Non-catalytic β- and γ-Subunit Isoforms of the 5′-AMP-activated Protein Kinase*

(Received for publication, December 20, 1995)

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The mammalian 5′-AMP-activated protein kinase (AMPK) is a heterotrimeric protein consisting of α, β, and γ-subunits. The α-subunit is the catalytic subunit and is related to the yeast Snf1p kinase. In this study, we report the cloning of full-length cDNAs for the non-catalytic β- and γ-subunits. The rat liver AMPK β-subunit clone predicts a protein of 30,464 Da, which is related to the Sip1p, Sip2p, and Gal83p subfamily of yeast proteins that interact with Snf1p and are involved in glucose regulation of gene expression. The AMPK β-subunit, when expressed in bacteria and in mammalian cells, migrates anomalously on SDS gels at an apparent molecular mass of 40 kDa. Rat and human liver AMPK γ-subunit clones predict a protein of 37,577 Da (AMPK-γs), which is related to the yeast Snf4p protein that copurifies with Snf1p and to a larger family of other human AMPK γ-isomers. The mRNAs for both AMPK-β and AMPK-γ are widely expressed in rat tissues, consistent with a broad role for AMPK in cellular regulation. These data reveal a mammalian multisubunit protein kinase strikingly similar to the multisubunit glucose-sensing Snf1 kinase complex. The identification of isoform families for the AMPK subunits indicates the potential diversity of the roles of this highly conserved signaling system in nutrient regulation and utilization in mammalian cells.

The mammalian 5′-AMP-activated protein kinase (AMPK) and its homologs are expressed in plants, yeast, and mammals

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* This work was supported in part by National Institutes of Health Grant DK 35712 (to L. A. W.) and by a grant from the National Heart Foundation (to B. E. K.). The costs of publication of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†† The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U42411, U42412, and U42413.

‡‡ Performed a portion of this work in partial fulfillment of a Ph.D. thesis at the St. Vincent’s Institute of Medical Research.

§§ Performed a portion of this work in partial fulfillment of a Dartmouth College undergraduate senior honors thesis supported in part by an undergraduate internship from the Howard Hughes Medical Institute.

¶¶ Supported by fellowships from the Alberta Heritage Foundation and the Heart and Stroke Foundation of Canada.

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† The abbreviations used are: AMPK, 5′-AMP-activated protein kinase; PCR, polymerase chain reaction; bp, base pair(s); MOPAC, mixed oligonucleotide-primed amplification of cDNA; RACE, rapid amplification of cDNA ends; HA, hemagglutininina.

1 AMPK, a member of the SNF1 (sucrose non-fermentor) kinase family (4–7), was first recognized as a regulator of fatty acid and sterol synthesis through its phosphorylation of acetyl-CoA carboxylase and hydroxymethylglutaryl-CoA reductase, respectively (8). In particular, AMPK mediates responses of these pathways to several metabolic or other cellular stresses, including glucose depletion, heat shock, and ATP depletion (9–12).

Purification of pig and rat liver AMPKs has revealed a heterotrimeric kinase structure consisting of a 63-kDa catalytic α-subunit and non-catalytic β (40 kDa)- and γ (38 kDa)-subunits (4, 6). The AMPK α-subunit is 64% identical in its catalytic core to the Saccharomyces cerevisiae Snf1p2 protein kinase, which is responsible for the glucose derepression response of the SUC1 gene (4, 5, 7, 13). In contrast to AMPK, Snf1p occurs as a heterodimer with Snf4p and does not purify with other identified interacting proteins (4, 6). We have recently found that multiple isoforms of AMPK-α (α1 and α2), which are products of distinct genes, are present in liver and other tissues (14). The AMPK α1-isoform accounts for ~90% of total AMPK activity in liver extracts, yet its corresponding mRNA level is low relative to that of the AMPK α2-isoform. Preliminary peptide sequencing and limited PCR product analysis of the non-catalytic subunits have indicated that the AMPK γ-subunit is related to the S. cerevisiae protein Snf4p (CAT3), whereas AMPK-β is related to the S. cerevisiae Sip1p/Sip2p/Gal83p family of proteins. These are known to associate with the Snf1p kinase and to participate in glucose-regulated gene expression (6).

