorylation was not shown and AMPK was added to a mitochondrial fraction of GPAT from rat liver that was from only partially purified rat liver. While the inspection of the mtGPAT sequence does reveal several likely AMPK sites in the 80-residue cytoplasmic sequence, future studies are required to determine whether the effects of AMPK on GPAT activity are direct and the potential phosphorylation sites involved. Interestingly, fasting that activates liver AMPK (543) inhibits both GPAT and DGAT activity; effects that are promptly reversed by refeeding, which terminates AMPK signaling (22). Several recent studies (83, 97, 297, 343, 571) have shown that DGAT1 and DGAT2 are also critical for regulating lipid esterification and in turn insulin sensitivity, suggesting that AMPK regulation of these enzymes may be a second point of regulation in this important pathway.

2. **Hormone-sensitive lipase**

The breakdown of TG is accomplished through sequential hydrolysis of three FA residues. While researchers have known for several decades that adipose tissue lipolysis is sensitive to catabolic stimuli (49, 200, 412), it was several decades before the protein responsible for much of this lipolytic activity, justly named hormone-sensitive lipase (HSL), was purified from rat epidydimal fat pads. While HSL is known to be classically activated by PKA phosphorylation at serines 563, 659, and 660 (17), experiments with adipocytes and skeletal muscle suggested that AMPK inhibits this activation through phosphorylation at Ser-565 (107, 159, 160, 415, 482, 528, 531). Indeed, recent studies in 3T3-L1 adipocytes expressing DN and CA-AMPK mutants or adipocytes from AMPK α1 knockout mice support the role of AMPK inhibiting β-adrenergic-stimulated lipolysis in adipose tissue (122). Since AMPK is regulated by a variety of hormonal stimuli (as discussed below), it will be important to test whether these hormones control lipolysis in vivo via AMPK. In addition, the recent finding that adipose tissue triglyceride lipase (ATGL) is an important TG lipase suggests that there may be opportunities to regulate lipid hydrolysis upstream of HSL.

AMPK also appears to negatively regulate HSL activity during muscle contraction. In both resting (354) and contracting (455) muscle, TG hydrolysis is suppressed in response to AMPK activation by AICAR. Consistent with reductions in TG hydrolysis, PKA-stimulated HSL activity is reduced in L6 myotubes treated with AICAR (531) or following overexpression of a constitutively active AMPK (532). In vivo evidence supporting a role of AMPK inhibition of HSL stems from findings in human skeletal muscle that enhanced activation of AMPK, due to glycogen depletion prior to exercise, prevents exercise-induced increases in HSL activity (531). Similarly, prolonged endurance exercise (90 min) that results in significant activation of AMPK inhibits HSL activity during exercise (455) muscle, TG hydrolysis is suppressed in response to AMPK activation by AICAR. Consistent with reductions in TG hydrolysis, PKA-stimulated HSL activity is reduced in L6 myotubes treated with AICAR (531) or following overexpression of a constitutively active AMPK (532). In vivo evidence supporting a role of AMPK inhibition of HSL stems from findings in human skeletal muscle that enhanced activation of AMPK, due to glycogen depletion prior to exercise, prevents exercise-induced increases in HSL activity (531). Similarly, prolonged endurance exercise (90 min) that results in significant activation of AMPK inhibits HSL activity during muscle contraction.

D. **Mitochondrial Biogenesis**

A reduction in mitochondrial density is believed to be a critical factor contributing to the accumulation of intra-
muscular lipids and insulin resistance (389). While it has been known for several years that one of the most rapid and robust responses to exercise training is an increase in mitochondrial content and capacity (201), Winder et al. (541) were the first to demonstrate that chronic AICAR treatment stimulated mitochondrial biogenesis. Similar results were also observed following treatment with the creatine analog β-guanidinopropionate (β-GPA), which competitively inhibits creatine uptake and lowers ATP contents (45). The critical role of AMPK in regulating mitochondrial biogenesis basally and in response to chronic AMPK activators such as β-GPA or AICAR was proven to be causally related, as these effects were eliminated in mice with decreased AMPK signaling (242, 245, 496, 584).

Transcription factors and coactivators control mitochondrial biogenesis. Nuclear respiratory factor-1 and -2 (NRF-1, NRF-2) are critical transcriptional regulators of nuclear genes encoding all five electron chain complexes (322). Importantly, NRF expression is increased by the chronic activation of AMPK using β-GPA (45). Another important regulator of mitochondrial content is the inducible coactivator of nuclear receptors, PGC1α, which is increased in response to activation of AMPK and is reduced in AMPKα2 null (222) and DN mice (531). Recent studies by Jager et al. (230) demonstrate that PGC1α coimmunoprecipitates with AMPKα2 and that it can directly phosphorylate PGC1α on Thr-177 and Ser-538 (Fig. 6). Increased phosphorylation is proposed to cause an increase in PGC-1α protein action on the PGC-1α promoter resulting in increased expression of PGC1α, as well as GLUT4 (as discussed previously) and mitochondrial oxidative genes such as cytochrome c and uncoupling protein (UCP) 1. Since PGC-1α activity and expression are reduced in type 2 diabetes (344), activators of AMPK, such as adiponectin, may reverse defects in mitochondrial content and potentially the insulin resistance observed in this population (98).

Despite the robust AMPK-dependent effects of AMPK activators at rest in stimulating mitochondrial biogenesis, Jorgensen and co-workers (243, 245) have shown that a lack of AMPKα2 does not attenuate training-induced mitochondrial biogenesis, suggesting that other pathways during exercise may be more critical. Similar results are also reported in LKB1 null mice (496). One possibility may involve calcium signaling, since the treatment of muscle with calcium ionophores rapidly induces mitochondrial biogenesis (372, 553); however, it should be noted that in both models α1 activation in response to endurance exercise is relatively normal. In addition to regulating mitochondrial biogenesis, AMPK has also been shown to specifically regulate the expression of UCP3, an effect which may be independent of changes in mitochondrial density. Zhou et al. (583) demonstrated that AICAR and hypoxia increased UCP3, suggesting a role for AMPK. These findings have also been confirmed with chronic AICAR treatment (245, 387, 480).

E. Cholesterol Synthesis

HMGR is the rate-limiting enzyme for isoprenoid and cholesterol synthesis and a substrate for AMPK. The geranylgeranyl and farnesyl groups derived from the mevalonate pathway play an important role in providing lipid anchors for many signaling proteins including the small GTP binding proteins Rap, Rac, and Rho which regulate vesicular transport and cytoskeletal membrane interactions (329). Deletion of HMGR is embryonic lethal at the blastocyst stage, indicating that the mevalonate pathway is indispensable for development (371). HMGR is highly regulated with its transcription and translation under negative-feedback control mediated by sterols and nonsterol metabolites derived from mevalonate. There is also diurnal and dietary-induced changes in cholesterol synthesis in the liver that follow changes in liver HMGR mRNA. The catalytic activity of HMGR is inhibited by phosphorylation (42) on Ser-872 by AMPK (871 in mouse or 872 in humans) (101). The crystal structure of the dephosphorylated catalytic portion of human HMGR (460–888) has been solved (228), and inspection of the structure indicates that phosphorylation may act by altering NADPH affinity since the adjacent Arg-871 is directly involved in binding NADPH. However, further work is required to confirm this mechanism kinetically (374). HMGR is inhibited by ATP depletion, and with the use of transfected cells, it has been found that mutation of HMGR Ser871Ala renders it insensitive to AMPK-mediated inhibition in response to ATP depletion. Phosphorylation of Ser-871 does not play a role in AMPK turnover (574) in vivo, and the posttranscriptional feedback downregulation of the Ser871Ala mutant HMGR is normal when cells are incubated with mevalonate, 25-hydroxycholesterol, or LDL (433). The finding that adiponectin (500, 561) activates AMPK and reduces cholesterol synthesis and atherosclerosis in ApoE-deficient mice (376) suggests that the hormonal regulation of HMGR via AMPK may be critical for regulating cholesterol metabolism in vivo.

VI. AMPK REGULATION OF PROTEIN METABOLISM, CELL POLARITY, GROWTH, AND APOPTOSIS

A. Protein Synthesis

Protein synthesis accounts for a large proportion of cellular energy use, and inhibition of this pathway is
therefore an important mechanism by which to maintain cellular ATP under metabolic stress. AMPK inhibits protein synthesis at multiple points (Fig. 9). It phosphorylates and activates eukaryote elongation factor 2 kinase on Ser-398, which in turn phosphorylates eEF2 and inhibits protein synthesis (62). It also inhibits the mammalian target of rapacyn complex (mTORC) pathway (for review, see Ref. 275). This pathway is regulated through phosphorylation of tuberous sclerosis 2 (TSC2), which acts as a GTPase-activating protein (GAP) for the small GTPase Rheb. Rheb in turn directly binds the kinase domain of mTOR, activating the complex which in turn activates the S6 kinase while inhibiting 4E-BP1 (Fig. 9). While insulin and insulin-like growth factor I (IGF-I) signaling stimulate protein synthesis and the activation of mTORC, AMPK counteracts these effects to inhibit protein synthesis in skeletal muscle (52, 535), liver (208, 405), and cardiac muscle (86, 207). This effect is mediated by AMPK via three distinct mechanisms. The first involves the phosphorylation of TSC2 at Ser-1387, which enhances Rheb GAP activity resulting in the inhibition of mTORC1 signaling (225). Subsequent studies have confirmed these findings by demonstrating that the deletion of LKB1 and, subsequently, AMPK signaling, results in a hyperactive mTORC1 (106, 444). The second mechanism by which AMPK impairs protein synthesis is by directly phosphorylating the mTOR binding partner raptor at Ser-722 and Ser-792 (175). This phosphorylation by AMPK leads to binding of raptor to 14-3-3 proteins and therefore causes inactivation of the mTOR complex. Lastly, mTOR is also inhibited through feedback inhibition by S6 kinase (7) in skeletal muscle in response to endurance and resistance exercise training (23). The recent findings that the mTORC pathway controls many aspects of metabolism in addition to protein synthesis, including glucose homeostasis (511), fat metabolism (7), and orexigenic peptide expression (109), suggest that AMPK modulation of mTORC may mediate many of the reported effects of AMPK in the regulation of metabolism, but future studies are indeed warranted to fully understand these interactions.

