Posttranslational Modifications of the 5′-AMP-activated Protein Kinase β1 Subunit*

(Received for publication, June 4, 1997, and in revised form, June 30, 1997)

Ken I. Mitchelhill, Belinda J. Michell, Colin M. House, David Stapleton, Jason Dyck‡§, James Gamble‡§, Christina Ullrich¶, Lee A. Witters‡, and Bruce E. Kemp‡

From the St. Vincent’s Institute of Medical Research, 41 Victoria Parade, Fitzroy, Victoria 3065 Australia and the Endocrine-Metabolism Division, Departments of Medicine and Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755

The AMP-activated protein kinase (AMPK) consists of catalytic α and noncatalytic β and γ subunits and is responsible for acting as a metabolic sensor for AMP levels. There are multiple genes for each subunit and the rat liver AMPK α1 and α2 catalytic subunits are associated with β1 and γ1 noncatalytic subunits. We find that the isolated γ1 subunit is N-terminally acetylated with no other posttranslational modification. The isolated β1 subunit is N-terminally myristoylated. Transfection of COS cells with AMPK subunit cDNAs containing a nonmyristoylatable β1 reduces, but does not eliminate, membrane binding of AMPK heterotrimer. The isolated β1 subunit is partially phosphorylated at three sites, Ser24/25, Ser182, and Ser108. The Ser24/25 and Ser108 sites are substoichiometrically phosphorylated and can be autophosphorylated in vitro. The Ser-Pro site in the sequence LSSGKPPCPG is stoichiometrically phosphorylated, and no additional phosphate is incorporated into this site with autophosphorylation. Based on labeling studies in transfected cells, we conclude that α1 Thr172 is a major, although not exclusive, site of both basal and stimulated α1 phosphorylation by an upstream AMPK kinase.

The 5′-AMP-activated protein kinase (AMPK) isolated from liver consists of three subunits, the catalytic α subunit (548 residues, Mf 62,497 ± 63 kDa) and two noncatalytic subunits, β (M, 30,378 ± 40 kDa) and γ (M, 37,429 ± 38 kDa) (1–3). The AMPK phosphorylates a number of protein substrates including key enzymes involved in the control of lipid metabolism, acetyl-CoA carboxylase, HMG-CoA reductase and hormone-sensitive lipase (reviewed in Ref. 4). It has been proposed that the AMPK functions in stress responses since it is activated by increasing intracellular AMP resulting from a variety of treatments including arsenite, heat shock (5–7), ischaemia (8), exercise (9), and electrical stimulation of skeletal muscle (10).

There are multiple isoforms of the AMPK subunits present in rat liver (2, 11). The AMPK α1 isoform (2) is encoded by a gene localized to chromosome 5p11 (12), whereas the AMPK α2 isoform gene is localized to chromosome 1p31 (13). The β1 and γ1 subunits genes are located on chromosome 12 (12). The AMPK α1 and α2 isoforms are 90% identical in their catalytic cores but only 60% identical in their COOH-terminal tails. Previously, we found that the β1 subunit of the AMPK was multiply phosphorylated in an intramolecular autophosphorylation reaction (14). The β1 subunit of the AMPK contains an N-terminal consensus sequence for myristoylation with glycerine at position 1 and serine (a small uncharged residue) at position 5 (15).

In view of the potential importance of posttranslational modifications of the AMPK subunits in its physiological function, we have characterized the native state of the β subunit. We find that the β subunit purified from rat liver is fully myristoylated and present as a mixture of di- and triphosphorylated species with small amounts of monophospho-β subunit. Autophosphorylation of the AMPK in vitro results in up to 6 or more moles of phosphate incorporated per mole of β subunit. The mass of the isolated N-terminally acetylated γ subunit is identical to the mass inferred from the cDNA sequence and is not phosphorylated.

EXPERIMENTAL PROCEDURES

AMPK Purification and Assay—The AMPK was purified from rat liver (14) and assayed (16) as described previously using the SAMS peptide substrate and 200 μM 5′-AMP. The enzyme was diluted in 50 mM Tris-HCl, pH 7.5, 0.05% (v/v) Triton X-100, and the reactions were initiated by adding enzyme. The reactions were stopped by withdrawing 30-μl aliquots for liquid scintillation counting as described (17). Protein concentration was assayed by the method of Lowry (18).

