Identification of a Novel AMP-activated Protein Kinase β Subunit Isoform That Is Highly Expressed in Skeletal Muscle*

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The AMP-activated protein kinase (AMPK) is a member of a growing family of related kinases, including the SNF1 complex in yeast, which respond to nutritional stress. AMPK is a heterotrimeric complex of a catalytic subunit (α) and two regulatory subunits (β and γ), and proteins related to all three subunits have been identified in the SNF1 complex. We have used the two-hybrid system in order to identify proteins interacting with the catalytic subunit (α2). Using this approach, we have isolated a novel AMPKβ1 isoform, which we designate AMPKβ2. The N-terminal region of β2 differs significantly from that of the previously characterized isoform (β1), suggesting that this region could play a role in isoform-specific AMPK activity. Comparison of the C-terminal sequences of β1 and β2 with their related proteins in yeast identifies two highly conserved regions predicted to be involved in binding of the α and γ subunits. The expression of β1 and β2 was examined in a number of tissues, revealing that the β1 isoform is highly expressed in liver with low expression in skeletal muscle, whereas the opposite pattern is observed for the β2 isoform. These results suggest that the β isoforms have tissue-specific roles, which may involve altered responses to upstream signaling and/or downstream targeting of the AMPK complex.

In mammals, the AMP-activated protein kinase (AMPK) plays a major role in the response to metabolic stress (1–3). AMPK activates AMPK via a number of independent mechanisms, including activation of an upstream kinase (AMPKK), which in turn phosphorylates and activates AMPK (4, 5). The effects of AMP are antagonized by high concentrations of ATP so that it appears that the kinase responds to the AMP/ATP ratio, rather than AMP itself (4). Once activated, AMPK phosphorylates a number of enzymes involved in biosynthetic pathways causing their inactivation and preventing further ATP utilization. These findings have led to the hypothesis that the AMPK system has evolved to monitor the energy status, or fuel supply, within the cell (2–4).

Molecular characterization of AMPK has revealed that it is composed of three distinct subunits: a catalytic subunit, α (molecular mass approximately 63 kDa); and two regulatory subunits, β (30 kDa) and γ (36 kDa) (6–10). In vitro binding studies indicate that the α and β subunits and the β and γ subunits interact directly, whereas the α and γ subunits do not form a stable interaction (10). The formation of the heterotrimeric complex may therefore be mediated, at least in part, by the β subunit. Proteins related to all three subunits have been identified in the SNF1 kinase complex in Saccharomyces cerevisiae, which is involved in the derepression of glucose-repressible genes (11). AMPKa is 47% identical to Snf1p (we refer to the individual subunits as Snf1p, etc., and the complex as SNF1) (6), AMPKy is 35% identical to Snf4p (10, 12), and AMPKβ is related to the Sip1p/Sip2p/Gal83p family of proteins (10, 12). In addition to their primary sequence similarities, the AMPK and SNF1 complexes are functionally related since SNF1, like AMPK, phosphorylates and inactivates acetyl-CoA carboxylase (13). Indeed, the similarities between the two complexes extend even further as it has recently been shown that active SNF1, e.g. under glucose derepressing conditions, most likely exists as a heterotrimeric complex of Snf1p, Snf4p, and one of the Sip1p/Sip2p/Gal83p family of proteins (14, 15).

A second α isoform has been isolated recently, which was termed, somewhat unconventionally, α1 (the isoform that was first identified becoming α2) (16). It was reported that the α1 isoform accounted for virtually all of the AMPK activity measurable in rat liver extracts and that α2 was virtually inactive (16), but a subsequent study has challenged this finding (17). Immunoprecipitation of specific isoforms from rat liver shows that both α1 and α2 contribute equally to AMPK activity and expression of recombinant enzyme demonstrate that both isoforms have comparable specific activities (17). The only difference that could be detected between the two isoforms was in their specificity for peptide substrates (17). Although subtle, this does raise the possibility that there could be differences in the downstream targets in vivo leading to different physiological roles for α1 and α2.

In an attempt to identify novel downstream targets of AMPK, we screened a two-hybrid library with AMPKα2 as bait. Here, we report the identification and characterization of a second isoform for AMPKβ, which is highly expressed in skeletal muscle. Comparison of the amino acid sequences of the two β isoforms with Sip2p and Gal83p reveal two highly conserved regions, which are predicted to interact with the α and γ subunits. The sequences of the β isoforms diverge at their N...
Characterization of AMPKβ Isoforms

termini, suggesting that this region may play an important role in conferring isoform specificity, either by targeting to different subcellular and/or intracellular locations or by responding to different stimuli.