In this study, we report the molecular cloning of full-length cDNAs for the mammalian AMPK β- and γ-subunits. These domes have been used to characterize the tissue distribution of subunit mRNA and to express subunit protein in both bacteria and mammalian cells. Knowledge of their complete sequences has also led to the identification of protein isoform families for each of these non-catalytic units.

EXPERIMENTAL PROCEDURES

AMPK Isolation and Peptide Sequencing—Porcine and rat liver AMPKs were isolated by a previously published method (4, 6). Peptide sequences derived from the rat liver β (40 kDa)- and γ (38 kDa)-subunits were obtained after subunit separation by SDS gel electrophoresis, band elution, and in situ protease digestion as described (4, 6). AMPK β-subunit cDNA Isolation—Peptide sequences derived from the AMPK β-subunit were used to generate partial-length AMPK β-subunit cDNAs by PCR as described (13). One product, a 309-bp cDNA, was used to screen a rat liver λZAPII cDNA library (Stratagene) as described (13). Filters were hybridized with [32P]cDNA labeled with Nomenclature use is, for example, Snf1p, the protein product of the SNF1 gene.

2 CAT3 and SNF4 are alternative terminologies for this yeast gene.

25, 26.)
rounds of plating and rescreening. Duplicate, and positive plaques were purified through three additional rounds of screening.

 Autoradiography was overnight at 80°C. All plates were lifted in 3 parts formamide,10 M NaCl,50 m M Tris-Cl (pH 7.5), and 100 µg/ml salmon sperm DNA at 42°C for 18 h. They were then washed at room temperature 3 x 10 min and then at 55°C for 15 min. Autoradiography was overnight at ~80°C. All plates were lifted in duplicate, and positive plaques were purified through three additional rounds of plating and rescreening.

AMPK γ-Subunit cDNA Isolation—For the AMPK γ-subunit, we initially generated a 67-bp cDNA by the MOPAC technique (15). Degenerate PCR primers were synthesized corresponding to the N- and C-terminal sequences of a 17-amino acid rat liver AMPK-γ peptide that yielded a full-length AMPK-γ cDNA (see below). The purified PCR product was digested with BamHI and EcoRI sites were added to the 5'-ends of these primers. The strategy was to create a non-degenerate nucleotide sequence corresponding to the middle portion of the peptide sequence that would be used in library screening. Total rat liver cDNA, prepared with oligo(dT) and random hexamers (pre-amplification kit, Life Technologies, Inc.), was used with PCR to amplify oligonucleotide corresponding to amino acid sequence (520 bp) obtained was subcloned in pCR-Script (Stratagene) and sequenced. This sequence (see Fig. 4), which actually predicted amino acid sequence corresponding to all three AMPK-γ peptides used in the PCR strategy, was then used for library screening as described above. Screening of 2 x 10^9 plaques with this larger PCR product yielded several positive clones, which were further characterized (see below); however, none of the rat cDNAs (1–1.3 kilobases) isolated corresponded to the 5'-end of the open reading frame, a primer extension library was constructed using rat liver cDNA as template. The largest product (192 bp) was then used to screen a human fetal liver library, as previously reported (6) and as completed for this study (new sequences include amino acids 55–57, 108–125, 161–181, and 183–199). Assignment of the start codon is explained under “Results.”

[16P]CTP (3000 mCi/mmol; DuPont NEN) by random priming (random primer cDNA labeling system, Life Technologies, Inc.), in 50% formamide, 10 x Denhardt's solution, 1 x NaCl, 50 mM Tris-Cl (pH 7.5), and 100 µg/ml salmon sperm DNA at 42°C for 18 h. They were then washed at room temperature 3 x 10 min and then at 55°C for 15 min. Autoradiography was overnight at ~80°C. All plates were lifted in duplicate, and positive plaques were purified through three additional rounds of plating and rescreening.

Non-catalytic Subunits of 5'-AMP-activated Protein Kinase

Fig. 1. Nucleotide and deduced amino acid sequences of rat liver AMPK-γ. Shown is the nucleotide and deduced amino acid sequences of the 1107-bp rat liver AMPK-γ clone. The underlined nucleotide sequences correspond to those determined by direct peptide sequencing of the isolated rat liver AMPK-γ protein, as previously reported (6) and as completed for this study (new sequences include amino acids 55–57, 108–125, 161–181, and 183–199). Assignment of the start codon is explained under “Results.”