AMPK Autophosphorylation—Purified AMPK was desalted into assay buffer (PD-10, Pharmacia Biotech Inc.) and incubated with 20 μM 5′-AMP and 20 μM [γ-32P]ATP at 30 °C for 30 min. The reaction mixture was made 0.1% SDS and desalted into 100 mM Tris-HCl, pH 6.8, 10% glycerol, 2 mM EGTA, 0.01% SDS by centrifugal ultrafiltration (Centricron 30, Amicon). The retentate was made 0.1% SDS prior to reduction with 10 mM dithiothreitol and SDS-polyacrylamide gel electrophoresis (10% acrylamide gel).

Protein Electrospray Mass Spectrometry—Purified AMPK (10–30 μg) was precipitated in 6% (w/v) trichloroacetic acid in the presence of 12.5 μg/ml sodium deoxycholate. The protein pellet was washed once in 6% (w/v) trichloroacetic acid then twice in ether/ethanol (80/20), before air-drying, resuspension in 10 μL of 50% (v/v) acetic acid and dilution in 10 μL acetonitrile for direct infusion into a PE Sciex API III electrospray mass spectrometer. The resultant positive ion spectra were deconvoluted using the hypermass calculation in the supplied software (MacSpec 3.3).

Phosphoprotein Digestion—Peptides for protein sequencing or mass analysis were derived from the rat β1 subunit of the AMPK separated by SDS-polyacrylamide gel electrophoresis by in situ proteolysis (19). Briefly, Comassie Blue-stained gel slices were excised and placed in the funnel compartment of a Hewlett Packard G1004B Chemstation where they were washed extensively in water, reduced in 5 funnel
volumes (approximately 5 ml) of 10 mM dithiothreitol in 0.2 mM Tris-HCl buffer, 1 mM EDTA, pH 8.5, at 45 °C over 3 h and alkylated with 5 funnel volumes of 1% 4-vinylpyridine in 0.2 mM Tris-HCl buffer, 1 mM EDTA, pH 8.5, at ambient temperature over 2 h. The gel slices were then destained in 7 funnel volumes of 50 mM ammonium bicarbonate, 50% (v/v) acetonitrile (50°C) over 2 h. The destained gels were sonicated again (30% duty, continuous) for 10 s. The dry gel slices were rehydrated completely in digestion buffer (50 mM Tris-HCl buffer, 10% acetonitrile, pH 9.3, approximately 30 μl of digestion buffer per slice from a single lane of a mini-gel) containing achromobacter endoproteinase Lys-C (Wako) with protease to substrate ratio approximately 1:10 (w/w). The rehydrated gel slices were covered with an equal volume of digestion buffer and incubated overnight at 37 °C. The peptide digestions were recovered by combining the digestion buffer with 1 h washes of the gel slices (in a tube floating on a sonicating water bath) as follows: 200 μl of 2% trifluoroacetic acid (TFA) v/v; 200 μl of 30% acetonitrile, 0.1% trifluoroacetic acid; and finally, 200 μl of 60% acetonitrile, 0.1% trifluoroacetic acid. The combined gel eluates were almost dried in a centrifugal freeze drier, reconstituted in 6 μl of 100% trifluoroacetic acid, and vortexed. 6 μl guanidine hydrochloride (300 μM) was added, and the mixture subjected to chromatography on Nucleosil C18 5 μm 300 A reversed phase material packed in a 1 x 250-mm glass-lined column. The chromatography was performed on a Pharmacia SMART system using a linear 0–80% acetonitrile, 0.1% trifluoroacetic acid gradient over 120 min at 40 μl/min and monitored at 214 nm, and 2 min fractions were collected.