EXPERIMENTAL PROCEDURES

Materials—A human skeletal muscle library in pGAD10 was obtained from CLONTECH. pYTH9 used to make a fusion of AMPKα2 with the DNA-binding domain of Gal4 was a gift from Dr. Julia White, GlaxoWellcome Research Group. Yeast strain Y190 was used for the two-hybrid screening, and standard methods were used for manipulation and growth of yeast (18). Oligonucleotides were obtained from Genosys. CCL13 cells were obtained from the American Type Culture Collection and grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum.

Plasmid Construction—In order to remove an internal XbaI site, AMPKα2 cDNA was amplified by the polymerase chain reaction (PCR) with the following oligonucleotides: AGATCGGACACTACGTG (forward primer) and CCTCATCATGATGGTTAAAAAGTCCGAAAG (reverse primer). The amplified product was digested with Clal and BglII and ligated with AMPKα2 (Clai/BglII). A second round of amplification was carried out on the modified AMPKα2 cDNA using the following primers: GAGCTCAGGTCGACGCCCATGGCTGAGAAGC and TCT-12444 dideoxy chain termination method (19) using Sequenase (version 2.0 a 1 min were used. Products were analyzed by electrophoresis either min at 95 °C, 35 cycles of 95 °C for 1 min, 56 °C for 1 min, and 72 °C for CG) flanking the cloning sites. Following an extended hot start of 10 °C. Immune complexes were collected by centrifugation at 6000 g for 5 min, washed extensively with buffer A (50 mM Tris-HCI, pH 7.5, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol, 1% (v/v) Triton X-100), and then analyzed for AMPK activity and by Western blotting.

Western Blot—Samples were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was blocked by incubation in 10 mM Tris-HCI, pH 7.4, 0.5 M NaCl, 0.5% Tween 20, 0.5% fat milk powder overnight at 4 °C. The membrane was then incubated with primary antibody in the same buffer for 2–4 h at room temperature. After extensive washing with 10 mM Tris-HCI, pH 7.4, 0.1% (v/v) Tween 20, the membrane was incubated for 1 h at room temperature with either protein A conjugated with horseradish peroxidase (for primary antibodies raised in rabbits) or protein G conjugated with horseradish peroxidase (for sheep antibodies). After further extensive washing, the membrane was developed using enhanced chemiluminescence (Boehringer Mannheim).

In Vitro Translations—cDNAs encoding AMPKβ isoforms were constructed in pCND3 (Invitrogen). RNA transcripts were synthesized using T7 polymerase and translated in reticulocyte lysates using a coupled transcription/translation system (TNT system, Promega) in the presence of [35S]methionine. Total labeled products of translation were analyzed by SDS-PAGE and fluorography.

Mammalian Cell Transfections—For mammalian expression all AMPK subunits were constructed in pCND3. cDNAs encoding α1 and β2 were constructed with a sequence encoding a 10-amino acid epitope tag derived from e-Myc (EQKLISEEDL; Ref. 22) immediately following the initiating methionine. CCL13 cells were transfected with plasmid (10 μg of each plasmid) by calcium phosphate precipitation (23). The precipitate was incubated with the cells overnight, followed by a 2-min incubation with phosphate-buffered saline containing 10% (v/v) dimethyl sulfoxide. Cells were harvested 60 h after transfection and lysed in buffer A. Insoluble material was removed by centrifugation and the supernatant analyzed by Western blotting or used for immunoprecipitation using anti-AMPK antibodies or an anti-Myc antibody (22).

AMPK Activity—Activity was measured by phosphorylation of the SAMS peptide as described previously (24).