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Plasmid Preparation and DNA Sequencing—Plasmid DNA was prepared using QIAGEN mini- or midi-columns according to the manufacturer's instructions. DNA was sequenced, with vector- or gene-specific primers, using an Applied Biosystems Prism™ ready reaction dye deoxy terminator cycle sequencing kit and cycled in a Perkin-Elmer PC Thermocycler according to the manufacturers' instructions. Dye terminators were removed from the resulting sequence reactions using a Centri-Step column (Princeton Separations, Inc.). The purified sequence was then synthesized for use in library screening (CTC-CAAGTTTATGTTATCAACC). Screening of ~10^9 plaques with this probe, however, did not yield any positive clones.

The non-degenerate 23-mer cDNA was then used in conjunction with degenerate primers constructed from two other peptide sequences to generate a larger AMPK-γ cDNA by PCR. Both sense and antisense oligonucleotide primers corresponding to the peptide sequences EELQIG and FPKPEFM were used together with the sense MOPAC-derived non-degenerate sequence to generate all possible PCR products, using rat liver cDNA as template. The largest product (192 bp) obtained was subcloned in pCR-Script (Stratagene) and sequenced. This sequence (see Fig. 4), which actually predicted amino acid sequence corresponding to all three AMPK-γ peptides used in the PCR strategy, was then used for library screening as described above. Screening of 2 x 10^9 plaques with this larger PCR product yielded several positive clones, which were further characterized (see below); however, none of the rat cDNAs (1–1.3 kilobases) isolated corresponded to the 5'-end of the open reading frame. In an effort to extend the sequence to the 5'-end of the open reading frame, a primer extension library was constructed using rat liver cDNA as template. The largest product (192 bp) obtained was subcloned in pCR-Script (Stratagene) and sequenced. Additional screening of this library, as previously reported (6) and as completed for this study, did not yield the start Met codon. The application of a 5'-RACE strategy with rat liver cDNA was also unsuccessful in attempts at sequence extension, although a 5'-RACE product from porcine liver was obtained (data not shown). The most 5' RACE product (520 bp) was then used to screen a human fetal liver library, which yielded a full-length AMPK-γ cDNA (see below).
Fig. 2. Bacterial and cellular expression of AMPK-β and AMPK-γ. In the upper panel is shown a Coomassie Blue-stained SDS-polyacrylamide gel (9% acrylamide) of fractions from a nickel affinity column obtained on elution of the bacterially expressed His₆-tagged polyacrylamide gel (9% acrylamide) of fractions from a nickel affinity chromatography under monoclonal antibody (Novagen). The fusion proteins were purified from the inclusion bodies of bacterially by both Coomassie Blue staining and immunoblotting with anti-T7 samples of the column wash just prior to elution (55°C; 2 h) (lane 5). The inclusion body extract applied to the column (Load), a sample of the column wash just prior to elution (Wash), and six elution fractions obtained after increasing the imidazole concentration to 300 mM. In the lower panel is shown a a composite anti-HA immunoblot following SDS-polyacrylamide gel electrophoresis of lysates obtained from COS-7 cells after transfection with pMT2-HA vector alone (lane 1), pMT2-HA-AMPK-γ (lane 2; truncated cDNA), pMT2-HA-AMPK-β (lane 3), pMT2-HA-human AMPK-γ (lane 4; full-length cDNA), and both pMT2-HA-AMPK-β and pMT2-HA-human AMPK-γ (lane 5). Each lane was loaded with lysate equivalent to 4% of total cells/well after transfection. The migration positions of prestained molecular mass standards are indicated to the right.

sequencing reactions were then dried in a Speed-Vac and analyzed on an automated DNA sequencer (Applied Biosystems Model 373).

Bacterial Expression of cDNAs—Full-length rat AMPK β-subunit cDNA and a partial-length rat AMPK γ-subunit cDNA (amino acids 33–331; see Fig. 5) were expressed in Escherichia coli using the pET vector system, which introduces polyhistidine (His₁₀) and T7 fusion epitope tag sequences (Novagen), according to the manufacturer’s protocols. Bacterial expression was induced with 1.0 mM isopropyl-1-thio-D-galactopyranoside at 37°C for 2 h; expressed protein was detected by both Coomassie Blue staining and immunoblotting with anti-T7 monomonal antibody (Novagen). The fusion proteins were purified from the inclusion bodies of bacteria by nickel affinity chromatography under denaturing conditions. His₀-AMPK-β or His₀-AMPK-γ was solubilized from the inclusion bodies in 6 M urea according to manufacturer’s instructions. After sample application, the column was washed extensively with 20 mM Tris-Cl (pH 7.9), 0.5 M NaCl, 20 mM imidazole, and 6 M urea. The His₀-protein was eluted with the same buffer containing 300 mM imidazole.

