Interaction of PKN with α-Actinin*

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PKN is a fatty acid- and Rho-activated serine/threonine protein kinase, having a catalytic domain homologous to protein kinase C family. To identify components of the PKN-signaling pathway such as substrates and regulatory proteins of PKN, the yeast two-hybrid strategy was employed. Using the N-terminal region of PKN as a bait, cDNAs encoding actin-cross-linking protein α-actinin, which lacked the N-terminal actin-binding domain, were isolated from human brain cDNA library. The responsible region for interaction between PKN and α-actinin was determined by in vitro binding analysis using the various truncated mutants of these proteins. The N-terminal region of PKN outside the RhoA-binding domain was sufficiently shown to associate with α-actinin. PKN bound to the third spectrin-like repeats of both skeletal and non-skeletal muscle type α-actinin. PKN also bound to the region containing EF-hand-like motifs of non-skeletal muscle type α-actinin in a Ca2+-sensitive manner and bound to that of skeletal muscle type α-actinin in a Ca2+-insensitive manner. α-Actinin was co-immunoprecipitated with PKN from the lysate of COS7 cells transfected with both expression constructs for PKN and α-actinin lacking the actin-binding domain. In vitro translated full-length α-actinin containing the actin-binding site hardly bound to PKN, but the addition of phosphatidylinositol 4,5-bisphosphate, which is implicated in actin reorganization, stimulated the binding activity of the full-length α-actinin with PKN. We therefore propose that PKN is linked to the cytoskeletal network via a direct association between PKN and α-actinin.

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1 The abbreviations used are: HuActSk1, human skeletal muscle type 1 α-actinin; aa, amino acid or amino acids; GST, glutathione S-transferase; HuActNm, human non-skeletal muscle type α-actinin; PCR, polymerase chain reaction; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; PI4,5P2, phosphatidylinositol 4,5-bisphosphate; HA, hemagglutinin.

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fusin protein or with 25 μg of GST alone in 400 μl of GST binding buffer (20 mM Tris/HCl at pH 7.5, 0.5 mM DTT, 150 mM NaCl, 0.05% Triton X-100, 1 mM EDTA, 1 μg/ml leupeptin) and incubated for 1 h at 4 °C. After addition of 25 μl of glutathione-Sepharose 4B pretreated with 10 mg/ml *Escherichia coli* extract to block nonspecific binding, the binding reaction was continued for an additional 30 min at 4 °C. The glutathione-Sepharose 4B was then washed four times in GST wash buffer (20 mM Tris/HCl at pH 7.5, 0.5 mM DTT, 1 mM EDTA, 1 μg/ml leupeptin) containing 0.5 M NaCl and 0.5% Triton X-100 and further washed with GST wash buffer. Bound proteins were eluted with GST elution buffer (100 mM Tris/HCl at pH 7.5, 10 mM glutathione, 120 mM NaCl, 1 mM EDTA, 0.5 mM DTT, 1 μg/ml leupeptin) and were subjected to SDS-PAGE.

For analysis of the effect of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) (Boehringer Mannheim) on the binding between HuAct and PKN, 2 μl of *in vitro* translated full-length HuActSk1 was mixed with 5 μg of GST-PKN1 fusion protein or with 25 μg of GST alone in 400 μl of buffer P (20 mM Tris/HCl at pH 7.5, 0.5 mM DTT, 120 mM NaCl, 1 mM EDTA) and incubated for 1 h at room temperature with or without PI(4,5)P2 as indicated in the figure legends. After addition of 25 μl of glutathione-Sepharose 4B pretreated with *E. coli* extract, the binding reaction was continued for an additional 30 min at 4 °C. The glutathione-Sepharose 4B was then washed four times in buffer P containing 100 mM NaCl. Bound proteins were eluted with GST elution buffer and were subjected to SDS-PAGE. The binding was visualized and quantitated by an imaging analyzer (FUJI BAS1000).

**Antiserum**—The anti-hemagglutinin (HA) monoclonal antibody 12CA5 was purchased from Boehringer Mannheim. αC6, a specific antiserum against PKN, was prepared by immunizing rabbits with the bacterially synthesized fragment of aa 863–946 of rat PKN.