RESULTS

Identification of a Novel AMPKβ Subunit Using the Two-hybrid System—In order to identify proteins interacting with the catalytic subunit of AMPK, we screened a human skeletal muscle two-hybrid library with AMPKα2 as bait. A total of 2.3 million clones were screened, and 136 positive clones were identified by blue/white selection from the lacZ reporter gene. Grouping of the clones by restriction digest mapping and partial sequencing of the cDNA inserts revealed that 50 clones, which we designate AMPKβ2, shared considerable sequence identity with the cDNA encoding the rat AMPKβ1 subunit. A representative clone was sequenced in its entirety, and the nucleotide and predicted amino acid sequences are shown in Fig. 1A. The nucleotide sequence of β2 is 66.7% identical to rat β1, whereas the predicted amino acid sequence is 70.6% identical to rat sequence. To determine whether the DNA we had isolated was the human homologue of rat β1, we searched the human EST data base with the rat β1 and human β2 sequences. A number of clones were identified with sequence, which, although highly related to rat β1, did not match β2. Two of these clones (with accession numbers H06094 and R20494) encoded the sequence corresponding to the N-terminal region of rat β1 (including the initiating methionine)
FIG. 1. Nucleotide sequence and predicted amino acid sequence of human AMPKβ1 and AMPKβ2. A, β2. The initiating and stop codons are shown in bold. Nucleotides are numbered on the right and amino acids on the left. B, β1. Arrows mark the limits of the 343-base pair deletion found in clone H06094.
and were obtained from the IMAGE consortium through the UK Human Genome Mapping Resource Center, Cambridge. Sequence analysis of these clones revealed that they were identical, except that clone H06094 had a 343-base pair deletion compared with clone R20494 (see Fig. 1B). The nucleotide and predicted amino acid sequence of clone R20494, which we designate human $\beta_1$, is shown in Fig. 1B. At the nucleotide level, human $\beta_1$ is 89% identical to rat $\beta_1$ (97% amino acid sequence identity) and 66% identical to human $\beta_2$. These results indicate that clone R20494 is the human homologue of rat $\beta_1$ and that $\beta_2$, therefore, is a novel $\beta$ subunit isoform.

The predicted amino acid sequences of human $\beta_1$ and $\beta_2$ are 71% identical and are shown aligned in Fig. 2A. The two sequences are most divergent in the region spanning the N-terminal 75 amino acids (40% identity), although a predicted N-myristoylation signal is conserved in both isoforms (25), while the remaining C-terminal sequence is highly conserved.

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We have previously shown that rat $\beta_1$ shares sequence identity with a family of yeast proteins that interact with Snf1p, the yeast homologue of AMPK$\alpha$ (10). The deduced amino acid sequences of human $\beta_1$ (top) and $\beta_2$ (bottom) were aligned using the GAP program in the University of Wisconsin package with a gap weight of 3.0 and a length weight of 0.1. Dots indicate gaps introduced to maximize the alignment. Identities between the two sequences are boxed. B, the amino acid sequences of $\beta_1$, $\beta_2$, Sip2p, and Gal83p were aligned as above, using the PILEUP program. Numbers on the left refer to amino acid residues within the full-length polypeptides. The N-terminal regions showed no significant homology and have been omitted. A solid bar beneath the sequences marks the two highly conserved regions, which are contained within the KIS and ASC domains of Sip2p and Gal83p (15).

**Tissue Distribution of AMPK$\beta_1$ and AMPK$\beta_2$—Poly(A)-rich RNA isolated from a number of human tissues was probed with cDNA encoding $\beta_1$ or $\beta_2$ and the results are shown in Fig. 3. $\beta_1$ mRNA is detected as a single band of approximately 3 kilobase pairs and is expressed in all tissues examined at approximately equal levels. A single band of approximately 7.5 kilobase pairs is detected with the $\beta_2$ probe, although in this case there is clearly a difference in the pattern of expression. Relatively high levels of expression are detected in skeletal and cardiac muscle with low levels in the kidney and lung.

Antibodies were raised against specific peptide sequences derived from either $\beta_1$ or $\beta_2$ and used to determine protein expression in a number of rat tissues. Preliminary experiments revealed that it was not possible to detect the subunits directly by Western blotting of tissue lysates (data not shown), and so tissue homogenates were first immunoprecipitated using the antibodies bound to protein A- or protein G-Sepharose and then the immune complexes were analyzed by SDS-PAGE, followed by Western blotting. Fig. 4A shows the expression pattern of the two $\beta$ isoforms. The very prominent band migrating at approximately 56 kDa, detected in both blots, is due to a strong cross-reaction of the IgG heavy chains, present from the immunoprecipitation, with the protein A/G-conjugated horseradish peroxidase used for detection of the blotting antibody. In each case, however, an additional band specific for the $\beta$ isoform,
migrating ahead of the IgG band, can be detected. β1 expression is highest in the liver and brain with low level expression in kidney and skeletal muscle, whereas β2 is most highly expressed in skeletal muscle with low level expression in kidney, liver, and lung. At the present time, we cannot explain why β2 appears as a distinct doublet in lung. Although the predicted molecular mass of each isoform is 30 kDa, both β1 and β2 migrate anomalously on SDS-PAGE. β1, as we have reported previously, migrates with an apparent molecular mass of approximately 38 kDa (Fig. 4A). In vitro translation of β1 and β2 in a rabbit reticulocyte lysate system results in a similar discrepancy in the migration of the isoforms on SDS-PAGE (Fig. 4B). We have not been able to determine the reason for the anomalous migration of the β isoforms on SDS-PAGE.