Cellular Expression of cDNAs—Full-length rat AMPK-β cDNA, a partial-length rat AMPK-γ cDNA (amino acids 33–331), and a full-length human AMPK γ-subunit cDNA were also expressed in COS-7 cells. cDNAs were cloned into a pMT2 vector in frame with a hemagglutinin (HA) epitope tag (pMT2-HA) (17). Transfection was done using Lipofectamine reagent (Life Technologies, Inc.) according to the manufacturer’s general protocol. Cells were plated at 3 × 10⁶/well in 6-well plates (Corning Inc.) in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and penicillin/streptomycin. The following day, the cells were switched to serum-free and antibiotic-free Dulbecco’s modified Eagle’s medium, and then Lipofectamine-DNA conjugates (2 μg of DNA; 10 μl of Lipofectamine/well) diluted in the same medium were added. After 5 h of incubation at 37°C, an equal volume of medium containing 20% fetal calf serum was added to each well. The following morning, the medium was switched to the original cell medium. Cells were harvested 48 h after transfection. After washing with phosphate-buffered saline, cells were lysed in buffer containing 50 mM Tris-Cl (pH 7.5), 100 mM NaCl, 50 mM NaF, 5 mM NaPPi, 1 mM EDTA, 2 mM dithiothreitol, and 0.5% Nonidet P-40 with several protease inhibitors (9). For complete lysis, cells were placed on ice for 15 min, followed by scraping and vigorous vortexing (15 s) of the lysate. After clearing of debris by brief centrifugation, this lysate was used for SDS gel electrophoresis and immunoblotting. Blots were probed with an anti-HA monoclonal antibody (derived from the 12CA5 hybridoma line). After secondary probing with a peroxidase-conjugated anti-mouse IgG antibody, blots were developed by ECL (Amersham Corp.).

Northern Blot Analysis—Total RNA was isolated from the tissues of male Sprague-Dawley rats (150–200 g of body weight; Charles River Laboratories) or from lactating mammary glands of female rats using a guanidium isothiocyanate/lithium chloride method (13). RNAs were fractionated on 1% formaldehyde-garose gels with capillary transfer to nitrocellulose (MSI, Westboro, MA). cDNA probes were labeled by random priming as described above. Hybridization was carried in 5 × Denhardt’s solution, 0.2 M Tris (pH 7.4), 1 M NaCl, and 0.1 mg/ml salmon sperm DNA at 42°C for 20 h. Filters were washed sequentially with 2 × SSPE, 0.1% SDS (room temperature; 2 × 15 min); 0.2 × SSPE, 0.1% SDS (room temperature; 2 × 15 min); and 0.2 × SSPE, 0.1% SDS (55°C; 2 × 15 min). Autoradiography on Kodak XAR film with enhancing screens was at −80°C for 18–48 h.

DNA Sequence Analysis and DNA Sequences—DNA sequences were analyzed using MacVector™ and the Genetics Computer Group software package. Sequences were compared to the data base using BLAST and Genetics Computer Group software; amino acid alignments were made using the Genetics Computer Group Pileup program. Sequences were formatted using an Excel™ macro (18). The DNA sequences in this report have been deposited in the GenBank™ Data Bank with the following accession numbers: U42411, rat liver AMPK-β; U42413, rat liver AMPK-γ; and U42412, human fetal liver AMPK-γ.

Materials—[α-³²P]CTP for cDNA probe labeling was purchased from DuPont NEN. Nitrocellulose for Northern blots and library screening was purchased from MSI and Schleicher & Schuell. Gene-specific primers for PCR and DNA sequence analysis were synthesized by Midland Certified Reagent Co. (Midland, TX).