![Fig. 9. AMPK inhibition of protein metabolism. Schematic illustrating the major pathways by which AMPK reduces protein synthesis and may induce apoptosis. AMPK inhibits the TORC1 complex by phosphorylating the tumor suppressor complex TSC1/TSC2, which negatively regulates TORC1 by inactivating the GTPase Rheb. Phosphorylation of TSC1/TSC2 may also mediate the effects of autophagy. TORC1 activity is also impaired through phosphorylation of Raptor, which leads to 14-3-3 binding and physical separation of the TORC1 complex. Lastly, AMPK inhibits the initiation of mRNA translation by inhibiting peptide elongation by activating the eEF-2 kinase. Secondary effects of TORC1 inhibition by AMPK may be to reduce S6 kinase activity, which is a negative regulator of IRS signaling, which may explain the positive effects of AMPK on insulin sensitivity. Green arrow, stimulation/activation of AMPK; red oval, inhibition/deactivation.](http://physrev.physiology.org/)
B. Cell Growth and Apoptosis

In addition to direct effects on protein synthesis, AMPK may also inhibit cell growth and proliferation. One mechanism by which this may occur appears to be through AMPK induction of G1/S phase cell cycle arrest. This effect is associated with the accumulation of the tumor suppressor p53 and of the cyclin-dependent kinase inhibitors p21 and p27, which act downstream of p53 and may be dependent on phosphorylation of p53 on Ser-15 (223, 239, 404, 555). A recent study has demonstrated that p27\textsuperscript{Kip1} may be the critical point of convergence in nutrient regulation of cell-cycle progression and the balance between autophagy and apoptosis. While AMPK directly phosphorylates p27\textsuperscript{Kip1} at Thr-198, this phosphorylation is very weak and unlikely to occur in vivo, suggesting that additional kinases downstream of LKB1-AMPK probably regulate p27 stability in vivo, which would be expected to inhibit cell cycle progression (293). In skeletal muscle, AMPK increases the expression of the ubiquitin ligases muscle atrophy F-box (MAFbx) and muscle RING finger 1 (MucRF1) protein, suggesting that AMPK may also be important for the atrophic transcriptional program executed under various conditions of skeletal muscle wasting (268). However, the physiological significance of these findings is unclear, since exercise training is known to prevent sarcopenia in the elderly. Taken together, these data suggest that the effects of AMPK on cell growth may be mediated by many different pathways, each with tissue explicit specificity; this will make it challenging to understand which key pathways are involved.

Another possible mechanism by which AMPK may regulate cell growth involves AMPK reducing the cytoplasmic-to-nuclear ratio of the RNA-binding protein HuR, which in turn reduces mRNA stability of critical cell cycle regulators such as cyclins A and B1 (523). Additional studies also implicate AMPK in the regulation of transcription factors and coactivators, which might regulate cell growth and proliferation, especially in cancer. In the prostate cancer cell line, DU145, activation of AMPK with hypoxia and blockade of AMPK activity with a dominant negative mutant demonstrated that AMPK was essential for the transcriptional activity of the hypoxia inducible factor 1 (HIF1) (281). The protein products of the HIF1 target genes function to increase oxygen delivery and to enhance metabolic adaptation to anaerobic conditions, requisites for tumorigenesis (281). AMPK has also been shown in vitro to phosphorylate the transcriptional coactivator p300 on Ser-89, reducing its affinity for nuclear receptors (564). The physiological significance of p300 phosphorylation by AMPK is still unknown; however, this event has the ability to influence transcription levels of a large set of genes, which may be critical for regulating cell growth and survival and may therefore be important under a number of conditions including cancer cell proliferation.

C. Regulation of Cell Polarity and Ion Flux

Cells maintain an electrical potential difference (voltage) across their plasma membranes so that the cytoplasm is usually electrically negative relative to the extracellular fluid. This resting membrane potential is essential for cell survival and is maintained through generation of electrical potentials between ions mediated through active and inactive transport. In quiescent cells, the maintenance of this resting membrane potential is by far the greatest consumer of cellular ATP, and the resting membrane potential plays a central role in the excitability of nerve and muscle cells. Thus far, AMPK has not been shown to play a role in maintaining membrane potential.

An important aspect of maintaining ionic equilibrium involves the regulation of cell volume. Hallows and colleagues have identified that an important role for AMPK is in the inhibition of the activity of the cystic fibrosis transmembrane conductance regulator Cl\textsuperscript{−} channel (CFTR) (180) and epithelial Na\textsuperscript{+} channel (eNaC) (74). Although AMPK directly phosphorylates CFTR to regulate its activity (180), the inhibition of eNaC involves upregulation of the ubiquitin Ligase Nedd4–2 which reduces EnaC expression in the plasma membrane (47). In the kidney, the Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{−} cotransporter (NKCC1 and NKCC2) is highly expressed in the distal nephron and is important for regulating whole body sodium balance. An increase in osmotic stress activates AMPK (148), increasing the activity of NKCC2 and thereby helping to maintain osmotic balance (149). AMPK also regulates the activity of sarcolemmal K (ATP) channels in cardiomyocytes (481).

In *Drosophila*, AMPK-null mutants are lethal with severe abnormalities in cell polarity and mitosis, similar to those of *lkb1*null mutants (280). Constitutive activation of AMPK restored many of the phenotypes of *lkb1*-null mutants, suggesting that AMPK mediates the polarity- and mitosis-controlling functions of LKB1. Two studies suggest that these effects may be mediated through AMPK's ability to promote transepithelial resistance and tight junction assembly through activation of a calcium switch which prevents tight junction disassembly (579, 580). The mechanisms and phosphorylation events implicated in this process are not known. Recently, it was reported the calcium-activated potassium channel KCa3.1, which is expressed at the basal lateral membrane of many epithelial cells, is inhibited by AMPK (255). On the basis of two-hybrid analysis, it is proposed that the AMPK γ1 subunit interacts with the COOH-terminal tail of the channel. However, it is not yet clear that AMPK directly phosphorylates KCa3.1 or another associated protein.
VII. INTEGRATIVE ROLE OF AMPK AS A REGULATOR OF WHOLE BODY ENERGY METABOLISM

A. Exercise

It is well documented that exercise or lack thereof is a modifiable risk factor for a multitude of common diseases ranging from diabetes and cardiovascular disease to certain cancers and even depression (129, 505). Despite the tremendous clinical importance of exercise in the prevention and treatment of these common diseases, the molecular mechanisms mediating the positive effects of exercise remained elusive for many years. Therefore, one of the most influential findings in AMPK research was the discovery that the enzyme was activated by muscle contractions (219, 514) and exercise in both rodents (546) and humans (90, 156, 547) in an intensity-dependent manner (90, 92, 125, 477, 546, 547). Studies in LKB1 null mice have shown that the activation of AMPKα2 by exercise is entirely dependent on phosphorylation by LKB1 (259, 427, 496), while rather surprisingly maintaining AMPK α1 phosphorylation (259).

During intense exercise, increased demands by contracting sarcomeres and Ca^{2+} pumps in the sarcoplasmic reticulum can increase ATP turnover by in excess of 100-fold. In humans, this dramatic increase in ATP demand is nearly precisely matched by rapidly increasing oxidative and nonoxidative phosphorylation of substrates derived from intra- and extracellular lipid and carbohydrate stores (460). Recent studies in AMPK α2 (245) and LKB1 (427) null mice have highlighted the importance of AMPK in the maintenance of energy charge during contraction, since these mice have an elevated AMP/ATP ratio in isolated muscles contracted ex vivo, independent of complications related to substrate delivery and perfusion. This reduction in the capacity to maintain ATP in response to muscle contraction may contribute to the reduced exercise capacity of LKB1 KOs (496) as well as muscle-specific AMPK dominant negative mice (347). Since AMPKα1 and α2 are also increased in the heart with exercise intensity (110), it is unknown whether a reduced exercise capacity is due principally to effects in skeletal muscle or is the result of secondary effects that limit blood flow and perfusion (347). Rather surprisingly, AMPK α2 DN mice do not have an elevated cardiac AMP/ATP ratio during treadmill running, suggesting this may not be the case (356). Taken together, these studies highlight the critical importance of AMPK for the maintenance of skeletal muscle energy balance during exercise; however, as discussed below, this may not be due to impaired metabolism of substrates.

1. Glucose uptake

During exercise and/or skeletal muscle contraction, glucose uptake is increased, an effect which is independent of the proximal part of the insulin signaling pathway (for review, see Ref. 241). The role of AMPK in regulating exercise-stimulated glucose uptake has been an area of considerable interest in recent years due to the therapeutic potential of stimulating glucose uptake via alternative pathways in insulin-resistant skeletal muscle. As discussed, many studies have examined the role of AMPK in mediating glucose uptake utilizing the pharmacological AMPK activator AICAR in resting (noncontracting) skeletal muscle. Based on the above studies that demonstrated positive associations between contraction, AMPK activity, and glucose uptake, it was somewhat surprising that in transgenic AMPK dominant negative mice (157, 348) and AMPK α2 null animals (244) that ex vivo contraction-stimulated glucose uptake was either normal (157, 244) or only modestly reduced (157, 233, 348) during tetanic muscle contractions. Even though all of the above models have some degree of residual AMPK signaling, e.g., some remaining α1 activity, these findings collectively point towards a system dependent on more than one signaling pathway.