**Peptide Sequencing and Phosphate Release—**Peptides were sequenced with a Hewlett Packard G1000A protein sequencer utilizing aminopeptidase M digestion (enzyme dilutions were tested for each treatment) on the MALDI target plate against a series of synthetic peptides. Alkaline phosphatase, chymotrypsin, and trypsin digestions were carried out at room temperature in a humidified atmosphere on the MALDI target. Alkaline phosphatase, sequencing grade chymotrypsin (Boehringer Mannheim), and modified sequencing grade trypsin (Promega) were desalted into 25 mM ammonium bicarbonate, 10% acetonitrile on a Pharmacia SMART reversed phase material packing column. Lyophilized aminopeptidase M (Sigma) was reconstituted, and all dilutions were carried out in the same solvent. Peptides in HPLC solvent (0.1% trifluoroacetic acid, variable acetonitrile concentrations) were added to equal volumes of diluted enzyme (a range of enzyme dilutions were tested for each treatment) on the MALDI target and allowed to react for 10–15 min. At the completion of this time, an equal volume of acetonitrile containing 2% trifluoroacetic acid was added, the sample was dried. The aminopeptidase M digestion was performed in a tube at 37 °C for 24–48 h, and an aliquot was removed and mixed with matrix prior to mass analysis.

**AMPK Subunit Mutagenesis and Cellular Expression—**Two mutant forms of AMPK subunits were used in these experiments. To generate an inactive AMPK α subunit, threonine 172 (a major autophosphorylation site) was mutated to alanine as described previously (20). This mutation rendered AMPK (when expressed as a heterotrimer) nearly inactive (2% of the activity of wild-type) and also created a dominant negative inhibitor of wild-type AMPK heterotrimer (data not shown), similar to the inactivating α1 K45R mutant (20). The β1 subunit glycine 2 was mutated to alanine to remove the myristoylation site. For cell labeling experiments with 32P, COS-7 cells were trypsinized and subjected to either wild-type GST-tagged α1 or GST-tagged T172A-α1 (both in pEG vector) in association with HA (hemagglutinin)-tagged α subunit of the AMPK by electrospray mass spectrometry. In contrast, there were multiple species of the β1 subunit (Fig. 1B). The form with M of 30,552 accounted for approximately 10% of the material corresponded to the mass of the myristoylated form of the protein with a single phosphate group. The M, expected of the unmodified species was 30,264 based on the CDNA-derived sequence reported by Woods (NCBI Seq. ID 1185269) (3). Our earlier reported cDNA sequence (11) (NCBI Seq. ID 1335858) was found to contain two errors, Glu for Gly at position 26 and a Pro deletion at position 137. These errors were confirmed by either direct Edman sequencing for position 26 or by mass spectrometry for position 137 (results not shown) and confirm the correct β1 cDNA sequence (3). The two most prominent species corresponded to the di-(M, 30,635) and triphosphorylated (M, 30,722) forms of the myristoylated protein (Fig. 1B). Depending on the enzyme preparation, the relative amounts of the di- and triphosphorylated species varied but were always much more prominent than the monophosphorylated form.

**β Subunit Phosphorylation Site and N Terminus Analysis—**β subunit was digested with endoproteinase Lys-C and the resultant peptides were chromatographed on reversed phase HPLC (Fig. 2). Each fraction was screened using MALDI-TOF mass spectrometry. The fractions labeled A–D contained peptides with masses that did not match the predicted Lys-C digest patterns that were further examined. Residue numbering is based on the protein sequence inferred from the cDNA sequence (3) with the inferred methionine at position 1 being absent from the mature protein.

Fraction A contained a phosphopeptide of M, 1242 that reduced to M, 1161 (Table I) on phosphatase treatment, which is consistent with a single phosphate addition to the predicted Lys-C fragment STRPRDSC24SGGTTC26. The corresponding dephospho-form of this peptide was observed in the adjacent fraction. Attempts to elude the site of phosphorylation by mass fragmentation techniques were unsuccessful. This peptide is also autophosphorylated in vitro with γ-32P[ATP. When the corresponding phosphoaminoacid and peptide was subjected to 32P phosphate release sequencing, most radioactivity was released at cycle 6, the primary phosphorylation site, but with significant phosphate release at cycle 7 (Fig. 3). Thus either Ser24 or Ser25 (but not both) were phosphorylated with a preference for Ser24.