*In Vivo Binding Assay*—HA-tagged cDNA for HuActSk1 (aa 333–894) was created by fusion of a 5′ coding sequence of the influenza HA to the C terminus of clone 4. A vector pHA-Act was constructed by subcloning this cDNA into pcDNA3 (Invitrogen). Empty pHA vector was constructed by subcloning a cDNA encoding only HA epitope into pcDNA3. A vector pHA-Act or empty pHA vector was cotransfected into COS7 cells with the expression vector pMhPKN3 (2) encoding the fusion protein or with 25 μl of 12CA5 for 2 h. After addition of 20 μl of 50% protein A-Sepharose, the mixtures were further incubated for 1 h. The immunoprecipitates adsorbed to protein A-Sepharose were washed twice with HA wash buffer (100 mM Tris/HCl at pH 7.5, 0.5 M LiCl) and twice with 100 mM Tris/HCl at pH 7.5. The resultant immunoprecipitates were resuspended in 50 μl of Laemmli’s sample buffer (9), and 25 μl aliquot of the extract was subjected to SDS-PAGE, following detection by immunoblotting with αC6.

**Preparation of Actin Cytoskeletal Proteins**—Actin was purified from rabbit skeletal muscle by the method of Mommaerts (10). α-Actinin was purified from bovine aorta by the method of Feramisco and Burridge (11). Filamin was purified from bovine aorta by the method of Koba-yama et al. (12). Caldesmon was partially purified from bovine aorta by the method of Abe et al. (13). Filamin, metavinculin, and talin were partially purified from bovine aorta as described (11).

**Kinase Assay**—The phosphorylation by PKN was carried out at 30 °C in an assay mixture containing 20 mM Tris/HCl at pH 7.5, 4 mM MgCl2, 100 μM ATP, 185 kBq of [γ-32P]ATP, phosphate acceptors, 20 ng/ml purified PKN from rat testes (13), and with or without 40 μM arachidonic acid as indicated in each experiment. Partially purified protein was boiled for 5 min to destroy endogenous kinase activity before use as a phosphate acceptor. After incubation for 5 min, the reaction was terminated by the addition of an equal volume of Laemmli’s sample buffer and separated on SDS-polyacrylamide gels. The gels were dried under vacuum, and the phosphorylation was visualized and quantitated by an imaging analyzer (FUJI BAS1000). When the δ protein kinase C peptide (1) was used as a phosphate acceptor, reactions were terminated by spotting a mixture onto a Whatman P81 paper and submerging it in 75 mM phosphatase and followed by three 10-min washes. Incorporation of [32P]phosphate into the peptide was assayed by scintillation counting.

**RESULTS**

**PKN Interacts with α-Actinin in the Yeast Two-hybrid System**—We screened a million yeast colonies transformed with both human brain cDNA library fused to Gal4 transcriptional activation domain and a bait construct encoding PKN1 fused to Gal4 DNA binding domain. The 82 plasmids were isolated representing 16 different cDNAs as judged by cDNA sequencing. Three positive clones (clones 4, 10, and 25) encoded the skeletal muscle type α-actinin (HuActSk1, designated in Ref. 7). The clone 4 encoded HuActSk1 from aa 333 to the C terminus, and both clone 10 and clone 25 encoded HuActSk1 from aa 344 to the C terminus. All these clones contained complete C terminus but lacked the N-terminal actin-binding domain (14) (Fig. 1B). These clones resulted in high β-galactosidase levels upon co-transformation with the PKN bait construct in the original yeast host strain YG11. The specificity of this interaction was tested further by measuring the stability of other combinations of two-hybrid constructs, LexAbd (instead of Gal4bd)-PKN and Gal4ad-α-actinin to support lacZ expression in L40 cells (MATa trpl1 leu2 his3 lys2::lexA-HIS3 URA3::lexA-lacZ). As shown in Fig. 2, high β-galactosidase activity was also developed in this system, suggesting a specific interaction between the N-terminal region of PKN and α-actinin. The two-hybrid method was employed to identify the region on PKN that interacted with HuActSk1, and this region was compared with the binding site for RhoA, protein already known to interact with PKN in *in vitro* and *in vivo* (4, 5). The RhoA-binding site has been mapped on the aa 33–111 of PKN that corresponds to the first leucine zipper-like sequence of PKN (15), whereas α-actinin very weakly interacted with this region of PKN (Fig. 1A). By contrast, α-actinin strongly interacted with aa 136–189 of PKN, whereas no interaction was detected between RhoA and this region of PKN (data not shown). This region corresponds to the second leucine zipper-like sequence and its immediate N-terminal region, which is conserved through evolution in vertebrates (16). Thus α-actinin binds most avidly to the region distinct from that which binds to RhoA. These results raise the possibility that PKN binds simultaneously to RhoA and α-actinin.

