Expression profiling of the γ-subunit isoforms of AMP-activated protein kinase suggests a major role for γ3 in white skeletal muscle

Margit Mahlapuu,1* Carina Johansson,1 Kerstin Lindgren,1 Göran Hjälm,2 Brian R. Barnes,3 Anna Krook,3 Juleen R. Zierath,3 Leif Andersson, and Stefan Marklund2

1Aresis AB, SE-413 46 Gothenburg; 2Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, and 3Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala Biomedical Center, SE-751 23 Uppsala; and 3Department of Surgical Sciences, Karolinska Institutet, SE-171 77 Stockholm, Sweden

Submitted 3 April 2003; accepted in final form 6 October 2003

Mahlapuu, Margit, Carina Johansson, Kerstin Lindgren, Göran Hjälm, Brian R. Barnes, Anna Krook, Juleen R. Zierath, Leif Andersson, and Stefan Marklund. Expression profiling of the γ-subunit isoforms of AMP-activated protein kinase suggests a major role for γ3 in white skeletal muscle. Am J Physiol Endocrinol Metab 286: E194–E200, 2004. First published October 14, 2003; 10.1152/ajpendo.00147.2003.—Expression patterns of the three isoforms of the regulatory γ-subunit of AMP-activated protein kinase (AMPK) were determined in various tissues from adult humans, mice, and rats, as well as in human primary muscle cells. Real-time PCR-based quantification of mRNA showed similar expression patterns in the three species and a good correlation with protein expression in mice and rats. The γ3-isoform appeared highly specific to skeletal muscle, whereas γ1 and γ2 showed broad tissue distributions. Moreover, the proportion of white, type IIb fibers in the mouse and rat muscle samples, as indicated by real-time PCR quantification of Atp1b2 mRNA, showed a strong positive correlation with the expression of γ3. In samples of white skeletal muscle, γ3 clearly appeared to be the most abundant γ-isofrom. Differentiation of human primary muscle cells from myoblasts into multinucleated myotubes was accompanied by upregulation of γ3 mRNA expression, whereas levels of γ1 and γ2 remained largely unchanged. However, even in these cultured myotubes, γ2 was the most highly expressed isoform, indicating a considerable difference compared with adult skeletal muscle. Immunoblot analysis of mouse gastrocnemius and quadriceps muscle extracts precipitated with a γ3-specific antibody showed that γ3 was exclusively associated with the α2- and β2-subunit isoforms. The observation that the AMPKγ3 isoform is expressed primarily in white skeletal muscle, in which it is the predominant γ-isoform, strongly suggests that γ3 has a key role in this tissue.

Adenosine monophosphate; real-time polymerase chain reaction; antibodies; mouse; rat; human

AMP-ACTIVATED PROTEIN KINASE (AMPK) is a fuel-sensing enzyme that plays a key role in regulation of carbohydrate and fat metabolism in response to cellular stress (10, 21). When activated by energy depletion, AMPK switches off ATP-consuming processes while switching on pathways that generate ATP. AMPK acts via phosphorylation of downstream metabolic enzymes as well as via effects on gene expression. AMPK is expressed in most mammalian tissues, but its actions have most widely been studied in skeletal muscle. The alternation in energy charge during exercise activates AMPK, which is associated with enhanced glucose uptake and fatty acid oxidation. Together, these mechanisms provide the working muscle with additional substrates for ATP-generating processes.