1 Fraction B contained a phosphopeptide of M, 3478 that re-
duced to $M_r$ 3399 (Table I) on phosphatase treatment, which is consistent with a single phosphate addition to the predicted Lys-C fragment $^{173}$BSDVSELSSS$^{182}$PPGPYHQEPYISKPEE-RFK$^{201}$ (where B is pyridylethyl cysteine). Chymotryptic digestion of the phosphopeptide yielded a peptide fragment of $M_r$ 1710 (Table I) consistent with the phosphate being located in the fragment $^{173}$BSDVSELSSS$^{187}$PPGPYHQEPYISKPEE-RFK. Aminopeptidase M digestion of this fraction resulted in a mass spectral ladder (Table I) containing fragments consistent with phosphorylation on Ser182. The corresponding dephospho-form was not observed, which is consistent with Ser 182 being stoichiometrically phosphorylated. Furthermore, autophosphorylation in vitro did not result in any incorporation into this site, and no dephosphorylated $\beta_1$ subunit was detected in the isolated enzyme (Fig. 1B).

Fraction C contained a phosphopeptide of $M_r$ 2862 that reduced to $M_r$ 2782 (Table I) on phosphatase treatment, which is consistent with a single phosphate addition to the predicted Lys-C peptide $^{103}$LPLTRSQNNFVAILDLPEGEHQYK$^{126}$. The corresponding dephospho-form was also present in fraction C. Tryptic digestion of this fraction (Table I) yielded a peptide fragment of $M_r$ 2281 consistent with phosphorylation at Ser108. Parallel autophosphorylation experiments show that Ser108 is the major autophosphorylation site on the AMPK $\beta$ subunit. A corresponding synthetic peptide analog, CKLPLTRSQNNFVAILDLPEGEHQYK (Cys101, Arg115/116) was readily phosphorylated by the AMPK with a $K_m$ of 117.5 $\pm$ 10.8 $\mu$M and a $V_{max}$ of 2.4 $\pm$ 0.4 $\mu$mol/min $^{-1}$mg $^{-1}$.

Fraction D contained a peptide of $M_r$ 2202 (Table I), which is N-terminally blocked by Edman sequencing. This mass is consistent with the predicted N-terminal Lys-C peptide myristoyl-$^5$GNTSSERAALERQAGHK$^{18}$ and a tryptic fragment of $M_r$ 961 (Table I) corresponding to the peptide myristoyl-$^5$GNTSSER and confirming the structural assignment.

Effect of $\beta$ Subunit Myristoylation on Subcellular Distribution—Mutation of the Gly2 myristoylation site or addition of a 12-amino acid HA-tag to the N terminus of the $\beta_1$ subunit (removing Gly2 as a target for myristoylation) alters the subcellular distribution of expressed AMPK subunits in COS-7 cells. As shown in Fig. 4 (upper panel) under the selected conditions of triple transfection of AMPK subunit cDNAs, cell lysis, and centrifugation, wild-type $\beta_1$ protein is almost entirely recovered in a membrane fraction ($M$), as are its associated $\alpha_1$ (middle panel) and $\gamma_1$ (lower panel) subunits. In expression of AMPK heterotrimers containing either the G2A $\beta_1$ mutant or with the addition of an HA-tag to the N terminus of the $\beta_1$ subunit, a substantial fraction of $\beta_1$ and $\gamma_1$ are recovered in the soluble cell fraction along with a small amount of $\alpha_1$. It should be noted that immunostaining of the expressed $\beta_1$ subunit reveals more than one protein band, particularly with the G2A mutant or HA-tagged construct (upper panel). This could reflect molecular heterogeneity due to other posttranslational modifications, such as phosphorylation. Taken together, these data indicate that the Gly$^2$ myristoylation site on $\beta_1$, in part, influences the subcellular localization of the transfected/expressed AMPK, but other factors must be involved.
TABLE I
Mass spectral analysis of phosphopeptides