**Binding of PKN to HuActSk1 in Vitro**—α-Actinin is composed of three domains, an N-terminal actin-binding domain, extended rod-shaped domain with four internal 122 aa repeats (spectrin-like repeats), and a C-terminal region containing a pair of presumptive helix-loop-helix Ca2+-binding motifs, often referred to as EF-hands (reviewed in Ref. 17). To investigate whether PKN binds directly to α-actinin and to clarify which part of α-actinin is necessary for binding to PKN, various truncated constructs of HuActSk1 were produced as GST fusion proteins in *E. coli* (Fig. 1B). As shown in Fig. 3, *in vitro* translated PKN2 strongly bound to each α-actinin fragment (aa 423–653, aa 653–837, and aa 486–607) but not to the truncated construct of HuActSk1 (aa 837–894), whereas no interaction was detected between RhoA and this region of PKN (data not shown). The region corresponding to the second leucine zipper-like sequence and its immediate N-terminal region, which is conserved through evolution in vertebrates (16). Thus α-actinin binds most avidly to the region distinct from that which binds to RhoA. These results raise the possibility that PKN binds simultaneously to RhoA and α-actinin.

**Preparation of Actin Cytoskeletal Proteins**—Actin was purified from bovine aorta by the method of Feramisco and Burridge (11). Vinculin was purified from bovine aorta by the method of Koba-yama et al. (12). Caldesmon was partially purified from bovine aorta by the method of Abe et al. (13). Filamin, metavinculin, and talin were partially purified from bovine aorta as described (11).
FIG. 1. Schematic representation of the various expression constructs and results of their interactions in the two-hybrid system. The schematic whole structure of each protein is represented at the top of each figure, and the deletion mutants of each protein are aligned below. The numbers preceding and following each line denote the positions of the most terminal aa residue of each clone, which is represented by solid or open box. The interaction in the two-hybrid system was examined by a filter assay for β-galactosidase activity. “+++” and “+” indicate the development of blue color within 20 min and 24 h from initiation of the assay, respectively. “±” indicates the development of faint blue color after 24 h from initiation of assay, and “-” indicates no development of color within 24 h. Gal4bd and LexAbd indicate the DNA binding domain of Gal4 and LexA, respectively. Gal4ad and VP16ad indicate the transcription activation domain of Gal4 and VP16, respectively. A, human PKN.
ized, including skeletal, smooth, and non-muscle α-actinins, from various kinds of cells and tissues. The only recorded functional difference among these α-actinins is that binding of the non-muscle isoform to F-actin is inhibited by Ca²⁺, whereas binding of the muscle isoform is Ca²⁺-insensitive (18–21). In human, only one clone of the non-muscle cytoskeletal isoform (HuActNm, designated in Ref. 7), having strong sequence homology with HuActSk1 (89% similarity and 80% identity for pairwise comparison), has been isolated (22, 23). Then we tested whether PKN could bind to the region of HuActNm corresponding to the PKN-binding site of HuActSk1. As shown in Fig. 4A, PKN could also bind to spectrin-like repeat 3 domain of HuActNm, whereas the binding to the EF-hand-like domain of HuActNm was not detected in the absence of Ca²⁺. However, PKN could effectively bind to the EF-hand-like region of HuActNm in the presence of 1 mM Ca²⁺ (Fig. 4B). Although it is uncertain at present whether this Ca²⁺ dependence is retained in the binding between PKN and the full-length HuActNm, the Ca²⁺ dependence may be one of the reasons why the cDNA clone encoding the non-muscle type α-actinin was not isolated in the two-hybrid screening of “brain” cDNA library. Beggs et al. (7) compared the sequences of EF-hand-like regions of HuActSk1 with the EF-hand consensus of Kretsinger (24) and indicated that the first EF-hand-like region of HuActSk1 has only 11/16 matches with either an arginine or lysine at the Y position and that these peptides would probably not be able to coordinate Ca²⁺ binding properly. Our results support this estimation from a different point of view.