It is clear from the Western blots that there is a marked difference in the relative expression of β1 and β2 in the liver compared with skeletal muscle. In order to investigate this difference in more detail, AMPK was partially purified from either rat liver or skeletal muscle by ion-exchange chromatography on DEAE-Sepharose (21) and immunoprecipitated using β1- or β2-specific antibodies. AMPK activity in the immune complexes was determined and is shown in Fig. 4C. AMPK activity is present in the immune complex following immunoprecipitation of liver or skeletal muscle extracts with either β1- or β2-specific antibodies. In liver, however, much higher activity is recovered with the β1-specific antibody, and in order to obtain a linear rate of phosphorylation it was necessary to use a 50-fold lower amount of liver extract when immunoprecipitated with β1 compared with β2. Despite using a lower amount of starting material, AMPK activity in the β1 immune complex is still greater than in the β2 complex (Fig. 4C), suggesting that nearly all the activity in liver is associated with the β1 isoform. In contrast, immunoprecipitation from a skeletal muscle extract with either β1 or β2 yields approximately equal activities. Following determination of AMPK activity, the immune complexes were analyzed by Western blotting using α1- or α2-specific antibodies. Both β isoforms form complexes with α1 and α2 in liver and skeletal muscle (Fig. 4D). Although the blots are not quantitative, it is possible to draw some general conclusions from them regarding the relative amounts of the different AMPK complexes present in the two tissues. In liver the β1 complexes (both α1β1γ and α2β1γ) are more abundant than the β2 complexes (compare lanes 1 and 3 in Fig. 4D), consistent with the finding that most of the AMPK activity can be immunoprecipitated with β1-specific antibodies. In skeletal muscle the reverse is true, with the β2 complexes being more abundant than the β1 complexes (compare lanes 2 and 4). In this case, however, AMPK activity following immunoprecipitation with either β1 or β2 is approximately the same, although significantly lower than the corresponding activities from liver (Fig. 4C).

Expression of Subunits in Mammalian Cells—cDNAs encoding α1/β1/γ, α1/β2/γ, α2/β1/γ, or α2/β2/γ were co-transfected into CCL13 cells. AMPK was immunoprecipitated from transfected cells with an anti-Myc antibody (a Myc epitope tag is present on both α1 and α2) and kinase activity in the immune complexes determined. No significant differences in AMPK activity between β1-containing complexes compared with β2-containing complexes were detected (Fig. 5A). As has been reported previously, complexes containing α1 were expressed at higher levels than those containing α2 and yield correspondingly higher activities (7). The immune complexes were analyzed by Western blotting using an antibody that recognizes both β isoforms (10) (Fig. 5B). Transfections with β1 yield a single band migrating with an apparent molecular mass of approximately 38 kDa, whereas transfections with β2 reveal a single band migrating with an apparent molecular mass of 34 kDa. No products could be detected in untransfected cells. There is no significant difference in the Western blots from immunoprecipitates of α1- or α2-transfected cells, indicating that β1 and β2 can form complexes with either α1 or α2, consistent with the results from liver and skeletal muscle. As we have reported previously, co-transfection of α, β, and γ is required in order to detect expression of recombinant protein or activity in this system (10) (data not shown).