2. Fatty acid oxidation

During exercise/muscle contractions, the activation of AMPK is associated with increased phosphorylation and deactivation of ACC2 and MCD (219, 247, 331, 413, 496a, 514, 540), which results in reductions in malonyl CoA levels (538). Despite the strong correlations between the activation of AMPK and the stimulation of fatty acid oxidation under many conditions, especially in rodents, there is a mismatch between rates of fatty acid oxidation and the activation of AMPK/ACC signaling. One example of this dichotomy is observed in LKB1-deficient mice, which despite markedly blunted resting and contraction stimulated phosphorylation of ACC (259, 427, 493, 496), malonyl-CoA levels and rates of resting palmitate oxidation are not altered and the ability of contraction to suppress malonyl-CoA is only marginally impaired (493). We have also demonstrated that although contraction-stimulated AMPK activity is reduced in AMPK DN mice, ACC phosphorylation and fatty acid oxidation are maintained (137). In addition, it has also been reported that low-intensity contraction can increase fatty acid oxidation independent of increased AMPK activity (402, 403) and that AICAR and contraction may have additive effects on fatty acid oxidation (455).

In exercising humans, there are also a number of specific examples in which there is a mismatch between AMPK signaling and fatty acid oxidation. One example involves observations following endurance exercise training, which increases lipid oxidation during exercise but...
results in the suppression of AMPK signaling (318). The inverse is also observed during sprint exercise, which maximally activates AMPK signaling (90, 92) but reduces fatty acid oxidation (416). Similarly, in exercising women who have higher rates of fatty acid oxidation than men at the same relative intensity, AMPK activity is markedly lower, although ACC phosphorylation is only marginally suppressed (414, 489). Taken together, these studies suggest that either 1) AMPK is not required and an alternative ACC kinase(s) exists which assists in the regulation of fatty acid oxidation during muscle contraction or 2) ACC2 is not required for the regulation of fatty acid oxidation and alternative enzymes are more critical for controlling malonyl-CoA production. Future studies in muscle-specific double-KO (α1α2 or β1β2) mice or those lacking the ability of AMPK to phosphorylate ACCS221 should be revealing as to which of these possibilities is most important and may lead to the identification of alternative regulators of fatty acid oxidation.

3. Blood flow

During exercise there is an increased demand for exogenous fatty acids and glucose by contracting muscle, which is met by increases in blood flow and capillary recruitment, ultimately resulting in improvements in muscle perfusion. Using L-NAME (a pharmacological NOS inhibitor) and AICAR, Shearer et al. (448) have shown that AMPK stimulation of skeletal muscle glucose but not fatty acid uptake is dependent on nitric oxide. This effect may be mediated by AMPK phosphorylation of eNOS which phosphorylates the eNOS at Ser-1177 to activate NOS both in vitro and during ischemia in rat hearts (93) and contracting human skeletal muscle (90). These data suggest that the phosphorylation of NOS by AMPK in endothelial cells and myocytes is an important link between metabolic stress and substrate delivery and muscle perfusion. However, direct studies measuring muscle perfusion in genetic models of AMPK deficiency in response to exercise and hormonal activators of AMPK are needed to fully address the physiological significance of this relationship.

B. Regulation of Appetite

The hypothalamus receives input from circulating nutrients such as glucose and fatty acids (438) as well as hormonal signals derived from the pancreas, adipose tissue, and the gut (438). The integration of these signals occurs within the arcuate nucleus (ARC)-containing neurons resulting in alterations in the expression of key orexigenic (neuropeptide Y, agouti related peptide) and anorexigenic (proopiomelanocortin) neuropeptides, which reciprocally regulate food intake, peripheral energy expenditure, and insulin sensitivity (438). Studies have demonstrated that AMPK is an important link in integrating these nutrient signals. AMPK catalytic subunits colocalize with neuropeptide Y (NPY)-expressing neurons and play a role in feeding control. Hypothalamic AMPK is activated by fasting and inhibited by refeeding (335). In elegant experiments where adenovirus encoding dominant negative (DN) and constitutively active (CA) mutations of AMPK were injected into the ventral medial hypothalamus, Minokoshi et al. (335) showed that a CA-AMPK increased body weight and food intake while a DN-AMPK had the opposite effect. The hyperphagia observed with CA-AMPK administration was related to increased expression of arcuate NPY and agouti-related peptide (AgRP) when in the fasted state while the reciprocal relationship was observed in the fed state with the DN-AMPK. The injection of AICAR intracerebroventricularly also increases AMPK and food intake (15). In a neuroblastoma cell line, the modulation of cellular ATP and AMPK by glucose, 2-deoxyglucose, pyruvate, or ATP synthesis inhibitors alters the expression of AgRP. Similarly, the fatty acid synthase inhibitor C75 was also found to suppress AMPK activity and NPY expression in arcuate-containing neurons, an effect that is reversed in the presence of AICAR (252). Additional anorexigenic signals, namely, insulin, glucose, and refeeding, also suppress AMPK activity in the brain, whilst the appetite-stimulating agouti-related protein activates hypothalamic AMPK (335).

The one caveat of all of the studies described above is that they have examined AMPK signaling in the context of hypothalamic sections, which contain multiple populations of neuronal (AgRP, POMC, NPY, etc.) and nonneuronal cell types, which may alter AMPK activity and appetite regulation in unknown ways. Surprisingly, mice with a whole body deletion of AMPKα1 or α2 have normal food intake and energy expenditure, suggesting that in the hypothalamus the α isoforms may substitute for one another in response to appetite control (519). However, recent studies by Claret et al. (99) utilizing a genetic approach to delete AMPKα2 from AgRp-containing hypothalamic neurons have found that the deletion of AMPK results in a lean phenotype, while the effects of deleting AMPK from POMC neurons has the opposite effect, as would be expected and surprisingly resulted in obesity. Importantly, these studies illustrate that AMPK is not required for leptin and insulin’s effects on K_ATP channel activation and appetite regulation, but is essential for glucose sensing. An important consideration of the current data in AMPK null mice is that compensatory pathways during development may be upregulated to compensate for the lack of AMPK signaling in specific neurons that were not present using transient infections using adenovirus. In addition, the experiments described by Claret et al. (99) were completed in mice with tissuespecific AMPKα2 deletion but on a whole body AMPKα1
background that has previously been reported to have hematopoietic defects; this may have influenced the findings (518, 519). Future studies using inducible models of AMPK deficiency or constitutive activation directly in AgRP and POMC-expressing neurons will be important to validate the role of AMPK in nutrient sensing and in response to hormonal stimuli in the hypothalamus.

It is currently unclear whether AMPK effects on neuropeptide expression are mediated directly or through inhibition of its downstream substrates ACC and mTOR. Surprisingly, whole body AMPKα1 and α2 null mice appear to have normal food intake, suggesting that the effects of AMPK on appetite may be indirect. The activation of AMPK would be expected to inhibit ACC, increasing rates of fatty acid oxidation and reducing long-chain fatty acyl-CoAs. Since the infusion of long-chain acyl-CoAs directly into the hypothalamus (369) or the chemical (368) and genetic (191) inhibition of hypothalamic CPT-1 leads to reduced food intake, this is consistent with the role of AMPK’s effects on orexigenic peptide expression. Another possibility is that AMPK regulation of mTOR may be critical seeing that the inhibition of mTOR using leucine, leptin, or rapamycin reduces food intake (109). While a recent proteomics screen has identified 12 in vitro substrates of AMPK in the brain, many of which were related to glycolysis (504), future studies are required to determine whether AMPK directly affects neuropeptide gene expression or whether this is via indirect mechanisms possibly involving ACC and mTOR.

1. Circadian clock

An emerging role of AMPK is in the regulation of circadian rhythm, which is set by a core of clock genes. These genes are regulated in a negative-feedback loop by casein kinase Iε (CKIε), which phosphorylates Per and Cry proteins, suppressing their own transcription. Since food intake and energy metabolism are synchronized to a circadian rhythm, it was hypothesized that AMPK may play a role in regulating this pathway (510). Treatment of cells with metformin shortened the circadian clock by reducing Per expression, suggesting that the activation of AMPK may be playing a role. In vitro phosphorylation confirmed that AMPK phosphorylated CKIε but not Per directly. Further analysis confirmed that AMPK phosphorylation of CKIε at Ser-389 dramatically increased CKIε activity inducing the degradation of mPer2, effects that were confirmed both in fibroblast cells and in vivo in mice treated with metformin. These data suggest that the circadian regulation of AMPK may be critical to understand the normal biological rhythms of metabolic enzymes and perhaps appetite warranting future studies in this area.