Specificity of the Interaction between PKN and α-Actinin—α-Actinin is a member of spectrin superfamily, including spectrin, dystrophin, and so on (17, 25, 26). Family members are characterized by the N-terminal actin-binding domain, central rod-shaped spectrin-like repeats, and the C-terminal EF-hand-like domain. α-Spectrin contains 21 rod-shaped repeats in the N-terminal to the EF-hand-like domain. The C terminus of α-spectrin is clearly related to α-actinin, and especially the repeat 20 of α-spectrin has extensive homology to the repeat 3 of α-actinin (27, 28), and the position of the repeat in each

**HuActSk1 (skeletal muscle type α-actinin)** (aa 423–894) was expressed as a fusion protein with VP16 transcription activation domain, and its interaction with the various deletion mutants of PKN expressed as fusion proteins with the LexA DNA binding domain was examined in the two-hybrid system. **LZ** indicates the leucine zipper-like motif. **BR** indicates the region rich in basic aa. **Solid box** indicates the bait construct. **B**, HuActSk1. The numbers (4, 10, and 25) on the right indicate the clone numbers isolated in the screening. The N-terminal region of PKN (aa 1–540; this region was designated as PKNN1) was expressed as a fusion protein with Gal4 DNA binding domain, and its interaction with the various deletion mutants of HuActSk1 expressed as a fusion protein with Gal4 activation domain or VP16 activation domain was examined in the two-hybrid system. **SR** indicates spectrin-like repeats. C, HuActNm (non-skeletal muscle type α-actinin). **SR** indicates spectrin-like repeats.
protein seems to be related to each other. Since PKN bound to the repeat 3 of α-actinin, we examined whether PKN can bind to the repeat 20 of α-spectrin. As shown in Fig. 5, in vitro binding between PKN and the repeat 20 of rat α-spectrin was not detected in the same condition in which PKN bound to the repeat 3 of α-actinin. These results indicate that PKN specifically binds to the spectrin-like repeat of α-actinin.

**Binding of PKN to α-Actinin in Vivo**—The interaction of α-actinin with PKN in vivo was examined by cotransfection experiment in COS7 cells (Fig. 6). An epitope-tagged α-actinin was generated by fusion of a 9-aa epitope from the influenza HA to the N terminus of clone 4 protein, enabling the selective immunoprecipitation of the tagged α-actinin polypeptide with anti-HA monoclonal antibody 12CA5 (29). This HA-tagged α-actinin contains the complete C-terminal region of α-actinin, whereas it lacks the N-terminal actin-binding domain. After co-expression of HA-tagged α-actinin with the full-coding region of PKN in COS7 cells, anti-HA immunoprecipitates contained substantially immunoreactive PKN. These results suggest that the C-terminal region of α-actinin can associate in vivo with PKN.