| Peptide fraction | $M_r$ obs. | Corresponding to | Residue | $M_r$ calc. |
|------------------|------------|------------------|---------|------------|
| Fraction A       | 1241.6     | TPRRDSSGGTK + PO$_4$ | 19–29   | 1240.6     |
| Phosphatase-treated fraction A | 1161.7 | TPRRDSSGGTK | 19–29 | 1160.6     |
| Fraction B       | 3478.0     | BSVDSELSSPPGFYQGYEPY1SKPEERFK + PO$_4$ | 173–201 | 3477.6     |
| Phosphatase-treated fraction B | 3399.3 | BSVDSELSSPPGFYQGYEPY1SKPEERFK | 173–201 | 3397.6     |
| Chymotrypsin-treated fraction B | 1709.9 | BSVDSELSSPPGFYQGYEPY + PO$_4$ | 173–192 | 1708.7     |
| Aminopeptidase M-treated fraction B | 3476.9 | BSVDSELSSPPGFYQGYEPY1SKPEERFK + PO$_4$ | 173–201 | 3476.7     |
| Fraction C       | 2862.1     | LPLTP5QNNFVAILDPEGEHQYK + PO$_4$ | 103–129 | 2861.4     |
| Phosphatase-treated fraction C | 2782.6 | LPLTP5QNNFVAILDPEGEHQYK | 103–129 | 2781.4     |
| Trypsin-treated fraction C | 2281.8 | 5QNNFVAILDPEGEHQYK | 108–129 | 2281.1     |
| Fraction D       | 2202.1     | myristoyl-GNTSSERAALEQAGHK | 2–18 | 2201.3     |
| Trypsin-treated fraction D | 960.9 | myristoyl-GNTSSER | 2–19 | 959.7      |

Fig. 3. Phosphate release sequencing of Peak A. Sequencing was as described under “Experimental Procedures.”

β Subunit Phosphorylation in Intact Cells—To study the phosphorylation of the β subunit in intact cells and the relative importance of AMPK autophosphorylation, AMPK heterotrimer was expressed in 32P-labeled COS-7 cells using heterotrimers with wild-type α or an inactive α subunit. The latter was generated by site-directed mutagenesis of Thr$^{172}$, initially predicted to be the major site of activating phosphorylation by an upstream kinase kinase, based on kinase structure (19). As recently confirmed by peptide sequencing, Thr$^{172}$ is indeed the site phosphorylated by such an AMPK kinase (21). The Thr$^{172}$ to Ala mutant has <2% of the activity of the wild-type subunit when expressed in the AMPK heterotrimer, indicating the importance of Thr$^{172}$ in regulation of activity (data not shown). As shown in Fig. 5, both wild-type α and β in AMPK heterotrimer isolated from labeled cells by glutathione adsorption are phosphorylated in control cells, and the 32P content of each increases on treatment of cells with sodium azide. No phosphorylation of the γ subunit is observed. However, expression of inactive AMPK heterotrimers containing the T172A α subunit reveals reduced phosphorylation of both α and β subunits in the basal state and resulted in no change in their 32P content on azide treatment. Taken together, these data indicate that Thr$^{172}$ is the major, though not exclusive site, of both basal and azide-activated α phosphorylation. Some β phosphorylation depends on the activity of the α catalytic subunit, but non-α, protein kinases active on β are likely to account for some of the residual phosphorylation observed in the presence of inactive α.

DISCUSSION

The N terminus of β (MGNTSSERAA... ) contains a penultimate glycine (Gly$^2$) in an appropriate context of downstream sequence (MGNXXS) of other known myristoylation sites (15). In some cases myristoylation is responsible for targeting proteins to the membrane, but may also facilitate protein/protein interactions (22). Myristoylation of proteins in the src family (23), the myristoylated alanine-rich protein kinase C substrate (MARCKS) (24), ADP-ribosylation factor 1 (25) and recoverin (26) has been shown to facilitate membrane binding, but for calcineurin-B and the cAMP-dependent protein kinase catalytic subunit, myristoylation does not affect membrane association (27).
Recurring basic amino acids and palmitoylation may also effect membrane binding either alone (27) or with myristoyl groups (28). A number of evenly spaced basic amino acids that could potentially interact with acidic phospholipids in membrane bilayers have been described for the src proteins (23) and there is a polybasic sequence in the N terminus of β (Arg6, Arg12, Lys18, Arg20, Arg21). The β1 subunit is not modified by palmitoylation. In other examples the myristoyl-dependent membrane association may also be modulated by protein phosphorylation (24) or calcium binding (26) and play a role in signal-induced protein localization. The present studies indicate that the presence/absence of the penultimate Gly2 myristoylation site in the native AMPK heterotrimer has a modest influence on the intracellular localization of AMPK heterotrimer, although other factors also seem to be involved. More studies are needed to examine the role/localization of particulate AMPK in the action of this kinase. It is not known if the presence of the myristate group contributes to the stability of the AMPK heterotrimer as it does for the cAMP-dependent protein kinase (29).