C. Regulation by Nutrients

1. Free fatty acids

Increasing fatty acid availability results in the up-regulation of fatty acid oxidation while concomitantly reducing endogenous rates of fatty acid oxidation and glucose metabolism (133) consistent with the Randle hypothesis (401). However, an unresolved question has been how increased extracellular fatty acid availability leads to this “feed-forward” activation of fatty acid metabolism. Recent studies in heart (100) and skeletal muscle (144, 307, 532) have revealed that AMPK may be sensitive to the “lipid status” of the cell and that activation may be influenced by intracellular fatty acid availability independent of the cellular AMP levels. The activation of AMPK by fatty acids is, alike to AMP, resulting in allosteric activation leading to an increased ability of LKB1 to phosphorylate the AMPK holoenzyme in vitro (532). Importantly, this effect is not observed when only AMPK α 1-312 is expressed, demonstrating that the effect of fatty acids requires interaction with the β or γ subunits of AMPK (532). It appears that at least in muscle cells the feed-forward activation of fatty acid oxidation by fatty acids is completely dependent on AMPK signaling, suggesting this is an important mechanism by which muscle responds to alterations in substrate availability (532). In support of these findings, genetic models that alter fatty acid handling as observed in mice null for steryl-CoA desaturase 1 (130) or the fatty acid binding protein AP2/MAL (73, 307) have increased AMPK in liver and skeletal muscle, respectively, and are protected from diet-induced obesity, suggesting a potentially important role for fatty acid sensing in vivo. During fasting, AMPK activity and fatty acid oxidation are increased, suggesting that an elevation of circulating fatty acids may mediate this effect.

2. High-density lipoprotein cholesterol

Circulating high-density lipoprotein (HDL) is often low in patients with the metabolic syndrome, and it is well documented that high levels of HDL are protective against atherosclerosis. The major apolipoprotein of HDL, apoAI, activates AMPK in endothelial cells and increases endothelial NOS phosphorylation and activity (132). Recent studies have shown APOA-I stimulation of AMPK signaling in C2C12 myotubes and that in skeletal muscle and liver of ApoAI null mice, AMPK phosphorylation is reduced (182). The authors suggest this as a potentially important role of HDL in regulating glucose and lipid metabolism in skeletal muscle and liver and that HDL may be important for protecting against diabetes. Future studies are required to determine whether the insulin-sensitizing effects of HDL are dependent on AMPK and to understand the mechanisms by which HDL may regulate AMPK signaling.
3. Amino acids

As discussed above, AMPK regulates protein translation via at least two mechanisms: phosphorylation and activation of the eukaryotic elongation factor 2 kinase (eEF2K), and inhibition of the mTOR-signaling pathway. Since the regulation of protein synthesis by mTOR is highly dependent on amino acid availability, it would seem logical that AMPK may also be regulated by amino acids. Recent studies by Gleason et al. (165) using MIN-6 cells have demonstrated that AMPK activity is suppressed by amino acids. This effect was found to be independent of mTOR, since treatment with rapamycin did not alter the ability of amino acids to inhibit AMPK phosphorylation. Subsequent studies using nonmetabolizable amino acids suggested that the inhibitory effect of amino acids on AMPK was indirect and was likely due to the well-documented stimulatory effects of amino acids on mitochondrial glutamate dehydrogenase, which results in enhanced tricarboxylic acid cycle flux, which would be anticipated to reduce cellular AMP content (165).

D. Endocrine Regulation

Adipocytes and resident macrophages secrete a wide variety of hormones and cytokines commonly called "adipokines." These signal changes in adipose tissue mass and energy status to other organs that control fuel usage, such as the brain, skeletal muscle, and liver. The secretion of adipokines such as leptin, adiponectin, interleukin-6, visfatin, TNF-α, retinol binding protein-4, and resistin are altered in obesity and are linked with many aspects of the metabolic syndrome. The discovery that AMPK is a central node utilized by hormones to regulate metabolism has been an exciting development in the field of AMPK biology. This is summarized in Figure 10 and is discussed below.

1. Leptin

Leptin signaling in the hypothalamus is essential for the regulation of body mass and neuroendocrine homeostasis (124a). However, leptin’s effects on metabolism are not limited to the hypothalamus as almost all tissues examined express leptin receptors (490). Leptin acts directly in isolated skeletal muscle to increase fatty acid oxidation (353) in an AMPK-dependent manner (336), and this process appears to involve an increase in the AMP/ATP ratio (336, 469, 526). The acute activation of AMPK is limited to α2-containing heterotrimeric (484) in oxidative muscle fibers (336), but the reason for this tissue-specific activation is still not clear.

In addition to the direct activation of leptin in skeletal muscle, there is also a delayed action of leptin that requires inputs from the central nervous system (CNS). The CNS-mediated activation of AMPK involves the stimulation of α-adrenergic signaling (336) and is dependent on the melanocortin (MC) system, since intracerebroventricular delivery of an MC4 receptor antagonist inhibits leptin activation of AMPK in skeletal muscle while a melanocortin agonist increases AMPK (487). Contrary to the situation in muscle, leptin administration results in suppression of AMPK activity in the paraventricular and arcuate sections of the hypothalamus (15, 335). The effects of leptin on AMPK signaling in the brain appear to be downstream of the MC receptor as intracerebroventricular administration of the MC4 receptor agonist MT-II inhibits AMPK activity in the paraventricular nucleus.
(PVN), while both refeeding and leptin fail to inhibit hypothalamic AMPK activity in MC4 receptor knockout mice (335). Future studies are required to delineate the upstream pathways mediating the acute and chronic effects of leptin on AMPK signaling and to understand how leptin mediates opposite effects on AMPK, activating in muscle but suppressing in hypothalamus.

Chronic leptin treatment is believed to help reverse insulin resistance independent of caloric restriction by reducing lipid storage in skeletal muscle and liver (451). Chronic leptin administration increases skeletal muscle AMPK α and β expression (472) and fatty acid oxidation (466). There is also evidence in cultured myotubes that AMPK activation by leptin increases AMPKα2 nuclear translocation and results in PPARα transcription (484). While leptin dramatically reduces lipid storage in the liver, this effect is independent of AMPK and involves the inhibition of PPARα (285) and SCD1 (102). Chronic adenovirus-induced hyperleptinemia rapidly reduces adipocyte size and mass, induces mitochondrial biogenesis, and concomitantly increases AMPKThr-172 phosphorylation in adipocytes (375, 522), but whether AMPK is required for this process is currently unknown. Skeletal muscle leptin resistance develops following high-fat feeding in rodents (467) and is also prevalent in obese humans (471, 473). The development of leptin resistance in obese skeletal muscle is characterized by suppressed rates of leptin-stimulated AMPK signaling (315, 469, 473, 526). Similarly, high-fat feeding inhibits the ability of leptin to suppress hypothalamic AMPK signaling (315, 474), an effect which may be mediated by the suppressor of cytokine signaling-3 (SOCS3) (469); however, this requires further study. In addition, while it is commonly believed that the insulin-sensitizing effects of leptin are mediated through activation of AMPK, further evidence in genetic models of AMPK deficiency is required to validate this hypothesis.

2. Interleukin-6

The interleukin-6 (IL-6) family, also known as “gp130 cytokines,” consists of IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (Osm), cardiophrin 1 (CT-1), ciliary neurotrophic factor (CNTF), and cardiophrin-like cytokine (CLC) (57). While IL-6 is elevated with obesity, the discovery that IL-6 can be produced and released from skeletal muscle during exercise (143) suggested a possible alternative role of IL-6 in regulating metabolism. Indeed, studies highlighting this possibility were findings that IL-6 can increase skeletal muscle fatty acid oxidation (63, 388, 513) and glucose uptake (75), effects observed both in vitro and in rodents and humans. Interestingly, basal AMPK signaling is markedly suppressed in liver, adipose tissue, and skeletal muscle of IL-6 KO mice, an effect which persists following swimming (248). Recent studies in myotubes (9, 75) have found that the activation of AMPK by IL-6 is essential for increases in fatty acid oxidation and glucose uptake. The mechanisms by which IL-6 increases AMPK are presently not understood.

3. TNF-α

TNF-α is directly implicated in the pathogenesis of insulin resistance in both humans and rodents, effects which have been linked to inflammatory signaling and serine phosphorylation of IRS1 (392, 512). TNF-α treatment downregulates fatty acid oxidation and inhibits AMPK signaling, an effect which is reversed following the siRNA of protein phosphatase 2C (PP2C) (470). TNF-α infusion into lean animals increases PP2C expression, resulting in a reduction in AMPK signaling, fatty acid oxidation, and increased intramuscular diacylglycerol accumulation, protein kinase C (PKC)-ε and -θ activation, and the development of skeletal muscle insulin resistance. Importantly, when TNF-α was neutralized in ob/ob mice, or when TNF-α signaling was genetically ablated (ob/ob TNFR−/− mice), the reduced AMPK activation observed in obese animals was reversed. These studies provide mechanistic evidence supporting a common link between inflammation and defective fatty acid metabolism in obesity while also highlighting an important role by which PP2C regulates AMPK signaling. Similar studies have also recently been reported in vascular smooth muscle (457). Future studies are required to determine whether TNF neutralization, which has been shown to improve insulin sensitivity in large-scale trials in patients with rheumatoid arthritis (170, 254), is associated with increased AMPK signaling and reductions in skeletal muscle lipid.

4. Resistin

Resistin (or FIZZ3) is an adipocyte-derived secretory factor first identified as a novel transcript produced exclusively by adipocytes (478). Despite the significant interest generated by the discovery of resistin in 2001, very little is known about the intracellular signaling pathways by which resistin induces its metabolic effects. A consistent finding in vivo is that resistin suppresses liver and muscle AMPK signaling (30, 398, 434), an effect also observed in L6 muscle cells (378). The mechanisms mediating this inhibition of AMPK signaling are still unclear, and it is unknown whether the observed in vivo effects are due to direct effects on AMPK signaling or may be mediated through indirect pathways. One possibility may be that resistin-induced increases in SOCS3 (479) may inhibit leptin signaling through to AMPK, but future studies are required to establish the mechanisms involved.
5. Ghrelin

Ghrelin is a gut-derived peptide hormone that promotes food intake (358) and has been shown to increase AMPK activity in the hypothalamus (15). Ghrelin has differential effects on AMPK activity depending on the tissue studied, with reports of ghrelin treatment suppressing AMPK activity in liver (31, 262) whilst activating AMPK in the heart (262). Ghrelin appears to have no effect on skeletal muscle AMPK. This may be related to the fact that ghrelin signaling is dependent on the G protein-coupled receptor (211) and therefore most likely on CAMKK signaling.