AMPK is a heterotrimeric complex comprising a catalytic α-subunit and two regulatory components (β and γ), all of which are required for full activity. The understanding of the AMPK system is complicated because of the existence of two α- and two β-isofroms (α1, α2, β1, β2) and three γ-isofroms (γ1, γ2, γ3), all encoded by different genes. Thus 12 different heterotrimeric combinations of AMPK may potentially form, and the physiological function of the AMPK holoenzyme depends on the particular isoforms present in the complex (7, 19). A clear tissue distribution profile and the relative expression of different isoforms can shed light on AMPK function in the specific metabolic pathways studied. Previously reported expression differences between subunit isoforms include tissue distribution and cellular location (6, 7, 15, 16, 18, 19). Northern blot analysis in human tissues has provided evidence that the mRNA expression of the γ3-isoform is restricted to skeletal muscle, whereas γ1 and γ2 show broad tissue distributions (7, 15). Furthermore, immunoprecipitation studies of AMPK with subsequent kinase assays have indicated that γ1 is the predominant isoform contributing to kinase activity in skeletal muscle (7, 9). This observation is surprising considering the high γ3 mRNA expression specifically directed to skeletal muscle and the critical role of γ3 in pig skeletal muscle, as indicated by the identification of a mutation causing a 70% increase of muscle glycogen (15). Of note is the finding that this mutation leads to an increase in glycogen content and influences enzyme activities in skeletal muscles with a large proportion of white muscle fibers (2, 14). This implies that, even within divergent skeletal muscle types, the γ-isoforms affects metabolic pathways differently. Additional data on tissue distribution and relative expression of different γ-isoforms can elucidate their specific contribution to AMPK function.

In this study, we have determined the expression patterns of the genes encoding AMPKγ isoforms in various human, mouse, and rat tissues, including skeletal muscles of different fiber type composition. Additionally, we have analyzed human primary muscle cells (HPMC) as a model system for studies of AMPK function. HPMC, originating from satellite cells, can be induced to differentiate in culture to form multinucleated myotubes (1), and they have been used as a model system for studies of insulin action and glucose metabolism. We report

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
real-time PCR-based analyses of the distribution and relative amounts of mRNA representing the three AMPK\(_\gamma\) isoforms as well as data on the tissue distribution of each isoform at the protein level. Finally, we show the \(\alpha\)- and \(\beta\)-subunit composition in \(\gamma3\)-containing AMPK complexes in mouse skeletal muscle.

**MATERIALS AND METHODS**

*Cell culture, tissues, and RNA isolation.* HPMC originate from satellite cells, a population of stem cells responsible for postnatal growth, repair, and maintenance of skeletal muscle (17). The HPMC used here were cultured from satellite cells isolated from biopsies of rectus abdominus muscle from three healthy subjects. The ethics committee at Karolinska Institutet approved the protocols, and informed consent was obtained from the subjects. Biopsies were obtained during scheduled abdominal surgery. Isolation of satellite cells and initiation of differentiation program was performed as previously described (1). Cultures were harvested on day 0 (corresponding to mononucleated myoblasts) or day 14 (corresponding to polynucleated myotubes) after initiation of differentiation, and preparation of RNA and protein was performed as described previously (1).

Fed C57Bl/6 mice (one male and one female for each tissue, 8–12 wk of age) and Wistar rats (2–4 males per tissue, 8 wk of age) were anesthetized with an intraperitoneal injection of either 2.5% avertin (0.02 ml/g body wt) for mice or pentobarbital sodium (5 mg/100 g body wt) for rats. Tissue samples, including liver, heart, extensor digitorum longus (EDL), gastrocnemius, quadriceps, soleus, and diaphragm muscles, brain, white adipose tissue (WAT), brown adipose tissue (BAT), lung, spleen, kidney, and testis were collected. Thus a variety of rodent hindlimb skeletal muscles were obtained that contained predominantly red, slow-twitch, type I fibers (e.g., the soleus) or predominantly fast-twitch fibers (e.g., the EDL, gastrocnemius, and quadriceps with fiber types IIa, IIb (2, 3)). In the case of the gastrocnemius and quadriceps muscles in rats, the red (predominantly fast-twitch, oxidative-glycolytic type IIa fibers) and white (predominantly fast-twitch, glycolytic type IIb fibers) parts of the muscle could be visually recognized and were analyzed separately. The Ethics Committee on Animal Research in Northern Stockholm approved all the experimental protocols. Poly(A\(^+\)) RNA was isolated from mouse and rat samples using the Quickprep Micro mRNA purification Kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK).