Extensive in vitro phosphorylation of noncatalytic subunits of protein kinases has been observed for a number of protein kinases including phosphorylase kinase and CK2 (30, 31) but the physiological significance of these phosphorylation events is uncertain. In the AMPK we have found three phosphorylation sites in the native β1 subunit, whereas no phosphorylation of the γ subunit has been observed. Of the three β1 phosphorylation sites, Ser125 and Ser168 are part of the intramolecular autophosphorylation cascade that are phosphorylated in vivo. There are (at least) three additional sites present in the hyperphosphorylated state following in vitro autophosphorylation that we have not characterized. We have observed these on two-dimensional phosphopeptide maps (32) of tryptic peptides derived from in gel digests of autophosphorylated β1 subunit. Phosphorylation at Ser167 seems likely to result from phosphorylation by a separate protein kinase with a Ser-Pro specificity. This site appears to be stoichiometrically phosphorylated in the native enzyme as no additional [32P]phosphate is incorporated into this site; the corresponding dephosphorylated peptide is not detected nor is any dephosphorylated β1 subunit detected.

We were unable to obtain electrospray mass spectrometry data on the native α subunit to determine its state of phosphorylation. Tryptic phosphopeptide mapping of in vitro and cell-labeled α subunits indicate that they are multiply phosphorylated (data not shown). The phosphorylation site Thr172 in the activation loop that is phosphorylated by the AMPK kinase has been recently characterized (21). Our intact cell studies with expression of AMPK heterotrimers indicate that α, Thr172 is critical for enzyme activity and that its absence diminishes phosphorylation of both the α and β1 subunits. Furthermore, upon activation of AMPK activity by the uncoupling of oxidative phosphorylation with sodium azide, no increase in 32P content in either α or β1 is observed when the T172A mutant is incorporated into the heterotrimer. These data indicate that Thr172 is the critical activating phosphorylation site under these conditions and that β1 subunit phosphorylation, in part, occurs through an intramolecular autophosphorylation. However, there is residual phosphorylation of both α and β1 subunits, even in the absence of an active catalytic subunit (we have been unable to detect any endogenous α subunit in COS-7 cells), suggesting that there may be distinct AMPK kinases active on both the α and β1 subunits.

Acknowledgments—We are grateful to Frosa Katsis for the preparation of synthetic peptides.