6. Adiponectin

Adiponectin is an abundant plasma protein that circulates as a range of multimers from low-molecular-weight trimers to high-molecular-weight dodecamers (409). Adiponectin levels are reduced in obesity (212), and overexpression has been shown to reverse skeletal muscle insulin resistance in models of genetic and diet-induced obesity (150, 316, 376, 560, 562). Adiponectin activates AMPK and stimulates fatty acid oxidation and glucose uptake in skeletal muscle (500, 561) and adipose tissue (554). Purification and characterization of adiponectin multimers from human plasma suggest that high-molecular-weight adiponectin most potently stimulates AMPK activity, at least in C2C12 cells (178). The activation of AMPK signaling is dependent on signaling through the adiponectin receptor 1 (AdipoR1) (563) and requires the adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif (APPL1); however, it is still unknown how APPL1 regulates AMPK signaling (312). The metabolic effects of adiponectin also involve the suppression of hepatic glucose output (103), an effect which is dependent on the activation of AMPK (16, 561). In agreement with these findings, the deletion of the AdipoR1 receptor in liver using siRNA results in reduced AMPK and increased gluconeogenesis and glucose intolerance (563).

In the hypothalamus, a recent study has found that adiponectin trimers and hexamers, but not high-molecular-weight forms, increase appetite (271) via AdipoR1 activation of AMPK. Importantly, Kubota et al. (271) demonstrate that the activation of hypothalamic AMPK by adiponectin reduces leptin sensitivity, leading to the proposal that through their central action leptin and adiponectin have opposite roles but when acting in concert these two adipokines work to maintain fat levels/energy stores through modulation of hypothalamic AMPK signaling (468).

7. Sex hormones: estradiol and testosterone

Estrogen and androgens play a critical role in regulating metabolism as highlighted by studies in mice bearing inactivating mutations in either aromatase, the enzyme responsible for estrogen biosynthesis (ArKO), or in the estrogen receptor α (ERKO). In both models mice develop age-onset obesity, hepatic steatosis, and insulin resistance (193, 197, 237, 338). Estrogen directly activates AMPK signaling and stimulates fatty acid oxidation in C2C12 myotubes (116), suggesting that in these models reduced AMPK signaling in muscle may contribute to the observed phenotype. However, a complicating factor in both the ArKO and ERKO mice is the finding of elevated circulating androgens. Recent studies by McInnes et al. (326) have shown that dihydrotestosterone infusion inhibits AMPK signaling in adipocytes, suggesting that increased adiposity in ArKO mice is due at least in part to the increase in androgen-to-estrogen ratio resulting in the inhibition of AMPK signaling rather than solely a lack of estrogen. Interestingly, AMPK expression levels and activity are not altered in human skeletal muscle (414). However, future studies are required to determine the mechanisms by which estrogen and androgens modulate AMPK activity and to determine the effects of these sex hormones in other tissues critical for regulating metabolism.

VIII. AMPK DYSREGULATION IN DISEASE

A. Aging and Longevity

Aging affects multiple aspects of metabolism, but it is generally well accepted, no matter what pathways are studied, that all organisms have a decreased ability to tolerate metabolic stress upon aging. Studies in lower organisms such as yeast and worms have indicated a potentially important role of AMPK homologs in regulating these age-related changes. Lin et al. (295) have used Snf1p, a yeast homolog of AMPK, to explore the genetic and biochemical link between glucose metabolism and aging. Snf1p is incorporated into a complex that contains Snf4p (relative to AMPK γ1), Sip1p, Sip2p, and Gal83 (relative to AMPK β1). Loss of the Snf1p activator Snf4p produces a 20% increase in the life span of yeast cells, whereas loss of Sip2p, a presumed repressor of the kinase, causes a rapid aging phenotype that appears to be related to increased Snf1p activity (21). The authors found that a prominent feature of the metabolic evolution of aging in yeast cells was activation of the Snf1p kinase pathway and increased gluconeogenesis and glucose storage. In Caenorhabditis elegans, the overexpression of AMPKα, designated aak-2, increases life span and appears to be required for increased longevity associated with signaling via daf-2/insulin and the deacetylase sir-2.1 (114). AMPK is also required for the long-term survival of the dau larva stage of C. elegans (360). In a followup study, it was found that AMPK signaling was required to
suppress the lipase ATGL and suppress the depletion of lipid stores in the daur larva (359). This seems likely to be a species-specific adaptation as *C. elegans* contains a non-conserved insert around Ser-303, the AMPK phosphorylation site. Glucose reduction or 2-deoxy-D-glucose-induced caloric restriction increases mitochondrial respiration, and Schults et al. (437) have suggested reactive oxygen/oxidative stress may be important for the longevity effects, but this view has been challenged (400). Greer et al. (173) also showed that dietary restriction-induced longevity required AMPK, and this involved multisite phosphorylation of the transcription factor FOXO/DAF-16 previously known to mediate longevity in *C. elegans*.

In higher organisms, the role of AMPK in the regulation of aging is not clear, with some reports demonstrating a significant reduction in AMPK signaling in muscle (309, 408) while others report no change (199, 379) or even increased AMPK activity (494, 495). The effects of metabolic stress such as hypoxia, caloric restriction, or muscle contraction on AMPK signaling in aging are equally equivocal with some studies reporting increased activation (169, 379), while others have reported a reduction (349, 408). Interestingly, resveratrol (3,5,4'-trihydroxy-stilbene), which extends the life span of organisms through Sir2, a conserved deacetylase, proposed to underlie the beneficial effects of caloric restriction, activates AMPK (121, 220, 384, 575). While recent studies suggest that the effect of Sir2 on longevity may have more to do with altered insulin and IGF-I signaling than AMPK (292), future studies examining longevity in AMPK null mice will be important.

**B. Obesity and the Metabolic Syndrome**

The World Health Organization estimates that of the more than 1 billion adults worldwide who are overweight, 300 million are clinically obese, defined as having a body mass index equal to or greater than 30 kg/m². Obesity is associated with a number of health problems that are often summarized together as the metabolic syndrome. Those include insulin resistance, type 2 diabetes, cardiovascular disease, and fatty liver disease. There are currently in excess of 170 million patients with type 2 diabetes, and this figure is expected to double by 2030 in line with trends in obesity. Much of the interest in AMPK has been generated because of the associations drawn between the metabolic syndrome and the pathways which AMPK regulates at the cellular level leading to the suggestion that AMPK signaling may be suppressed with obesity and that therapeutic activation of AMPK may therefore be beneficial (419, 539). Evidence supporting or refuting this conclusion will be discussed below.

In peripheral tissues such as heart, skeletal muscle, and liver, AMPK activity is reduced in most genetic models of rodent obesity (33, 298, 462, 470, 521, 570). However, it appears that AMPK activity is not altered in skeletal muscle (315, 526) or the hypothalamus (315, 474) of animals made obese by a high-fat diet, which is believed to more directly mimic the development of human obesity and insulin resistance. In obese human muscle, AMPK protein expression and activity are unaltered (473), although others have reported modest reductions (29). In human type 2 diabetic skeletal muscle, AMPK expression and activity are also unaltered relative to subjects with a similar body mass (199, 355). The sensitivity of AMPK to allosteric activation by AMP (473) and LKB1 activity/expression (88) is also unchanged in obesity and in obese type 2 diabetic skeletal muscle, suggesting that the AMPK system is indeed intact at least in moderate obesity. Similarly, AICAR has been demonstrated to increase AMPK activity to a similar degree in obese skeletal muscle from rodents (44, 68, 222, 394, 570) and humans (260, 327, 469, 473) relative to lean controls, although a recent study suggests that there may be reduced sensitivity of AMPK to aerobic exercise (461). Importantly, this activation of AMPK by AICAR increases glucose uptake and fatty acid oxidation in obese diabetic rodents (32, 44) and humans (88, 115, 260, 469, 473), validating the therapeutic potential of an AMPK activator for bypassing skeletal muscle insulin resistance. Taken together, these data suggest that the downregulation of AMPK signaling may not be a primary defect preceding metabolic aberrations associated with the development of obesity-related insulin resistance. However, it does appear that suppressed AMPK signaling in severe obesity, as seen in obese genetic models, is likely to exacerbate aspects of the metabolic syndrome. Therefore, therapies that prevent or reverse this reduction in AMPK signaling or artificially elevate it may have therapeutic value as will be discussed in the following sections.

1. **Hypertension, diabetic myopathy, and lipotoxicity**

Regular physical exercise has advantageous effects on blood pressure level in humans and also beneficially influences blood lipid profile and glucose homeostasis in individuals displaying features of the metabolic syndrome. Chronic AICAR treatment for 7 wk reduces blood pressure in obese Zucker rats, but it is currently unknown whether this effect is direct or is secondary to improvements in glucose and lipid homeostasis (68). Lipotoxic cardiomyopathy is another prominent component of the metabolic syndrome and is characterized by ectopic fat accumulation in the heart. Wang and Unger (521) have demonstrated that AMPK phosphorylation in cardiac muscle of *ob/ob* mice and obese Zucker rats is reduced relative to lean controls, an effect associated with increases in PP2C expression and cardiac lipid deposition,
suggesting that reduced AMPK signaling may contribute to this condition in obesity.