**Relative quantification of mRNA using real-time PCR.** Quantification of AMPK\(_\gamma\) mRNA from adult mouse, rat and human tissues, as well as from HPMC cultures, was performed using real-time PCR with the ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Warrington, UK) and SYBR-green (human samples) or Taqman chemistry (mouse and rat samples). To indicate the relative proportion of different fiber types in each mouse and rat muscle sample, real-time PCR was used to quantify the relative amounts of *Atp1b1* and *Atp1b2* mRNA, which encode the ATPase, Na\(^+\)/K\(^+\) transporting, \(\beta_1\)- and \(\beta_2\)-polypeptides, respectively. Predominant expression of *Atp1b1* and *Atp1b2* in red, slow-twitch, type I fibers and fast-twitch, glycolytic type IIb fibers, respectively, has been shown previously (11). The relative quantites of different mRNA transcripts were calculated after normalization of the data against a housekeeping gene (see below) by use of the comparative \(C_T\) method (20).

**Real-time RT-PCR on human samples.** Human tissue-specific Poly(A\(^+\)) RNA (pooled from 3–20 male/female Caucasians; Clontech Laboratories, Palo Alto, CA) and RNA from HPMC were used as a template for cDNA synthesis using Superscript II RNaseH\(^{-}\) Reverse Transcriptase and oligo(dT) primers (Invitrogen, San Diego, CA). All samples were analyzed in triplicate, and the data were normalized using the peptidylprolyl isomerase A (PPIA) and \(\beta\)-actin (ACTB) genes as internal controls. One of the primers in each amplicon was spanning an exon/intron boundary. The forward (F) and reverse (R) primer sequences were as follows, 5’-3’: for the *PRKAG1* gene (encoding AMPK\(_\gamma1\); GenBank NM_002733) TGGAAAGCAGGCTTCACCGA (F) and TCTCACCAGTGACCCACCA (R); for *PRKAG2* (encoding AMPK\(_\gamma2\); GenBank AF249976) CCAGATGGAAGCCTCTTCGA (F) and GTGCTTATTTGCAAAGTATGTC (R); for *PRKAG3* (encoding AMPK\(_\gamma3\); GenBank NM_017431) CCGCCTTGTTGATTGGCTAC (F) and GGGTCTTCCACCA CTA (R). Real-time RT-PCR on mouse and rat samples. Reverse transcription on mouse and rat mRNA samples was performed using the First-Strand cDNA Synthesis Kit (Amersham Biosciences) with random hexamer priming in 15-μl reactions, as recommended by the manufacturer. The cDNA samples were then purified using the QIAquick PCR purification Kit (Qiagen, Hilden, Germany) and used in 25-μl TaqMan PCR reactions with 17.5 pmol of each PCR primer, a 6.25-pmol probe, and 1× TaqMan Universal PCR Master Mix (PE Applied Biosystems, Foster City, CA). In each Taqman amplicon, either the probe or the 3’ end of one of the primers included sequences from adjacent exons. The primers and probes were (P) as follows, 5’-3’: mouse *Prkag1* (GenBank AH10076) CAAGTTCTCAAGTTGTGATTGGTTATG (F), CGAACACCATTTGCTCCACCA (R), TET-CACCTCACAGTTGAAGAACAGGA (P); rat *Prkag1* (GenBank U42413) GCTCAACAGTTCGTTATTTGG (F), GCACGCAGCAAGACGGTATTA (R), TET-TACTTCTGCTGAGAAAGAAAGCTCATCTT (P); mouse/rat *Prkag2* (two primers described previously) (12): mouse (F), TATGTCTGACATGCCAA-TAMRA (P); rat (used on mouse samples), AAACGCCACTCTGATG (F), CGGCTTGGGTTGAGAG (R), and (used on rat samples), TET-CCTGGAGAAAATTGGTATTA (P); mouse/rat *Prkag3* (GenBank AF252500 and XM_237293) AGAGCCTAGTTGGAATGCATTGACA (F), AGATGGCTTGGGTTGAG (R, used on mouse samples), GCTGTCCTCACCACCA (F), AACAAGCCTCTGAGTTCTCTT (R, used on rat samples), TET-CCTGGAGAAATTGGTATTA (P); mouse/rat *Atp1b1* (GenBank NM_009721.1 and NM_013181.3) ATCCTGGAACCTGGAGAAAG (F), AATACCTGTAAGAAGAGATCTTA-AAC (R), FAM-AACCTACCGGCTCTGACCAAAACT-AMT; mouse/rat *Atp1b2* (GenBank NM_013145.1 and NM_012507.1) GGGACCGACTGGCC (F), AGACATTACCACTGGTGGAGG (R), FAM-CCTCTTCATACCTGCTTCTGATGTTCCTCAG-TMRA. All samples were analyzed in duplicate, and the data were normalized using the *Acid* (\(\beta\)-actin) gene as an internal control.