REFERENCES

1. Stapleton, D., Gao, G., Michell, B. J., Widmer, J., Mitchellhill, K. I., Teh, T., House, C. M., Witters, L. A., and Kemp, B. E. (1994) J. Biol. Chem. 269, 29343–29346
2. Woods, A., Cheung, P. C., Smith, F. C., Davison, M. D., Scott, J., Beri, R. K., and Carling, D. (1996) J. Biol. Chem. 271, 611–614
3. Woods, A., Cheung, P. C., Smith, F. C., Davison, M. D., Scott, J., Beri, R. K., and Carling, D. (1996) J. Biol. Chem. 271, 10282–10290
4. Hardie, D. G. (1992) Biochim. Biophys. Acta 1125, 251–258
5. Moore, F., Weekes, J., and Hardie, D. G. (1991) Eur. J. Biochem. 199, 691–697
6. Gillespie, J. G., and Hardie, D. G. (1992) FEBS Lett. 306, 59–62
7. Sato, R., Goldstein, J. L., and Brown, M. S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9261–9265
8. Kudo, N., Barr, A. J., Barr, R. L., Desai, S., and Lopashchuk, G. D. (1995) J. Biol. Chem. 270, 17513–17520
9. Winder, W. W., and Hardie, D. G. (1996) Am. J. Physiol. 270, E299–E304
10. Varvai, D., Apazidis, A., Saha, A. K., Gamble, J., Patel, A., Kemp, B. E., Witters, L. A., and Ruderman, N. B. (1997) J. Biol. Chem. 272, 12055–12061
11. Gao, G., Fernandez, C. S., Stapleton, D., Auster, A. S., Widmer, J., Dyck, J. R. B., Kemp, B. E., and Witters, L. A. (1996) J. Biol. Chem. 271, 8675–8681
12. Stapleton, D. A., Woollatt, E., Mitchellhill, K. I., Nicholl, J. K., Fernandez, C. S., Michell, B. J., Witters, L. A., Power, D. A., Sutherland, G. R., and Kemp, B. E. (1997) FEBS Lett. 409, 452–456
13. Beri, R. K., Marley, A. E., See, C. G., Sowpath, W. F., Aguain, K., Carling, D., Scott, J., and Carey, F. (1994) FEBS Lett. 356, 117–121
14. Mitchellhill, K. I., Stapleton, D., Gao, G., Michell, B. J., House, C. M., Katsis, C. M., Katsis, F., Witters, L. A., and Kemp, B. E. (1996) J. Biol. Chem. 271, 28445–28450
15. Artken, A. (1992) Lipid Modification of Proteins. A Practical Approach (Hawke, N. M., and Turner, A. J., eds) pp. 63–88, IRL Press, Oxford
16. Davies, S. P., Carling, D., and Hardie, D. G. (1989) Eur. J. Biochem. 186, 123–128
17. Pearson, R. B., Mitchellhill, K. I., and Kemp, B. E. (1993) Protein Phosphorylation: A Practical Approach (Hardie, G. D., ed), pp. 265–291, Oxford University Press, New York
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
19. Mitchellhill, K. I., Stapleton, D., Gao, G., House, C. M., Michell, B., Katsis, F., Witters, L. A., and Kemp, B. E. (1994) J. Biol. Chem. 269, 2361–2364
20. Dyck, J. R. B., Gao, G., Widmer, J., Stapleton, D., Fernandez, C. S., Kemp, B. E., and Witters, L. A. (1996) J. Biol. Chem. 271, 17795–17803
21. Hawley, S. A., Davison, M., Woods, A., Davies, S. P., Beri, R. K., Carling, D., and Hardie, D. G. (1996) J. Biol. Chem. 271, 27879–27887
22. Towler, D. A., Gordon, J. I., Adams, S. P., and Glaser, L. (1988) Annu. Rev. Biochem. 57, 69–99
23. Resh, M. D. (1994) Cell 76, 411–413
24. Taniguchi, H., and Manenti, S. (1993) J. Biol. Chem. 268, 9960–9963
25. Franco, M., Chardin, P., Chabre, M., and Paris, S. (1996) J. Biol. Chem. 271, 1573–1578
26. Dizhoor, A. M., Chen, K. C., Olekhovskaya, E., Sineinikova, V. V., Philippov, P., and Hurley, J. B. (1993) Science 258, 829–832
27. Zhu, D., Cardenas, M. E., and Heitman, J. (1995) J. Biol. Chem. 270, 24831–24838
28. Sigal, C. T., Zhou, W., Buser, C. A., McLaughlin, S., and Resh, M. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12255–12257
29. Yonemoto, W., McGlone, M. L., and Taylor, S. S. (1993) J. Biol. Chem. 268, 2348–2352
30. Wang, J. H., Stull, J. T., Huang, S. T., and Krebs, E. G. (1976) J. Biol. Chem. 251, 4521–4527
31. Desjardins, P. R., Lue, P. F., Liew, C. C., and Gornall, A. G. (1973) Can. J. Biochem. 50, 1249–1259
32. van der Geer, P., and Hunter, T. (1994) Electrophoresis 15, 544–554