C. Cardiovascular Disease and Reperfusion Injury

In the normal, healthy heart, ~10–40% of ATP utilization is produced via pyruvate oxidation, while the remaining 60–90% of the ATP is derived from the oxidation of fatty acids. The regulation of cardiac energy metabolism is altered under many pathological conditions such as cardiac hypertrophy and myocardial ischemia-reperfusion injury, and since AMPK plays a critical role in regulating substrate use in the heart, its role in these pathologies has been examined.

1. Cardiac hypertrophy

Cardiac hypertrophy can occur due to a chronically increased cardiac work load. Since the activation of AMPK inhibits protein synthesis, it seems logical that overt activation may protect against hypertrophy, although evidence supporting this conclusion is equivocal with some studies presenting positive associations between AMPK activation and the development of cardiac hypertrophy (18, 498) while others suggest that AMPK activation can inhibit hypertrophic growth (86, 449). Since Akt plays a critical role in regulating cardiac hypertrophy, Dyck and colleagues (86, 265) have demonstrated that overt activation of this pathway and subsequently the AMPK activation can inhibit hypertrophic growth. Based on these findings, it would be expected that mouse models of AMPK deficiency would have increased heart size, but in most cases heart size is normal or even slightly reduced (428), suggesting that compensatory pathways may have developed or that these models must be studied under conditions of increased cardiac work load.

2. Myocardial ischemia-reperfusion injury

Both AMPK α1 and α2 activities are elevated during ischemia (272, 273), although only α2 phosphorylation appears to be dependent on LKB1 signaling (428), possibly downstream of TAK1 (557). Increasing AMPK signaling results in GLUT4 translocation and the stimulation of glucose uptake (421, 567) and fatty acid oxidation (272, 273), but whether or not this is beneficial for the recovery of the heart during reperfusion is unknown with reports utilizing a variety of different genetic models reporting either positive (422, 450) or detrimental effects (558). As discussed in detail in two recent reviews (131, 136), these equivocal findings may be due to the availability of fatty acids as fatty acids may directly inhibit recovery following ischemia. Therefore, it may be possible that AMPK may protect against ischemia-reperfusion injury by increasing glucose uptake if the potential negative consequences of increased fatty acid oxidation are not present.

D. Cancer

The AMPK upstream kinase LKB1 is a tumor suppressor that is mutated in Peutz-Jegher syndrome, a rare genetic disease leading to a predisposition towards intestinal polyps (hamartomas) and increased incidence of epithelial cancers in many other tissues including breast (234). While LKB1 mutations are uncommon in most cancers, somatic LKB1 inactivation is estimated to occur in 30–50% of sporadically occurring lung adenocarcinomas, ~20% of squamous cell carcinomas, and ~10% of large cell carcinomas of the lung, illustrating its tumor suppressor properties (234). LKB1 nonsense or frame shift mutations resulting in abnormal or truncated protein can occur in both primary lung adenocarcinomas or in lung adenocarcinoma cell lines (81, 430). In the case of human endometrial cancer, downregulation of LKB1 expression has been detected by immunohistochemistry and was inversely correlated with both tumor grade and stage (144).

LKB1 (−/+ ) mice have been reported to have enhanced sensitivity to chemical carcinogen 7,12-dimethylbenz(a)anthracene (DMBA)-induced squamous cell carcinoma (SCC) of the skin and lung (174). In this study, restoration of wild-type LKB1 caused senescence in tumor-derived cell lines that was dependent on the Rb pathway, but not on p53 or AMPK, indicating that AMPK is not an obligate mediator of LKB1’s SCC suppressor activity.

The role of LKB1 in activating AMPK has highlighted the possibility that AMPK may be important in mediating LKB1 tumor suppression pathways. Accordingly, activation of AMPK by pharmacological or other means might be associated with reduced cancer incidence. Activation of AMPK by AICAR treatment of the breast cancer cell line MDA-MB-231 with AICAR blocks proliferation and colony formation in culture as well as reducing tumor growth in nude mice (485). Subsequently, these investigators have reported that the antiproliferative effects of AICAR can be further enhanced with methotrexate treatment to reduce AICAR metabolism (39). AICAR has also been found to inhibit cell proliferation and promote apoptosis in multiple acute lymphoblastic leukemia cells, CCRF-CEM (T-ALL), NALM6 (Bp-ALL), REH (Bp-ALL, TEL/AML1), and SupB15 (Bp-ALL, BCR/ABL) (443). The adenosine kinase inhibitor 5’-iodotubercidin abolished the effect of AICAR (443), indicating nucleoside phos-
phorylation to ZMP was required. Thus studies showing different activators of AMPK, namely, AICAR and metformin (discussed in detail below), can block growth of breast cancer cells in culture are in line with the epidemiology data showing a reduced risk of cancer in diabetic patients treated with metformin (142).

It is recognized that tumor development and progression are accompanied by marked changes in the expression and activity of key enzymes in lipid metabolism. These include FAS whose transcription is regulated by AMPK (as discussed previously) and is thought to play a critical role in lipogenesis for cancer cell energy storage. FAS is highly expressed in breast cancer and many common human carcinomas. Indeed, FAS is now regarded as an important anticancer drug target (330, 515). Inhibition of earlier steps in fatty acid synthesis such the AMPK substrate ACC by the macrocyclic polyketide inhibitor soraphen A has been shown to inhibit proliferation of prostate cancer cells (38). Thus from this perspective any loss of AMPK signaling to ACC in cancer cells would be expected to promote survival. There is a further important link in breast cancer metabolism because BRCA1 associates with phosphorylated ACC1 (308, 345) and suppresses its dephosphorylation and activation (i.e., equivalent to augmenting the AMPK signal). Deletion mutations in BRCA1 fail to block ACC1 dephosphorylation, thereby contributing to elevated fatty acid synthesis in tumor cells with BRCA1 mutations (65). AMPK readily associates with ACC1, and this raises the possibility that a larger signaling complex with BRCA1 may be present with other yet unrecognized tumor suppressor functions.

Statins have been reported to have antitumor activity, and the relationship between statin use and breast cancer risk has been investigated. One large study detected an 18% reduction in breast cancer incidence with statin therapy (hydrophobic statins only) targeting HMGR (84). AMPK phosphorylates and inactivates HMGR, thereby inhibiting flux through the mevalonate pathway and production of cholesterol and isoprenoid groups for the posttranslational modification of many signaling proteins (e.g., GTP-binding proteins that are either farnesylated or geranylgeranylated). While there may be a possible link between tumor suppression and AMPK regulation of HMGR, there is a lack of evidence to support a mechanism at this point. Transgenic mice carrying Ser/Ala mutations in the AMPK phosphorylation sites of ACC1 and HMGR have now become available, making it possible to test whether blocking AMPK signaling to these enzyme targets has an impact on tumor development or progression.

E. Dementia, Neurogenesis, and Stroke

Both AMPKα1 and -α2 are highly expressed in the brain (465), exhibiting a predominant neuronal localiza-

ix. OPPORTUNITIES FOR THERAPEUTICS

A. Metformin

Metformin is an oral biguanidine that reduces plasma glucose and lipids and improves insulin sensitivity in patients with type 2 diabetes (129). Although biguanidines became available for diabetes therapy in 1957, and have since become the most widely used treatment for the disease, the mechanisms by which they improve insulin sensitivity is incompletely understood. Interest in AMPK as a therapeutic target for the treatment of metabolic syndrome gained rapid momentum after experiments showed that AMPK was activated by metformin (357, 582). Metformin activation of AMPK is dependent on up-

Physiol Rev • VOL 80 • JULY 2009 • www.prv.org
take by the organic cation transporter (OCT1) (452), and although original studies suggested that metformin activated AMPK independent of changes in nucleotides (153, 187), more recent studies utilizing γ mutants which are insensitive to AMP have demonstrated that nucleotide binding is essential for metformin activation of AMPK (441). Both phenformin and metformin treatment of isolated rat hearts results in increased calculated cytosolic AMP based on 31P-NMR measurements of phosphocreatine, ATP, and intracellular pH (578). In this study, HPLC-based measurement of the total AMP/ATP ratio showed no change with metformin treatment (578), perhaps indicating that a local pool of AMP associated with the mitochondria may be responsible for AMPK activation. These findings are consistent with the well-documented effects of metformin, which is known to inhibit respiratory chain complex I, leading to an inhibition of mitochondrial respiration and the inhibition of β-oxidation of fatty acids which requires NAD as a cofactor (138, 377, 585). A secondary mechanism by which metformin may activate AMPK involves the accumulation of reactive nitrogen species that in turn stimulate the c-Src/PI3K pathway generating a metabolite or other molecule inside the cell to promote AMPK activation by the LKB1 complex (585). However, identification of this factor remains elusive.