*Quantitative analysis of protein expression.* Approximately 20 mg of tissue from male mice/rats were ground to powder under liquid nitrogen and homogenized in an ice-cold lysis buffer (50 mM HEPES, 1% Triton X-100, 1 mM DTT, 10% glycerol, 1 mM EDTA, 5 mM NaPP, 50 mM NaF, and proteolytic enzyme inhibitors) by use of a motor-driven pestle in a 1.5-ml microcentrifuge tube. The homogenate was kept on ice for 30 min, and insoluble material was removed by centrifugation (9,000 rpm, 10 min, 4°C). Supernatants were removed, and total protein content was measured by the bicinchoninic acid method (Pierce Chemical, Rockford, IL). The samples were run on 4–12% precast polyacrylamide gels (Invitrogen) with 40 μg protein loaded by incubation in Immobilon-P membranes (Millipore, Bedford, MA) by semidy in transfer (20 V, 45 min). Membranes were blocked by incubation in 5% nonfat dry milk (TOYOBO, Osaka, Japan) in Tris-buffered saline containing 0.1% NaCl, 20 mM Tris, pH 7.6) containing 5% low-fat milk powder for 1 h at room temperature and probed with primary antibodies for 1–18 h in TBS containing 2.5% low-fat milk powder. Antibodies against the
γ1-subunit were raised using the synthetic peptide CALENEH-FQETPESN (residues 12–25 of γ1), against the γ2-subunit using the CKRRSLVHPIPLDLSS peptide (residues 29–42 of γ2) or CLTPAGAKQKETETE peptide (residues 556–569 of γ2), and against γ3 using the MSVGEALRQRTL CLEG peptide (residues 415–431 of γ3). The antibodies were affinity-purified using the SulfoLink Kit (Pierce Chemical, Rockford, IL) and following the manufacturer’s instructions. After three washing steps with TBS-Tween (0.5% Tween 20 in TBS) for 10 min each, the membrane was incubated for 1 h with horseradish peroxidase-conjugated secondary antibody in TBS containing 2.5% low-fat milk powder. After further extensive washing in TBS-Tween, the blots were developed using enhanced chemiluminescence (Amersham Biosciences) and captured on X-ray film. Densitometry-derived values reflecting band intensities were used for quantification by Image Gauge V3.01 software (Fujifilm, Tokyo, Japan). Quantification was based on Western blot analysis of three different individuals, and the expression level of corresponding protein in EDL muscle was taken as 100%. As size markers in the Western analysis, bacterially expressed AMPK γ-subunit proteins were included in each SDS-PAGE. Briefly, synthetic oligos designed for amplification of the full-length open-reading frames of the different γ-subunits (GenBank nos. AF036535, AJ249976, and AJ249977) were used in RT-PCR with first cDNA strands synthesized with mouse or human skeletal muscle RNA as template. PCR fragments were cloned into the pQE-32 vector (Qiagen) in frame after an NH2-terminal His6 tag. Fusion protein from isopropyl-β-D-thiogalactopyranoside-induced TOP10F’ bacteria (Invitrogen) was purified by a nitrilotriacetic acid chromatography matrix (Qiagen).

**Immunoprecipitation of γ3-containing complexes.** For immunoprecipitation studies, 50-mg samples from mouse gastrocnemius or quadriceps muscles were homogenized, and lysate was prepared as described above. AMPK γ3-containing complexes were immunoprecipitated from equal amounts of muscle lysate by incubation with an anti-γ3 antibody prebound to protein A-Sepharose for 2 h on a roller mixer at 4°C. The immune complexes were washed four times with an ice-cold lysis buffer containing 0.5 mM NaCl and resolved using SDS-PAGE, followed by Western blotting with affinity-purified rabbit antibodies as described above. Antibodies against the α1-subunit were raised with the synthetic peptide CRHTLDELNPKQJKH (residues 377–389 of α1), against α2 with the CDDSAMHIPPGLKPH peptide (residues 353–366 of α2), against β1 with the CKAPEKEELAWQHD peptide (residues 53–66 of β1) and against β2 with the CSVFSLPDSKLPGDK peptide (residues 44–57 of β2). Protein A conjugated to horseradish peroxidase (Amersham Biosciences) was used for detection.