**DISCUSSION**

We report here the identification of a second isoform of AMPKβ (termed β2), which we isolated from a two-hybrid screen for proteins interacting with the catalytic subunit of AMPK (α2). cDNA encoding the human homologue of rat β1 was identified from a search of the EST data base, and a clone (accession number R20494) containing the entire coding sequence was obtained from the IMAGE consortium. In a recent paper, it was reported that at least three distinct gene products for AMPKβ were represented in the EST data base (16). Our results do not support this claim, indicating that all of the β sequences in the EST data base correspond to either β1 or β2. It is worth noting, however, that most of the entries in the data base do not match exactly the sequences of β1 and β2 reported here, differing by a number of single base changes. We believe that these differences probably represent errors in the automated DNA sequence analysis used to generate the EST sequences. The majority of the discrepancies occur at the beginning and end of the EST sequences, which are more prone to reading errors. At the present time, therefore, we believe that only two β isoforms can be identified, although we do not exclude the possibility that additional isoforms exist (see also “Addendum”). In addition to the β subunit isoforms, two isoforms of the α subunit have been characterized (16). Searching of the EST data base, combined with preliminary results obtained from two-hybrid screening using the β1 subunit as bait, demonstrate that there are at least three genes coding for isoforms of the γ subunit. The existence of different isoforms for each of the AMPK subunits suggests that a large subfamily of AMPK complexes is present in mammals (see below).

The amino acid sequences of β1 and β2 share considerable identity with a family of related proteins in yeast, which interact with the SNF1 protein kinase complex (10, 11, 16, 27, 28). Amino acid sequence alignment of β1, β2, Sip2p, and Gal83p reveals two highly conserved regions (Fig. 2B), which have been shown recently to be important in mediating the interac-
determined. In order to obtain a linear rate of phosphorylation, 50-fold less protein (10 μm) acts with both α- or β-specific antibodies. The blotting antibody was detected with either protein G (β1) or protein A (β2) conjugated to horseradish peroxidase and visualized by enhanced chemiluminescence. Bands corresponding to b membrane and probed with either

\[ \text{migration of molecular mass standards is indicated.} \]

\[ \text{C} \]

\[ \text{D} \]

**FIG. 4. Expression of β isoforms in rat tissues.** A. Western blot analysis. Immune complexes, following immunoprecipitation of the indicated tissue extracts using antibodies specific for either β1 or β2, were resolved by SDS-PAGE. Proteins were transferred to a polyvinylidene difluoride membrane and probed with either β1- or β2-specific antibodies. The blotting antibody was detected with either protein G (β1) or protein A (β2) conjugated to horseradish peroxidase and visualized by enhanced chemiluminescence. Bands corresponding to β1 and β2 are marked by an arrow. Migration of molecular mass standards is indicated. B. [35S]methionine-labeled β1 or β2 was translated in vitro in rabbit reticulocyte lysate programmed with RNA synthesized from β1 or β2 cDNA in pcDNA3. Proteins were resolved by SDS-PAGE and labeled products detected by fluorography. Migration of molecular mass standards is indicated. C. AMPK was immunoprecipitated from partially purified extracts of rat liver (open symbols) or skeletal muscle (closed symbols) using antibodies specific for β1 (squares) or β2 (circles) and activity in the immune complexes determined. In order to obtain a linear rate of phosphorylation, 50-fold less protein (10 μg) was used for immunoprecipitation of β1 from liver. D, the immune complexes from C were resolved by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and probed with either α1- or α2-specific antibodies.

Characterization of AMPKβ Isoforms

- **Region 1**: The conserved C-terminal region, termed the ASC domain, interacts with Snf4p, while the conserved internal region, termed the KIS domain, interacts with Snf1p (15). We have shown previously that the β subunit plays an important role in the formation of the ternary AMPK complex in vitro since it interacts with both α and γ, whereas α and γ do not appear to interact directly (10). The simplest interpretation of these results is that the α and γ subunits bind independently to the β subunit, probably through the two regions related to the KIS and ASC domains in the yeast proteins. It remains to be determined whether binding of the α and γ subunits to β then allows them to interact directly with one another, although there is evidence that this may be the case with the homologous subunits in the yeast complex (15). Studies in our laboratory are currently under way to examine these interactions in more detail. It is unlikely that the sole purpose of the β subunit is to mediate the formation of the AMPK complex, since both isoforms appear equally competent to perform this function in vitro and in vivo. Most of the amino acid sequence variation between β1 and β2 occurs within the N-terminal region, and this is also the case for Sip2p and Gal83p (data not shown). It seems likely, therefore, that functional differences between the β isoforms will be attributable to the divergent N-terminal regions and will not entail assembly of the heterotrimeric complex.