**PI4,5P2-dependent Binding between PKN and α-Actinin**—α-Actinin in vivo bound to various amounts of endogenous PI4,5P2, and the specific interaction between α-actinin and PI4,5P2 regulates the F-actin-gelating activity of α-actinin (30). This indicates that PI4,5P2 causes a conformational change in α-actinin. Exogenously added PI4,5P2 can bind to α-actinin strongly, and the binding is tight and stable (30). Then we examined the binding activity of PKN with α-actinin in the presence or absence of PI4,5P2. Since PI4,5P2 binding region resides in the actin-binding domain of α-actinin (14), in vitro translated full-length α-actinin containing actin-binding domain was used in this in vitro binding experiment (Fig. 1B).
Interestingly, the full-length α-actinin very weakly but specifically bound to PKN in the absence of PI4,5P2. However, addition of 10 μM PI4,5P2 stimulated the binding of the full-length α-actinin to PKN (Fig. 7A). Therefore PI4,5P2 appears to influence the conformation of α-actinin and discloses the partially cryptic binding region for PKN, although the other possibility cannot be ruled out that PI4,5P2 functions as a bridge between α-actinin and PKN. This binding activity was elevated with increased PI4,5P2 concentration up to 2.5–10 μM and then was lowered to 100 μM (Fig. 7B). This two-phase pattern of PI4,5P2 dependence was also reported in the binding of α-actinin with PKIN-kinase (31). Fukami et al. (30) reported that the effect of PI4,5P2 on gelating activity of α-actinin is increased up to 5–10 μM of PI4,5P2 and that a further increase in concentration of PI4,5P2 gives a reduction in gelating activity to the basal level due to the formation of large PI4,5P2 micelles. The two-phase pattern of PI4,5P2-dependent binding of α-actinin with PKN also may be explained by the same reason.

**Effects of α-Actinin on the PKN Kinase Activity.—** We investigated whether the binding of α-actinin to PKN directly altered PKN regulation or catalytic function. The purified α-actinin from bovine aorta neither activated PKN autophosphorylation nor affected PKN-catalyzed protein kinase C pseudosubstrate peptide phosphorylation when added at >100 molar excess to PKN (data not shown). When assayed in the presence of 10 μM PI4,5P2, peptide phosphorylation activity of PKN was stimulated ~1.5-fold; however, addition of α-actinin to this assay mixture slightly inhibited the phosphorylation activity toward the basal level. Thus, α-actinin does not seem to be a direct activator of PKN purified from the soluble fraction of rat testis in vitro.

**Phosphorylation of α-Actinin and Other Actin Cytoskeletal-associated Proteins by PKN.—** Since PKN bound to α-actinin, we tested whether α-actinin itself could be a substrate for PKN. In the absence of modifiers, PKN purified from rat testis did not phosphorylate α-actinin purified from bovine aorta. However, in the presence of 40 μM arachidonic acid, PKN phosphorylated purified α-actinin with a stoichiometry of ~0.02 mol of P per protein monomer by image quantitation (Fig. 8A). The bacterially expressed C-terminal region of α-actinin (amino acid 333–894) was not phosphorylated at all by PKN, but PKN phosphorylated the bacterially expressed N-terminal region of α-actinin (amino acid 1–332) that was lacking in the originally isolated clone 4 in the presence of arachidonic acid (data not shown), suggesting that phosphorylation of α-actinin by PKN occurred in the N-terminal region. We searched for PKN substrates among other actin cytoskeletal proteins, including filamin, metavinculin, vinculin, talin, caldesmon, and actin. Among them, caldesmon and G-actin were relatively preferred substrates for PKN. (The maximal phosphorylation by PKN per mol of protein subunit was estimated by image quantitation to be ~0.3 mol of P per mol of caldesmon and ~0.05 mol of P per mol of G-actin, respectively.) As shown in Fig. 8B, phosphorylation of G-actin and caldesmon was stimulated up to ~2-fold and >6-fold in the presence of arachidonic acid, respectively.