**RESULTS**

**mRNA expression.** The real-time PCR measurements of the relative expression levels of mRNA representing different AMPKγ isoforms in human, mouse, and rat tissues are presented in Fig. 1. The expression of the γ3 isoform appeared highly specific for skeletal muscle in all species examined. Conversely, expression of γ1 and γ2 showed a broad tissue distribution. ANOVA was performed with the mouse and rat data but did not show any significant effect of sex or covariation between the mRNA levels of the three isoforms (data not shown).

The mRNA expression of AMPKγ isoforms in the HPMC established from rectus abdominus muscle showed severalfold higher mRNA expression of γ2 than of γ1 and γ3 (Figs. 1A and 2). Moreover, differentiation of HPMC from myoblasts into poly nucleated myotubes was accompanied by an approximately fivefold increase in mRNA levels of γ3, with only minor changes in γ1 and γ2 (Fig. 2).

In mice, an 8- to 33-fold higher γ3 mRNA expression was observed in skeletal muscles containing mostly fast-twitch (type II) fibers (EDL, gastrocnemius and quadriceps) compared with the soleus muscle, which is composed mostly of slow (type I) fibers (Fig. 1B). Furthermore, when red (type IIA) and white (type IIB) portions of rat gastrocnemius and quadriceps muscles were analyzed separately, γ3 mRNA expression was found to be three- to ninefold higher in the white vs. red portions of the muscle (Fig. 1C). In these white muscle por-
tions, the γ3/γ1 and γ3/γ2 mRNA expression ratios were in the range 9-13 and 27-44, respectively. The proportion of fast-twitch glycolytic (type IIb) fibers in the mouse and rat muscle samples, as indicated by real-time PCR quantification of Atp1b2 mRNA, showed a highly significant correlation with the expression of γ3 mRNA (Pearson coefficient = 0.84, P < 0.001; Fig. 1, B and C). The γ1 and γ2 expression levels did not vary between red and white portions of rat skeletal muscle. However, in mice and rats, we observed slightly lower γ2 expression in the soleus muscle, with a greater proportion of type I fibers compared with skeletal muscle composed predominantly of type II fibers.

Expression of γ2 mRNA showed a broad tissue distribution in all of the three species tested. In mice and rats, we detected the highest levels of γ2 mRNA in testis and BAT, whereas we found the highest human γ2 mRNA expression in heart (Fig. 1). In mice and rats, real-time PCR analysis was also used to quantify the relative proportions of the two different γ2 transcripts previously observed in humans [PRKAG2-a and PRKAG2-b (7, 13)], which showed that Prkag2-a is the highly predominant transcript in most tissues (Table 1). In fact, the highest Prkag2-b proportions of the total Prkag2 were found in skeletal muscle (≤48% in mice) and brain (~16%).

Protein expression. To complement the data on the relative abundance of γ mRNA with data at the protein level, we performed Western blot analysis on mouse and rat tissues. This showed a positive correlation between mRNA and protein expression in different skeletal muscles and confirmed that γ3 is expressed primarily in white skeletal muscle (Fig. 3, A and B). For example, the levels of γ3 mRNA and γ3 protein were both highest in white parts of gastrocnemius and quadriceps muscles in rats (Figs. 1C and 3B). By using an anti-γ2 antibody, which detects both the longer (γ2a) and the shorter (γ2b) variants of the protein, we also confirmed highly predominant expression of γ2 protein encoded by the Prkag2-a transcript in relation to the Prkag2-b (Fig. 3C).

Some discrepancies between our mRNA and protein expression data could be noted. For example, although very low γ1 mRNA expression was observed in mouse brain and WAT, the amount of the γ1 protein in these tissues reached similar levels to what was observed for heart and skeletal muscle. Likewise, the relative abundance of γ2 protein in mouse heart appears to be lower than one would predict on the basis of our mRNA expression analysis (Figs. 1B and 3A). This may reflect a true biological difference; however, we cannot exclude the possibility that these differences were caused by minor variations between samples used for mRNA and protein isolation.