- **Region 2**: Both β isoforms are expressed in a wide range of tissues, but there is a marked difference in their expression patterns. β1 is most highly expressed in liver with low level expression in skeletal muscle, and this pattern is reversed for β2. These findings suggest that the β isoforms may play tissue-specific roles in regulating the activity and/or function of AMPK. To begin to address these questions, we examined the effect of expression of the different β isoforms on AMPK activity in a recombinant system. Co-expression of the α and γ subunits with either β1 or β2 in CCL13 cells did not reveal a significant difference in AMPK activity between the two β isoforms, although, as reported previously, expression with α1 led to substantially higher activity than with α2 (7). Furthermore, β1 and β2 both interact with α1 and α2, and we did not detect any obvious difference in the association of the different α and β isoforms in vitro. These results imply that the β isoforms do not directly alter the activity of AMPK per se, but they do not exclude a less direct role in the regulation of AMPK. We decided therefore to examine the relative expression and activity of β1- and β2-containing AMPK in vitro, and since there was an obvious difference in the expression of the isoforms between the liver and skeletal muscle, we concentrated on these tissues.
Complexes containing all four possible combinations of the α and β isoforms were detected in liver and skeletal muscle, indicating that there are no constraints on isoform-specific subunit composition in vivo. In liver the β1-containing complexes account for virtually all of the AMPK activity, and this may simply reflect the relative abundance of the β1 complexes compared with β2. Consistent with this result is the finding that purification of AMPK from rat liver yields a preparation in which the β subunit is almost exclusively β1, as judged by migration on SDS-PAGE and amino acid sequencing (9, 10, 26). It is clear from our results, however, that a proportion of AMPK from rat liver contains the novel β2 isoform. In skeletal muscle, although there is more β2 complex compared with β1, AMPK activity associated with the different β isoforms is approximately the same. These results suggest that the β2 complex, or a proportion of it, is in a relatively inactive state compared with the β1 complex. We reported previously that AMPK isolated from skeletal muscle is in a relatively inactive state and suggested that this could be due to lack of association of the α subunit with the β and γ subunits (29). The results of our present study appear to rule out this possibility, and we are currently investigating the basis for the low activity state of AMPK in muscle.

We have detected the γ subunit in all of the complexes isolated in vivo, although we have not been able to determine which particular isoform is present in the different complexes. AMPK isolated from liver contains predominantly the γ1 isoform, as judged by amino acid sequencing (9, 10, 26), although the γ2 and γ3 isoforms are also expressed in liver (25). It is likely that, at least in liver, some complexes will contain the γ2 and γ3 isoforms.

We believe that our results provide valuable clues regarding the possible role of the β subunit in the regulation of AMPK. The marked difference in the expression patterns of β1 and β2 in liver and skeletal muscle strongly suggests a tissue-specific role for the isoforms. It was reported recently that AMPKα2, but not AMPKα1, is activated in response to increased contraction in rat skeletal muscle (30). Although we have not assessed the relative amounts of the α isoforms in skeletal muscle, the predominant β isoform is β2. Interestingly, however, both β1 and β2 account for approximately equal AMPK activity in muscle. It is possible that association with the different β isoforms could play a part in an altered response to the upstream signaling pathways that lead to AMPK activation. This could explain the differential activation of α2 in skeletal muscle during contraction if there were a pool of inactive α2β2-containing AMPK. Subtle differences in substrate recognition have been reported between α1 and α2, suggesting that the isoforms could phosphorylate different proteins, at different rates, within the cell (17, 31). The ability of the different isoform complexes to have a varying response to specific stimuli, coupled with the differing substrate specificity between α1 and α2, would allow an enormous degree of flexibility within the AMPK cascade.

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Addendum—During the preparation of this manuscript, Stapleton et al. reported the partial sequence of a mouse cDNA identified in the EST data base (accession number W07176), which corresponds to the mouse homologue of β2 (26).

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**Fig. 5. Expression of β isoforms in mammalian cells.** A, AMPK activity determined in immune complexes of CCL13 cells following transient transfection with cDNAs encoding α1β1γ1 (open circles), α1β2γ1 (closed circles), α2β1γ1 (open squares), or α2β2γ1 (closed squares) is shown. Note the difference in scale between the activities of α1 and α2. B, the immune complexes from A were resolved by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and probed with an antibody that recognizes both β isoforms. Migration of molecular mass standards is indicated.
Characterization of AMPKβ Isoforms

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