**DISCUSSION**

The actin cytoskeleton plays a critical role in a number of cellular processes including motility, chemotaxis, and cell division (32–35). Members of Rho family of small GTP-binding proteins have been implicated in the regulation. Rho promotes the formation of actin stress fibers and focal adhesions (36, 37), although the mechanism by which Rho mediates the effect on the actin cytoskeleton is unclear. Recently it has shown that Rho interacts physically and regulates the activity of PI(4)P5-kinase in mouse fibroblasts and thereby regulates the cellular levels of PI4,5P2 (38, 39). PI4,5P2 can regulate Rho kinase in mouse fibroblasts and thereby regulates the cellular processes including motility, chemotaxis, and cell division (32–35). Members of Rho family of small GTP-binding proteins have been implicated in the regulation. Rho promotes the formation of actin stress fibers and focal adhesions (36, 37), although the mechanism by which Rho mediates the effect on the actin cytoskeleton is unclear. Recently it has shown that Rho interacts physically and regulates the activity of PI(4)P5-kinase in mouse fibroblasts and thereby regulates the cellular levels of PI4,5P2 (38, 39). PI4,5P2 can regulate in vitro the interactions of a number of actin-binding proteins, such as α-actinin (30), profilin (40), gelsolin (41), cofilin (42), and p38 MAP kinase (43). It has also been shown that the decrease in PI4,5P2 bound to α-actinin and vinculin by treatment with platelet-derived growth factor correlates with the depolymerization of actin (44). Recently, Glima and Burridge (45) have reported that microinjection of antibodies against PI4,5P2 into Balb/c 3T3 cells inhibits assembly of stress fibers and focal adhesions by serum stimulation. One possibility is that PI4,5P2 synthesis could mediate some of the effects of Rho on the actin cytoskeleton (45). In our experiment, Rho directly
interacts with and activates PKN, and PKN could directly associate with α-actinin in PI4,5P2-dependent manner in vitro. On the other hand, phosphoinositides such as PI4,5P2 have been reported to affect directly the kinase activity of PKN, although the stoichiometries were low in our in vitro assay condition. Thus one might further speculate that PKN mediates the effects of Rho and phosphoinositides by phosphorylating these proteins, although it is not known whether these proteins are physiologically relevant substrates of PKN. Further investigation will be required to clarify the role of PKN in the cytoskeletal network.