The main site of metformin action appears to be the liver, where AMPK is activated and is required to suppress gluconeogenic and lipogenic gene expression (582). Studies in human HepG2 cells using a constitutively active or dominant negative AMPKα have shown that AMPK is also required for the lipid-lowering effects of metformin (576). Metformin is ineffective in lowering blood glucose in high-fat diet fed mice lacking LKB1 in the liver (446), suggesting that the LKB1-AMPK pathway is required for metformin action. Interestingly, metformin also reduces inflammatory markers such as TNF-α (294) and C-reactive protein (179), effects that are likely mediated by AMPK activation and was reversed by siRNA against AMPK. The growth inhibition of MCF7 breast cancer cells, an effect dependent on the presence of AMPK (573). The growth inhibition was associated with decreased mTOR and S6 kinase activation and was reversed by siRNA against AMPK. The growth suppressor effects were specific, as shown by the fact that HeLa cells, which are deficient in LKB1, were unaffected by the metformin treatment. Metformin-treated cells have suppressed oxidative phosphorylation and compensate for this by increasing their rate of glycolysis, which is p53-dependent and raises the possibility that p53-deficient tumor cells are unable to make this metabolic switch in the presence of metformin (70). On the other hand, a recent study has identified reductions in cyclin D1 and Rb phosphorylation in response to metformin treatment as important for this drug’s antitumor action on prostate cancer cells (424). Importantly, inhibition of AMPK using siRNAs to the α subunits did not prevent the antiproliferative effects of metformin, strongly indicating that AMPK is not essential in this case (424).

B. Thiazolidinediones

PPAR-γ is a nuclear hormone receptor that is predominantly expressed in adipose tissue but also has important roles in skeletal muscle (194), liver (162), and macrophages (195). Despite intensive research into the role of PPARγ, the mechanisms by which their synthetic ligands, the thiazolidinediones (TZDs), cause insulin sensitization are incompletely understood. TZDs improve hepatic insulin sensitivity (196), but interestingly, they do not consistently lower lipid content (288). TZDs activate AMPK in skeletal muscle cells by increasing cellular adenine nucleotide levels (153, 277). In vivo, chronic treatment with TZDs increases skeletal muscle and cardiac AMPK, but it is difficult to discern whether this effect is direct or due to the potent effects of TZDs in stimulating adiponectin production (521). Supporting the latter conclusion is the finding that rosiglitazone is unable to improve glucose tolerance or activate AMPK in ob/ob adiponectin null mice (270, 362). In light of recent studies demonstrating an increased incidence of myocardial ischemic events with rosiglitazone treatment (204, 417), it is interesting to speculate that this may be due to chronic
activation of AMPK in the heart, which may lead to
glycogen storage disease, cardiac hypertrophy, and poten-
tially greater cardiac ischemia (306).

C. Life-style Interventions

Cross-sectional and retrospective epidemiological
studies have provided direct evidence that a lack of phys-
ical activity is strongly associated with impaired glucose
tolerance in obesity (390). Physical inactivity is consid-
ered an independent risk factor for insulin resistance and
type 2 diabetes and exercise (150 min/wk), with moderate
weight loss (−7%) reducing the incidence of diabetes by
58% in individuals with elevated fasting plasma glucose
(129). Importantly, this effect was greater than that
achieved by metformin treatment alone (−31%). Petersen
et al. (390) have demonstrated that improved glucose
tolerance with exercise training is related primarily to the
reversal of hepatic steatosis and a reduction in fasting
hepatic glucose production, while other studies also re-
port a reduction in skeletal muscle lipid metabolites (64).
While exercise activates AMPK signaling in muscle and
liver, it remains to be tested in genetic models of AMPK
deficiency whether AMPK is sufficient and/or essential for
this effect.

D. Ciliary Neurotrophic Factor

CNTF is a member of the IL-6 family of cytokines and
was initially identified as a neurotrophic factor to poten-
tially treat severe neurodegenerative disorders such as
amyotrophic lateral sclerosis (ALS). CNTF rapidly in-
duces weight loss in diet-induced (276) and genetic
(ob/ob, MC4R−/−) obesity (167, 454) at low doses without
causing the typical deleterious effects of other related
cytokines. This effect has been confirmed in recent clinical
trials (141). CNTF increases AMPK activity in skeletal
muscle by acutely decreasing the ATP/AMP ratio, result-
ing in increased fatty acid oxidation (526). The adminis-
tration of CNTF, at doses demonstrated to induce weight
loss, stimulated the expression of mitochondrial oxidative
genes in skeletal muscle, an effect which may involve the
activation of AMPK. Importantly, the stimulatory effect of
CNTF on AMPK activation and fatty acid oxidation re-
versed insulin resistance induced by acute exposure to
high fatty acid levels in rodents in vivo (527) and in vitro
(526); this effect was not maintained when cells were
infected with a dominant negative AMPK.

In the brain, CNTF and leptin display similar expres-
sion patterns and signaling homology within hypotha-
lamic regions involved in food intake (14, 276). Therefore,
in many ways, it was not surprising that like leptin CNTF
reduces AMPK signaling in hypothalamic sections con-
taining ARC neurons. Importantly, and unlike leptin, the
ability of CNTF to suppress AMPK in the hypothalamus
(474) and activate AMPK in skeletal muscle (526) persists
in leptin resistant mice fed a high-fat diet highlighting its
potential role in the therapeutic treatment of human obe-
sity. CNTF also induces hypothalamic remodeling/neuro-
genesis, which may contribute to the resistance to re-
bound weight gain (261), although whether this involves
AMPK as has been shown for leptin (118) is unknown. A
recent study (139) has found that like skeletal muscle
AMPK is also activated by CNTF in neurons/astrocytes
leading to increases in fatty acid metabolism and mito-
chondrial gene expression. This effect may serve to pro-
tect neurons in time of metabolic stress. CNTF also acti-
vates AMPK in hepatocytes (214) and adipocytes (111).
Future studies are required to delineate the mechanisms
by which CNTF can have diverse effects in different as-
pects of the brain and to determine whether the effects of
CNTF on feeding are dependent on AMPK signaling.

E. Natural Compounds

1. α-Lipoic acid

The naturally occurring short-chain fatty acid α-li-
poic acid (α-LA) is an essential cofactor of mitochondrial
respiratory enzymes and is best known for its powerful
effects as an antioxidant. However, recent studies have
demonstrated impressive effects of α-LA on many aspects
of metabolic regulation; these effects appear to be largely
dependent on AMPK signaling. For example, in the arcu-
te region of the hypothalamus, α-LA reduces AMPK sig-
naling and food intake (253), while in skeletal muscle it
activates AMPK enhancing glucose transport and fatty
acid oxidation (283); these effects are maintained in lep-
tin- and insulin-resistant obese rodents. AMPK activation
by α-LA has also been observed in the heart (284) and the
endothelium (282) where it has been shown to prevent
and improve endothelial dysfunction by normalizing lipid
metabolism. Consistent with its role in enhancing insulin
sensitivity in vivo, it appears to concomitantly reduce
insulin secretion and content, as well as cell growth in
β-cells (488).

2. Polyphenols and resveratrol

Polyphenols have been suggested to be the primary
active ingredients found in green tea or red wine, which
help mediate their reported beneficial effects on dyslipi-
demia, cardiovascular disease, and longevity. However,
the mechanism(s) of action of these compounds has re-
ained largely a mystery, limiting their therapeutic po-
tential. Several recent studies have demonstrated that
polyphenols such as resveratrol stimulate AMPK activity
in a variety of cell types including liver (575), skeletal
muscle (36, 384), neurons (121), and cancer (220). The
activation of AMPK by resveratrol may be mediated by AMP as resveratrol inhibits the mitochondrial F1 ATPase (166). In muscle cells, the activation of AMPK by resveratrol has been linked to the stimulation of glucose transport (384) and mitochondrial biogenesis (36), while in hepatocytes AMPK activation reduces lipid accumulation due to high glucose. This effect is inhibited by a DN-AMPK (575). Future studies in models of AMPK deficiency are needed to test in vivo whether the beneficial effects of polyphenols do in fact require AMPK. At present, this seems unlikely given the potent effects of a SIRT1 activator, which improves insulin sensitivity in obese Zucker rats without altering AMPK activity (334).

3. The traditional Chinese medicine berberine

Berberine is a naturally occurring bright yellow botanical isoquinoline alkaloid that has been commonly used in traditional Chinese and Korean medicines as an antimicrobial/fungal agent and as a treatment for type 2 diabetes. It has also been used historically to treat a plethora of ailments as well as a fluorescent indicator for heparin in mast cells. The clinical efficacy of this compound has been demonstrated in clinical trials where it has been shown to restore euglycemia in type 2 diabetic patients (364) and to reduce hypercholesterolemia (263); however, the mechanisms mediating these effects are only now being explored. Large oral doses of the drug are required, and derivatives, including dihydroxyberberine, with approximately fourfold greater potency are being explored (508). Recent studies have demonstrated that berberine potently activates AMPK in hepatocytes (66) as well as skeletal muscle, L6 cells (94), and adipose tissue (286). The activation of AMPK in these tissues was associated with the suppression of lipid synthesis and increases in both fatty acid oxidation and glucose uptake, effects which may have contributed to the significant improvements in insulin sensitivity in obese mouse models treated chronically with the extract (286). The activation of AMPK, analogous to other natural compounds such as metformin, is thought to be due to berberine inhibiting respiratory-complex I of the mitochondria, resulting in an elevated AMP/ATP ratio (508, 566). Berberine activation of AMPK is not upstream kinase selective, since activation in L6 cells is independent of CaMKKβ inhibition by STO-609, presumably because LKB1 is acting as the upstream kinase whereas in LKB1−/− MEFS the berberine-dependent activation is blocked by STO-609 pretreatment (508). In these experiments AICAR behaved in the same way as berberine with respect to STO-609 inhibition, leading the authors to make the point that inhibition of phosphatase activity by AMP or ZMP binding to the γ subunit was driving AMPK activation (508).