RESULTS

AMPK-β cDNA Isolation and Characterization—PCR amplification of pig and rat liver cDNAs with degenerate oligonucleotides corresponding to selected AMPK-β peptide sequences yielded two major PCR products (6). One product, a rat 309-bp partial-length cDNA, was used to screen a rat liver cDNA library, yielding a 1107-bp clone, shown in Fig. 1. The screening PCR probe corresponded to nucleotides 279–588 of this sequence. Shown in Fig. 1 (underlining) are the endoproteinase Lys-C peptide sequences obtained by direct sequencing of the purified rat liver AMPK-β protein. These account for 60% of the cDNA-derived sequence and provide strong evidence that the identity of the clone is correct.

This clone contains an open reading frame encoding for a 270-amino acid peptide, which contains all of the 15 independent (some overlapping) peptide sequences obtained from extensive sequence analysis of the purified protein. The translational start methionine codon is assigned from the typical Kozak sequence present for an initiation codon (19) and the lack of any other upstream in-frame methionine codons. While no in-frame stop codon is present in this 5′-upstream sequence, a human expressed sequence tag cDNA (GenBank™ accession number T78033; see below) in the data base contains such a stop codon preceding the same assigned start methionine codon. This reading frame, however, predicts a protein of 30,464 Da, well below the estimated molecular mass of 40 kDa evident on SDS gel electrophoresis (4, 6).

To clarify the size of the protein product that could be synthesized from this cDNA, the AMPK-β clone was expressed both in bacteria and mammalian cells. As shown in Fig. 2, in both expression systems, the protein product migrates at a higher than predicted molecular mass. When purified as a His₀-tagged fusion protein from E. coli, the isolated protein...
migrates on SDS gels with an apparent molecular mass of 
43,000 Da (the same as the ovalbumin standard). This corre-
sponds to a AMPK-β polypeptide product of 40 kDa with an
additional 3 kDa of fusion tag sequence derived from the pET
vector. When expressed in mammalian cells from an HA-
tagged expression vector, two polypeptides are evident, with
the major product corresponding to a 40-kDa species (after
correction for the size of the HA epitope tag). A second product
of 42–43 kDa is also evident using this expression system.

Taken together, these data demonstrate that the protein prod-
uct of this AMPK-β migrates on SDS-polyacrylamide gel elec-
trophoresis with an anomalously high molecular mass.

Comparison of the rat liver AMPK-β sequence to the data
base reveals that it is highly homologous to three yeast proteins
(Sip1p, Sip2p, and Gal83p) and with two partial-length human cDNAs in
the data base (GenBank™ accession numbers T78033 and F11147), as
matched by BLAST searching. Sequences were aligned with the Pileup program of
the Genetics Computer Group software package and are gapped with reference to
the Sip1p sequence. Residues identical to AMPK-β are boxed. The NCBI data base
also contains four other human sequences that are either very similar or identical to
the two human sequences indicated (data not shown); these sequences are accessible
as R14746, H06094, R20494, and R25722.

Using the MOPAC procedure and other
PCR amplification protocols, a 192-bp cDNA corresponding to
rat liver AMPK-γ sequence was obtained and used for library
screening. Despite exhaustive attempts, only a partial-length
rat liver cDNA of ~1.3 kilobases could be obtained from this
library; this sequence did not contain either a start methionine
codon or all the peptide sequences obtained from the purified protein. Attempts to extend this sequence to the 5'-end by the
use of a primer extension library and 5'-RACE only succeeded in
adding ~200 nucleotides to this sequence without identifi-
cation of the start codon. A partial-length rat cDNA was then
used to screen a human fetal liver library, which did yield the
full-length done shown in Fig. 4. This clone contains deduced
amino acid sequence corresponding to all of 22 independent (some overlapping) peptide sequences obtained from the puri-
fied rat and porcine liver AMPKs-γ (shown by underlining in
Fig. 4), confirming clonal identity.

A typical Kozak translation initiation sequence surrounds
the assigned methionine start codon; this start is also in frame
with a 5'-upstream stop codon. The assigned start methionine
codon is followed by an open reading frame predicting a protein
of 331 amino acids and of 37,546 Da, which corresponds to the
molecular mass observed on SDS gel electrophoresis of the
protein as purified from rat and porcine liver (4, 6). Expression
of a truncated rat AMPK-γ cDNA (amino acids 33–331) and the
full-length human AMPK-γ (331 amino acids) in COS-7 cells
yields products consistent with the molecular mass predicted
for each cDNA (34,081 and 37,577 Da, respectively) (Fig. 2).