To define the subunit composition of γ3-containing complexes in the skeletal muscle, we immunoprecipitated extracts from mouse gastrocnemius and quadriceps muscle with γ3-specific antibody and performed immunoblot analysis to determine α- and β-subunit isoforms (Fig. 3D). In both of these muscles types, the γ3-subunit was detected only in association with the AMPK α2- and β2-isofoms.

**DISCUSSION**

In this study, we demonstrate that expression of the AMPKγ3 isoform shows a high degree of specificity for white skeletal muscle and clearly appears to be the most highly expressed γ-isoform in this muscle type. The expression profile described fits well with the dramatic effect on glycogen content in pig skeletal muscle caused by a mutation in the PRKAG3 gene encoding γ3 (15). The increased glycogen levels found are particularly pronounced in white skeletal muscles, which is in good correlation with our data (14). The results are also consistent with previously published Northern blot results in humans, showing that γ3 is specifically expressed in skeletal muscle, whereas γ1 and γ2 are more widely distributed (7, 15).

**Table 1. Relative expression of the Prkag2-a and Prkag2-b transcripts* in mouse and rat tissues as measured by real-time PCR**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>657.9</td>
<td>618.7</td>
</tr>
<tr>
<td>Heart</td>
<td>25.2</td>
<td>38.9</td>
</tr>
<tr>
<td>EDL</td>
<td>1.2</td>
<td>9.4</td>
</tr>
<tr>
<td>Gastrocnemius†</td>
<td>1.1</td>
<td>NA</td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>NA</td>
<td>4.5</td>
</tr>
<tr>
<td>White gastrocnemius</td>
<td>NA</td>
<td>4.4</td>
</tr>
<tr>
<td>Quadriceps†</td>
<td>1.2</td>
<td>NA</td>
</tr>
<tr>
<td>Red quadriceps</td>
<td>NA</td>
<td>15.1</td>
</tr>
<tr>
<td>White quadriceps</td>
<td>NA</td>
<td>4.0</td>
</tr>
<tr>
<td>Soleus</td>
<td>7.4</td>
<td>73.5</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>9.8</td>
<td>40.7</td>
</tr>
<tr>
<td>Brain</td>
<td>5.4</td>
<td>5.2</td>
</tr>
<tr>
<td>WAT</td>
<td>82.8</td>
<td>236.7</td>
</tr>
<tr>
<td>BAT</td>
<td>538.7</td>
<td>418.3</td>
</tr>
<tr>
<td>Lung</td>
<td>19.0</td>
<td>19.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.7</td>
<td>36.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>85.7</td>
<td>32.8</td>
</tr>
<tr>
<td>Testis</td>
<td>822.7</td>
<td>1104.8</td>
</tr>
</tbody>
</table>

*The Prkag2-a and Prkag2-b transcripts tested here correspond to the human PRKAG2-a and PRKAG2-b transcripts previously described (13). †The samples of mouse gastrocnemius and quadriceps were taken without discrimination between white and red fractions. NA, not applicable. WAT and BAT, white and brown adipose tissue, respectively.
The data presented here show the mRNA expression ratios between the three γ-isofoms in several tissues, with a high degree of consistency between the three mammalian species investigated. Moreover, our observations of protein expression in mice and rats largely confirm the tissue distribution of each γ-isofom observed at the mRNA level. Yet our data on γ3 expression in skeletal muscle are obviously in contrast to previous data showing relatively high γ3-related activity in rat skeletal muscle (7, 9). In fact, we found γ1/γ3 and γ2/γ3 mRNA expression ratios higher than 150 in brain samples from rats, mice, and humans. The situation was similar in testsis, where the human and rat/mouse γ3 mRNA fractions of the total γ-mRNA were <2% and 0.02%, respectively. The reason(s) for these conflicting data may have to do with the different anti-γ3 antibodies used, their specificity, and location in the peptide sequence. For example, one possibility is the existence of more or less tissue-specific splice variants affecting an epitope for antibody binding. The anti-γ3 antibody used in this study binds a carboxy-terminal epitope whereas the antibodies used in the previous studies (7, 9) bind NH2-terminal epitopes. Although the reason(s) for the conflicting data discussed above
is still unclear, the protein expression data in mice and rats presented here are fully consistent with our mRNA data. Also, the specificity of our anti-γ3 antibody has been confirmed using a recently developed γ3 knockout mouse line that showed positive staining for γ3 expression in wild-type mice and no staining for γ3 expression in knockout mice when tested with this antibody in Western blot analysis (data not shown).