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REFERENCES

1. Mukai, H., Kitagawa, M., Shibata, H., Takanaga, H., Mori, K., Shimakawa, M., Miyahara, M., Hiroo, K., and Ono, Y. (1994) Biochem. Biophys. Res. Commun. 204, 348–356
2. Mukai, H., and Ono, Y. (1994) Biochem. Biophys. Res. Commun. 199, 897–904
3. Landschulz, W. H., Johnson, P. F., and McKnight, S. L. (1988) Science 240, 1759–1764
4. Watanabe, G., Saito, Y., Madaule, P., Ishizaki, T., Mori, N., Mukai, H., Ono, Y., Kakizuka, A., and Narumiya, S. (1996) Science 271, 645–648
5. Amano, M., Mukai, H., Ono, Y., Chihara, K., Matsui, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) Science 271, 648–650
6. Mukai, H., Toshimori, M., Shibata, H., Kitagawa, M., Shimakawa, M., Miyahara, M., Sunakawa, H., and Ono, Y. (1996) J. Biol. Chem. 271, 9816–9822
7. Begg, A. H., Byers, T. J., Knoll, J. H. M., Boyce, F. M., Bruns, G. A. P., and Kunkel, L. M. (1992) J. Biol. Chem. 267, 9281–9288
8. Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K., and Nishizuka, Y. (1988) J. Biol. Chem. 263, 6927–6932
9. Laemmli, U. K. (1970) Nature 227, 680–685
10. Mommaerts, W. F. H. M. (1951) J. Biol. Chem. 208, 559
11. Feramisco, J. R., and Burridge, K. (1980) J. Biol. Chem. 255, 1194–1199
12. Kobayashi, R., and Tashima, Y. (1990) J. Muscle Res. Cell Motil. 11, 465–470
13. Abe, M., Takahashi, K., and Hiwada, K. (1990) J. Biochem. (Tokyo) 107, 507–509
14. Fukami, K., Sawada, N., Endo, T., and Takenawa, T. (1996) J. Biol. Chem. 271, 2646–2650
15. Shibata, H., Mukai, H., Inagaki, Y., Homma, Y., Kimura, K., Kaibuchi, K., Narumiya, S., and Ono, Y. (1996) FEBS Lett. 385, 221–224
16. Mukai, H., Mori, K., Takanaga, H., Kitagawa, M., Shibata, H., Shimakawa, M., Miyahara, M., and Ono, Y. (1995) Biochim. Biophys. Acta 1261, 296–300
17. Blanchard, A., Ohanian, V., and Critchley, D. (1989) J. Muscle Res. Cell Motil. 10, 280–289
18. Burridge, K., and Feramisco, J. R. (1981) Nature 294, 565–567
19. Bennett, J. P., Zaner, K. S., and Stossel, T. P. (1984) Biochemistry 23, 5081–5086
20. Duhaime, A. S., and Bamburg, J. R. (1984) Biochemistry 23, 1600–1608
21. Landon, F., Gaech, Y., Toustiu, H., and Olomuc, A. (1985) Eur. J. Biochem. 153, 231–237
22. Millake, D. B., Blanchard, A. D., Patel, B., and Critchley, D. R. (1989) Nucleic Acids Res. 17, 6725
23. Youssoufiou, H., McAfee, M., and Kwiatkowski, D. J. (1990) Am. J. Hum. Genet. 47, 62–71
24. Kretsinger, R. H. (1982) Ann. N. Y. Acad. Sci. 356, 14–19
25. Dubreuil, R. R. (1991) BioEssays 13, 219–226
26. Bennett, V. (1990) Physiol. Rev. 70, 1029–1065
27. Wasenius, V. M., Saraste, M., Salven, P., Eramaa, M., Holm, L., and Lehto, V. P. (1989) J. Cell Biol. 108, 79–93
28. Hong, W., and Boyle, D. (1989) J. Biol. Chem. 264, 12758–12764
29. Field, J., Nikawa, J., Broek, D., MacDonald, B., Rodgers, L., Wilson, I. A., Lerner, R. A., and Wigler, M. (1988) Mol. Cell. Biol. 8, 2159–2165
30. Fukami, K., Furuhashi, K., Inagaki, M., Endo, T., Hatanu, S., and Takenawa, T. (1992) Nature 359, 150–152
31. Shibasaki, F., Fukami, K., Fukui, Y., and Takenawa, T. (1994) Biochem. J. 302, 551–557
32. Bretscher, A. (1993) Curr. Opin. Cell Biol. 5, 653–660
33. Stossel, T. P. (1993) Science 260, 1086–1094
34. Lasa, E. J., and Hitt, A. L. (1992) Science 255, 955–964
35. Salmon, K. D. (1989) Curr. Opin. Cell Biol. 1, 541–547
36. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1992) Cell 70, 401–419
37. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
38. Ren, X. D., Bokoch, G. M., TraynorKaplan, A., Jenkins, G. H., Anderson, R. A., and Schwartz, M. A. (1996) Mol. Cell. Biol. 7, 435–442
39. Chong, L. D., TraynorKaplan, A., Bokoch, G. M., and Schwartz, M. A. (1994) Cell 70, 507–513
40. Lassam, I., and Lindberg, U. (1985) Nature 314, 472–474
41. Janmey, P., and Stossel, T. P. (1987) Nature 325, 362–364
42. Yonezawa, N., Homma, Y., Yahara, I., Sakai, H., and Nishida, E. (1991) J. Biol. Chem. 266, 17218–17221
43. Yu, F. X., Johnston, P. A., Sudhoff, T. C., and Yin, H. L. (1990) Science 250, 1413–1415
44. Fukami, K., Endo, T., Imamura, M., and Takenawa, T. (1994) J. Biol. Chem. 269, 1518–1522
45. Gilmore, A. and Burridge, K. (1996) Nature 381, 531–535
46. Palmer, R. H., Dekker, L. V., Woscholski, R., Le Good, J. A., Gigg, R., and Parker, P. J. (1995) J. Biol. Chem. 270, 22412–22416

FIG. 8. Phosphorylation of actin and actin-binding proteins by PKN. Phosphorylation was detected by an autoradiograph of SDS-PAGE. White arrowhead indicates the position of autophosphorylation of PKN. Molecular mass markers are indicated in kDa. A. 100 ng of purified α-actinin was incubated with assay mixture without (lane 1) or with (lanes 2 and 3) PKN purified from rat testis in the absence (lanes 1 and 2) or presence (lane 3) of 40 μM arachidonic acid. Black arrowhead indicates the position of α-actinin. B. 100 ng of purified G-actin (lane 1–3) or caldesmon (lane 4–6) was incubated in the assay mixture without (lanes 1 and 4) or with (lanes 2, 3, 5, and 6) PKN purified from rat testis in the absence (lanes 2 and 5) or presence (lanes 3 and 6) of 40 μM arachidonic acid. Black arrow indicates the position of caldesmon, and black arrowhead indicates the position of G-actin.