The rat liver AMPK-γ product expressed in bacteria also dis-
mired on SDS gels with an apparent molecular mass of
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Comparison of the human and rat liver AMPK-g amino acid sequences to the data base yields a significant alignment of this protein with *S. cerevisiae* Snf4p (Fig. 5). In addition, our human (and rat; data not shown) full-length cDNA also aligns with several other human partial-length expressed sequence tag cDNA sequences from brain, breast, placenta, liver, and heart, recently reported in the data base (Fig. 6). Inspection of these sequences reveals that there are multiple isoforms of the human AMPK-g protein. There are likely also similar AMPK-g isoforms expressed in rat and pig. This latter expectation is based on sequence analysis of 14 other MOPAC-derived partial AMPK-g cDNA sequences, as identified on colony hybridization of the AMPK-g MOPAC products with 32P-labeled degenerate oligonucleotides (see “Experimental Procedures”). These products showed at least two reproducible patterns of nucleotide heterogeneity within the non-degenerate core (data not shown).

**DISCUSSION**

Mammalian AMPK, as isolated from rat and porcine liver, contains three polypeptide subunits termed AMPK-α, AMPK-β, and AMPK-γ. The α-subunit contains the kinase catalytic domain sequence and is homologous to several members of the SNF1 kinase family (4, 6, 7, 13, 14). There are multiple isoforms of the α-subunit, with α1 being responsible for 90% of the AMPK activity detected in liver extracts (14). The present report, based on very extensive peptide sequence and on predicted amino acid sequence from cDNA clones, establishes that full-length AMPK-β- and γ-subunits are likewise homologous to two classes of proteins in *S. cerevisiae*. This
extends information previously available from limited peptide sequence analysis and from smaller PCR-derived cDNAs (6). The present work further demonstrates, both by cDNA cloning and by direct peptide sequencing, which isoforms of AMPK β- and γ-subunits interact with the catalytic α1-subunit in liver. This work also establishes that these non-catalytic subunits, like the α-subunit isoforms, have a wider tissue distribution, as evidenced by mRNA content of several rat tissues, than expected from the AMPK activity distribution previously reported (13, 21).

The AMPK β-subunit is a mammalian homolog of a class of proteins in yeast, represented by Sip1p/Sip2p/Gal83p. The GAL83 gene product is known to affect glucose repression of the GAL genes (22). All of these proteins have been shown to interact with the Snf1p protein kinase either in the two-hybrid system or by immunoprecipitation (20, 23). It has been proposed that these proteins serve as adaptors that promote the activity of Snf1p toward specific targets (23). Based on analysis of yeast mutants, it has been suggested that these proteins may facilitate interaction of Snf1p with unique and different targets. Of interest is the demonstration of a highly conserved domain of 80 amino acids in the C terminus of Sip1p/Sip2p/Gal83p, termed the ASC domain (association with Snf1p complex) (23). As studied in the two-hybrid system, the ASC domain of both Sip1p and Sip2p interacts strongly with Snf1p (23). However, the interaction of Sip2p with Snf1p is not entirely lost on deletion of this domain, suggesting that the ASC domain is not solely responsible for this protein-protein interaction. A putative ASC domain is also highly conserved in the C terminus of rat liver AMPK-β (amino acids 203–270), suggesting that this region may be responsible, in part, for binding to the AMPK α-subunit.

AMPK-β, like Sip2p and Gal83p, is phosphorylated in vitro when associated with a catalytic subunit (AMPK-α or Snf1p, respectively) (4, 6, 23). Mutations of Gal83p can abolish most of the Snf1p kinase activity detectable in immune complexes, precipitated with anti-Snf1p antibody (23). A Sip2p Gal83p mutant shows reduced Snf1 protein kinase activity, which is restored following expression of either Sip2p- or Gal83p-LexA fusion proteins in the mutant strain (23). Taken together, these data suggest that the possibility that AMPK-β may also serve as an adaptor molecule for the catalytic AMPK α-subunit and will positively regulate AMPK activity. This possibility is being tested experimentally.