The striking difference in γ3 expression between white and red skeletal muscle fiber types probably accounts for most of the variation in the γ3/γ1 expression ratio between skeletal muscle samples studied. For example, a first glance at our data may give the impression that the γ3/γ1 expression ratio in skeletal muscle is much higher in human than in mouse skeletal muscle. Although we cannot exclude the possibility of a true expression difference between these species, this observation is rather likely to reflect differences in fiber composition between the samples compared.

In this study, the mRNA expression in skeletal muscle appeared to be generally higher for γ1 than for γ2. Interestingly, in human heart, the γ2 expression level was close to fivefold higher than the γ1 expression level. Several mutations in the PRKAG2 gene (encoding AMPKγ2) causing cardiac hypertrophy have been reported (4, 8). In rats and mice, however, we found that the γ2/γ1 mRNA expression ratios were considerably lower (1.3 and 0.6, respectively). If this reflects a general difference in γ1 and γ2 protein expression between rodents and humans, it may raise doubts for the use of rodents as model animals for functional analysis of γ2 in heart.

Our investigation of AMPKγ mRNA expression in HPMC, a possible model for functional studies of AMPK in adult skeletal muscle, showed that γ2 remains the most abundant γ-isof orm in these cells throughout the differentiation program. Thus γ2 expression reaches its full level very early during muscle cell differentiation, whereas γ1 and γ3 are upregulated at a later stage. This indicates a dramatic difference between HPMC and adult skeletal muscle regarding the γ1/γ2/γ3 mRNA expression ratio and may raise caution for using HPMC as a model for studies of regulation and action of AMPK in adult muscle.

We found that the γ3-subunit of AMPK, in mouse gastrocnemius and quadriceps, was associated solely with the α2- and β2-isoforms. This finding may reflect the relative abundance of α- and β-subunit isoforms in white skeletal muscle. Previous studies have shown that α2- and β2-subunits have considerably higher levels of expression in white compared with red skeletal muscle (21) and that interaction can occur between γ3- and all α- and β-isoforms (7). In this study, however, we did not detect any α1 or β1 protein, as assessed by immunoblot analysis after precipitation with our anti-γ3 antibody, despite the strong corresponding signals obtained with the α2 and β2 immunoblots. Thus the relatively small amount of α1 and β1 expected to be present in these muscles does not seem to associate with γ3. Therefore, our data indicate that γ3 preferentially forms a complex with α2 and β2.

Both exercise and the AMPK activator 5-aminimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) are known to display a fiber type-specific effect on AMPK activity, with the most marked increase in skeletal muscle composed predominantly of type IIb fibers (12). Glucose uptake is also stimulated by AICAR in a fiber type-specific manner, with the largest effects noted in white skeletal muscle (5). It is therefore intriguing that our data reveal αβ2γ3 as the major AMPK heterotrimer in skeletal muscle composed predominantly of white fibers. This indicates that targeting γ3-containing AMPK complexes might give an opportunity to promote glucose uptake specifically in skeletal muscle and thereby reverse some metabolic abnormalities in type 2 diabetes patients.

ACKNOWLEDGMENTS

We thank Lubna Al-Khalili for help with the analysis of HPMC, Elisabeth Nilsson for help with bioinformatics, and Björn Löwenadler for valuable comments during the preparation of this manuscript.

Current address of G. Hjälm: Dept. of Molecular Biosciences, Swedish University of Agricultural Sciences, Uppsala Biomedical Center, SE-751 23 Uppsala, Sweden.

GRANTS

This study was supported by the Arexis Research and Development Fund, the Swedish Medical Research Council, and the Torsten and Ragnar Söderbergs Foundation. G. Hjälm and S. Marklund were funded by the AgriFunGen program, Swedish University of Agricultural Sciences.

REFERENCES