4. Gut flora and AMPK

Despite the accepted role of both genetics and environmental factors in predicting the development of obesity, recent studies have demonstrated that rather surprisingly the type and number of bacteria colonizing the gut and intestines are also critical (27, 289, 290, 507). A recent study from the Gordon group (28) has shown that germ-free mice, which are protected from diet-induced obesity, have elevated AMPK signaling in skeletal muscle and liver, an effect most likely attributable to a 50% increase in muscle AMP content. Importantly, these effects are reversed following recolonization into the germ-free animals. Future studies are required to identify whether alterations in AMPK signaling are required for the lean phenotype and to determine whether changes in the hormonal milieu, related to a reduced caloric balance due to incomplete digestion or the bacteria themselves may be secreting a factor, which directly modulates cellular AMP levels. This raises the exciting prospect of identifying bacterial flora derived AMPK signaling suppressor molecule. In this event, antagonists would be expected to block the bacterial flora impact and promote weight loss. Alternatively, irrespective of the mechanisms involved, these findings suggest that it may be possible to manipulate AMPK signaling and therefore weight gain through the manipulation of gut flora by ingesting food enriched with selected probiotics.

F. Small Molecule Activators

While metformin and to a lesser degree TZDs appear to mediate many of their effects on lipid profiles and insulin sensitivity through activation of AMPK, these agents activate AMPK indirectly by altering nucleotide levels and thus are nonspecific for AMPK. In addition, these current treatments may also activate AMPK in tissues such as heart and brain where the activation of AMPK is not desired. The great breadth of AMPK’s roles linking numerous physiological events to energy metabolism makes a “drug target siren.” Nevertheless, there are compelling arguments favoring the development of agonists that target AMPK specifically in skeletal muscle and liver. Abbott Laboratories has pioneered this area and identified a thienopyridone AMPK activator. This compound resulted from a chemical library screen of 700,000 compounds resulting in a single hit, nonnucleoside thienopyridone (A-592107, EC50 38 μM) AMPK activator. Optimization of this compound resulted in A-769662 with substantially improved potency (EC50 0.8 μM) (105). A-769662 inhibits fatty acid synthesis in isolated hepatocytes and reduces liver malonyl-CoA levels in rats. It also decreases PEPCK, G-6-Pase, and FAS expression in ob/ob mice, lowers plasma glucose, body weight, and plasma and liver triglyceride levels. Although these properties
were encouraging, A-769662 did not activate AMPK in skeletal muscle and had poor bioavailability. The A-769662 activation mechanism has turned out to be novel and does not compete with AMP. However, A-769662 does mimic the effects of AMP in allosterically activating AMPK and preventing dephosphorylation (171). It does not involve the AMP binding sites on the γ subunit, since mutations that abolish AMP activation do not block A-769662 activation (431). Instead, A-769662 activation requires the β subunit (431). Truncation mutagenesis of the carbohydrate-binding domain (CBD) abolishes A-769662 activation but not AMP activation. While the CBD is important for A-769662 activation, it does not appear to involve the glycogen/sugar-binding site, since it does not inhibit CBD glycogen binding (171, 442). There is a puzzling requirement for β-subunit Ser-108 since mutation to Ala abolishes drug activation (431, 442). A-769662 does not activate the isolated catalytic domain (α 1-312) or the autoinhibited fragment (α 1-333) (171), suggesting that it is primarily reversing the β-subunit autoinhibitory actions rather than those of the α subunit. A screen of 76 kinases indicates that A-769662 is neither a promiscuous activator nor inhibitor of other kinases (171). In terms of upstream kinase requirements, A-769662 activates AMPK in HeLa cells, lacking LKB1 as well as in TAK1 null MEFs (171). Like AMP, A-769662 inhibits dephosphorylation of AMPK by phosphatase PP2C, which is interesting from a mechanistic aspect because both ligands appear to have distinct mechanisms of activation. We have recently found that A-769662 is selective for the β1 isoform and does not activate AMPK heterotrimers containing β2 (442) nor AMPK in tissues from β1 null mice.

Pang et al. (381) have recently reported a second class of small molecule AMPK activator, termed PT1. This compound was identified by a more targeted chemical library screening of 3,600 diverse compounds with the autoinhibited α-subunit fragment [α1 (1-394)] and has an EC50 8 μM for α1 and 12 μM for the corresponding α2 (1-398) fragment. Since the constitutively active AMPK fragment α1 (1-312) is not activated by the PT1 compound, it is proposed that PT1 acts by reversing the autoinhibition of the AIS (313-335). This is supported by molecular modeling and mutagenesis studies showing that PT1 activation depends on Glu-96 in the small lobe and Lys-156 in the large lobe near the catalytic DFG motif (381). However, these mutations also dramatically reduce basal activity so are not necessarily fully informative. PT1 appears to activate the native αβγ heterotrimer since it activates AMPK in L6 and HeLa cells (381). Both cholesterol and triglyceride synthesis are inhibited by PT1 in HepG2 cells (381). Considerable further work is required to determine the crystal structure of PT1 complexed with AMPK as well as chemical optimization, target specificity, and in vivo evaluation of the drug. This work sets the stage for exploiting drug screening of other proteins, the activity of which is regulated by autoinhibitory mechanisms (258).

X. CONCLUSIONS AND FUTURE DIRECTIONS

AMPK is present in all tissues as an αβγ heterotrimer, and its expression is regulated by multiple genes encoding each of the subunits (α1, α2, β1, β2, γ1, γ2, γ3) with differential tissue-specific expression and activity. AMPK regulates cellular energy metabolism through direct effects on gene transcription and key metabolic enzymes. This typically occurs at multiple points in metabolic pathways as is illustrated by AMPK’s regulation of lipid metabolism. At the whole body level, AMPK helps control appetite, energy expenditure, and substrate utilization in response to exercise, nutrients, and cytokines and may be linked to many physiological processes. AMPK has now fully emerged from its historical orphan status as a cellular metabolic stress sensor to a highly integrated signaling junction for many biochemical pathways. Nevertheless, despite AMPK’s ascent to “super metabolic regulator” status, it is becoming increasingly clear AMPK is just one of a number of players. Indeed, AMPK can suffer from the “Heisenberg uncertainty” phenomenon where the harder you look the less it seems to be essential, as appears to be the case for AMPK’s role in exercise-induced glucose uptake. AMPK signaling may be altered in a number of disease conditions; however, despite the convincing associations between AMPK signaling under a number of different conditions and treatments, there is nevertheless an urgent need for studies using genetic models of AMPK deficiency/functional titration to fully evaluate the quantitative role of AMPK in regulating physiological responses in vivo. We can look forward to an exciting vista of future discoveries that answer the following pressing questions: how significant is AMPK to the etiology of type 2 diabetes? Will future drug development lead to a repertoire of β1 and β2 specific antagonists and agonists? Will AMPK targeted therapy have a role in obesity treatment? Is AMPK a practical target for cancer therapy? Can AMPK-activating drugs mimic the beneficial metabolic effects of diet and exercise? Can AMPK therapy improve the quality of life in the elderly?

ACKNOWLEDGMENTS

We are indebted to Drs. Jonathan S. Oakhill and Sebastian B. Jorgensen for assistance in preparing the figures and to Anne Johnston for editing the manuscript.

G. R. Steinberg is a Canadian Research Chair in Metabolism Obesity and Type 2 Diabetes. Current address: G. R. Steinberg, Dept. of Medicine, McMaster University, 1200 main St. W, Hamilton, Ontario, Canada L8N 375 (e-mail: gsteinberg@mcmaster.ca).
REFERENCES


72. Camacho RC, Pencek RR, Lacy DB, James FD, Donahue EP, Wasserman DH. Portal venous 5-aminomimidazole-4-carboxamide-1-beta-t-ribosfuiranose infusion overcomes hyperinsulinemic sup-
73. Cao H, Maeda K, Gorgun CZ, Kim HJ, Park SY, Shulman GI, Kim JK, Hotamisligil GS. Regulation of metabolic responses by adipocyte/macrophage fatty acid-binding proteins in leptin-defi-
78. Carretero J, Medina PP, Pio R, Montuenga LM, Sanchez-

81.
83.
80.
82.
84.
85.
86.
87.
88.
89.
90.
91.
92.
93.
94.
95.
96.
97.
98.
99.
100.
101.
102.
103.
104.
105.
106.
107.
108.
109.
110.
111.
112.
113.
114.
115.
116.
117.
118.
119.
120.
121.
122.
123.
124.
125.
126.
127.
128.
129.
130.
131.
132.
133.
134.
135.
136.
137.
138.
139.
140.
141.
142.
143.
144.
145.
146.
147.
148.
149.
150.
151.
152.
153.
154.
155.
156.
157.
158.
159.
160.
161.
162.
163.
164.
165.
166.
167.
168.
169.
170.
171.
172.
173.
174.
175.
176.
177.
178.
179.
180.
181.
182.
183.
184.
185.
186.
187.
188.
189.
190.
191.
192.
193.
194.
195.
196.
197.
198.
199.
200.
201.
202.
203.
204.
205.
206.
207.
208.
209.
210.
211.
212.
213.
214.
215.
216.
217.
218.
219.
220.
221.
222.
223.
224.
225.
226.


284. Lee Y, Naseem RH, Park BH, Garry DJ, Richardson JA, Schaf-


286. Lemieux K, Konrad D, Klip A, Marette A.


288. Ley RE, Turnbaugh PJ, Klein S, Gordon JI.


AMPK IN HEALTH AND DISEASE 1071


502. Watt MJ, Holmes AG, Steinberg GR, Mesa JL, Kemp BE, Febbraio MA. Reduced plasma FFA availability increases net triglyceride degradation, but not GPAT or HSL activity in human.