AMPK-β appears to migrate anomalously on SDS gels, with the polypeptide migrating at a molecular mass ~10 kDa larger than the size predicted from the cDNA. This slower migration is evident for both the bacterially expressed His6 fusion protein and the protein expressed in COS-7 cells. These observations suggest that the AMPK-β subunit migrates as a protein containing higher order structure. AMPK-β is autophosphorylated in vitro (4, 6); this suggests that the enzyme may be responsible for the anomalous migration on SDS-polyacrylamide gel electrophoresis. The AMPK-β subunit is autophosphorylated in vitro (4, 6); this suggests that the two AMPK-β bands expressed on transfection of mammalian cells with AMPK-β cDNA may result from a similar post-translational modification giving rise to smaller mobility shifts. Interestingly, this aberrant migratory behavior of AMPK-β is similar to that of its yeast homolog,
Gal83p. The LexA fusion protein(s) of Gal83p, as expressed in yeast, also migrate at greater than the expected molecular mass and display more than one band on SDS gels, consistent with the known phosphorylation of Gal83p by Snf1p (23).

Rat and human liver AMPK-γ are mammalian homologs of S. cerevisiae Snf1p (CAT3) (24–26). Snf1p was shown to interact with the Snf1p protein in the first reported use of the two-hybrid system and also communoprecipitates with it (26). Indeed, an isolation of the Snf1p kinase from yeast, Snf4p, but not the other Snf1p-interacting proteins, opurifies in a 1:1 stoichiometry with the Snf1p polypeptide (4). Analysis of Snf4 mutants in yeast suggests that Snf4p also positively regulates the activity of its associated catalytic subunit, Snf1p (24, 27). By analogy, our prediction is that AMPK-γ will also have such a positive influence on the AMPK α-subunit.

Examination of the data base reveals that, in addition to the homology of AMPK-γ to Snf4p, there are two or three different human proteins highly homologous or identical to our human and rat liver AMPK-γ sequences. However, some of these data base sequences, as predicted from expressed sequence tag (ESTs) in brain, heart, breast, and placenta, are distinct from each other and from our clones; some, for example, have a C-terminal extension. This indicates that there is a mammalian isoform family of potential AMPK γ-subunits, each perhaps with different tissue expression and regulatory roles. We propose that these different γ-isomers be designated γ1, γ2, γ3, etc., as their full-length sequences are delineated. We have designated the rat liver/human liver AMPK-γ sequence reported herein as AMPK-γ1. The isoform diversity of both the α- and γ-subunits of AMPK underscores the need for complete characterization of the translation products of the enzyme, as isolated from various sources, in order to properly identify the relevant isoforms that make up the heterotrimeric complex. To what extent various species of AMPK heterotrimers with varying composition of individual subunits could exist in vivo is not yet known.

AMPK was first recognized as a protein kinase active on enzymes of lipid metabolism (acyl-CoA carboxylase, hydroxymethylglutaryl-CoA reductase, and hormone-sensitive lipase) (1–3). However, as has been observed for the AMPK α-subunit (5, 13, 14), the AMPK β- and γ1-subunits have wider tissue distribution than might be expected for a protein active only in the regulation of lipid metabolism. While mRNAs for each are detectable in “classic” lipogenic tissues like liver, white adipose tissue, and lactating mammary gland, high concentrations of mRNA in non-lipogenic tissues like heart, brain, spleen, and lung, for example, suggest that these proteins have roles that extend beyond the regulation of fatty acid and steryl metabolism. Of note are the relatively low amounts of AMPK-β and AMPK-γ mRNAs in skeletal muscle; this observation is consistent with the relatively low levels of AMPK activity reported by others in this tissue and with the failure of skeletal muscle AMPK-α to immunoprecipitate with detectable β- and γ-subunits (28).

The wide tissue distribution of the mRNAs for all three subunits for AMPK raises the question of other potential roles for AMPK beyond lipogenic regulation. The striking homology of all three subunits to yeast proteins that are involved in nutrient (glucose) responses raises the possibility that the three mammalian proteins may be involved in glucose (or nutrient) regulation of gene expression in mammalian tissues or in other adaptive responses to a changing nutrient environment. We (9) and others (12) have presented evidence that AMPK may be an important “metabolic sensor” linked to oxidative fuel choice in the heart and to glucose sensing in the pancreatic beta cell, perhaps being important for insulin secretion. There is every reason to believe that further study of the AMPK subunits may shed light on multiple aspects of cellular regulation.

Acknowledgments—We thank Drs. John Kyriakis and Joseph Avruch (Massachusetts General Hospital) for provision of the pMT2-HA expression vector and for anti-HA antibody.

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