Characterization of a Protein Kinase that Phosphorylates Serine 189 of the Mitogen-activated Protein Kinase Homolog ERK3*

(Received for publication, January 25, 1996, and in revised form, March 4, 1996)

Mangeng Chenqi, Erzhen Zhen, Megan J. Robinson, Doug Ebertt, Elizabeth Goldsmith, and Melanie H. Cobb‡

From the University of Texas Southwestern Medical Center, Departments of Pharmacology and Biochemistry, Dallas, Texas 75235-9041

A novel protein kinase activity present in nuclear and cytosolic extracts has been identified and partially purified as a consequence of its tight binding to and phosphorylation of the extracellular signal-regulated protein kinase (ERK) 3. This novel protein kinase is inactivated by treatment with phosphoprotein phosphatase 2A. The ERK3 protein kinase was immunologically distinct from mitogen-activated protein (MAP) kinase/ERK (MEK) 1 and 2 which phosphorylate the ERK3-related MAP kinases ERK1 and ERK2. This ERK3 kinase phosphorylated a single site on ERK3, Ser189, comparable to Thr183, one of the two activating phosphorylation sites of ERK2. To test the specificity of the ERK3 kinase, mutants of ERK3 and ERK2 were made in which the phosphorylated residues were exchanged. The double mutant SI89T,G191Y ERK3, in which the phosphorylated residues from ERK2 replaced the comparable residues in ERK3, was phosphorylated by the ERK3 kinase but only on threonine. The ERK3 kinase did not phosphorylate ERK2 or ERK2 mutants. These findings indicate that although the ERK3 kinase is highly specific for ERK3, it does not recognize tyrosine, a feature that distinguishes it from MEKs that phosphorylate other ERK/MAP kinase family members.

The ERK/MAP kinase pathway is stimulated by numerous hormones and growth factors and its activation is associated with increased proliferative and differentiated functions of cells (1–5). The importance of intracellular processes thought to be regulated by the MAP kinases has focused attention on understanding the control of this pathway. The MAP kinase kinases, also known as MAP/ERK kinases or MEK1 and MEK2, originally discovered by Ahn and Krebs, are dual-specificity protein kinases known to activate the MAP kinases ERK1 and ERK2 in a highly selective manner (6–8). The MAP kinases, on the other hand, are pleiotropic, phosphorylating many substrates throughout the cell (reviewed in Ref. 3). Kinase cascades containing a MEK and an ERK/MAP kinase are present in multiple pathways in yeast and have been reterated in mammalian cells (1, 9). Although mechanisms regulating the similar, but parallel mammalian pathways are less well characterized, the activation of a multipotential ERK/MAP kinase by a highly specific MEK is the common feature of all the related cascades.

ERK1 and ERK2 are phosphorylated on two sites separated by a single residue in the phosphorylation lip at the mouth of their active sites (10, 11). Phosphorylation of both Tyr185 and Thr183 on ERK2 and comparable residues on ERK1, catalyzed by the dual specificity protein kinases, MEK1 and MEK2, is required for high activity (10, 12–15). Because of their exquisite specificity, MEK1 and MEK2 are not able to phosphorylate other MAP kinase-related enzymes such as Jun-N-terminal kinase/stress-activated protein kinase (JNK/SAPK) or p38 MAP kinase, even though the phosphorylation sites are in comparable positions in the sequence (1).

Much less is known about the protein kinase ERK3. It was cloned in the same cDNA library screen as ERK1 and ERK2 (16) and has greater sequence identity to ERK1 and ERK2 than do the JNK/SAPKs or p38 MAP kinase. However, three important features distinguish ERK3 from the other family members. First, it lacks the tyrosine phosphorylation site that is absolutely conserved among those other related kinases. Second, ERK3 is a constitutively nuclear protein kinase (17). Third, it apparently has a very restricted substrate specificity, because it does not phosphorylate any of the known MAP kinase substrates. As no ERK3 substrates are known, its regulation has been difficult to define.

To understand more about the regulation of ERK3, we have examined the phosphorylation of ERK3 by MEK family members, and find that ERK3 is a poor substrate for MEK1, MEK2, MKK4, and MEK5 (18). We have identified a novel protein kinase activity in nuclear and cytosolic extracts that binds very tightly to the catalytic domain of ERK3 and phosphorylates it selectively. This ERK3 kinase phosphorylates a single site on ERK3, Ser189, which is comparable to Thr183, one of the activating phosphorylation sites of ERK2.

MATERIALS AND METHODS

Cell Culture—PC12 and 293 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Prior to mitogen stimulation, PC12 and 293 cells were maintained in Dulbecco’s modified Eagle’s medium without serum for 4 h and 18–20 h, respectively, then treated with nerve growth factor (NGF, 100 nm, 15 min), epidermal growth factor (100 ng/ml, 5 min), or phorbol ester (100 nm, 20 min, Sigma) as indicated.

Bacterial Expression of ERK3, ERK2, and ERK Mutants—Histidine-tagged and glutathione S-transferase (GST) fusion proteins of ERK3,
ERK3, without the C-terminal domain (ERK3ΔCt), and the following ERK3 mutants, S189A ERK3, S189E ERK3, D171A ERK3, and S189T,G191Y ERK3, were expressed and purified as described elsewhere (15, 17). Histidine-tagged ERK2 and K52R ERK2 were expressed as described previously (15). T183S ERK2 and the double mutant T183S,Y185G ERK2 were constructed using methods described earlier (15). The mutant Y185G ERK2 was made using the Chameleon double-stranded DNA mutagenesis kit (Stratagene, La Jolla, CA).

Subcellular Fractionation—Extracts from rabbit muscle and from NGF-stimulated PC12 cells were prepared according to the method of Seger et al. (14) for analysis of ERK1 and ERK2 phosphorylating activities. PC12 cells and 293 cells were fractionated into cytosolic and nuclear fractions as described by Dignam et al. (19) with modifications described previously (17). ERK3 was highly enriched in the nuclear fraction.

Purification of the Kinase Activity That Phosphorylates ERK3—Extracts were fractionated by chromatography on Q Sepharose, S Sepharose, Mono Q, or Mono S. Protein kinase activities in fractions eluted previously (17). ERK3 was highly enriched in the nuclear fraction. Fractions 20–42, which contained the major peak of activity phosphorylating ERK3, without the C-terminal domain (ERK3ΔCt), and the following ERK3 mutants, S189A ERK3, S189E ERK3, D171A ERK3, and S189T,G191Y ERK3, were expressed and purified as described elsewhere (15, 17). Histidine-tagged ERK2 and K52R ERK2 were expressed as described previously (15). T183S ERK2 and the double mutant T183S,Y185G ERK2 were constructed using methods described earlier (15). The mutant Y185G ERK2 was made using the Chameleon double-stranded DNA mutagenesis kit (Stratagene, La Jolla, CA).

Subcellular Fractionation—Extracts from rabbit muscle and from NGF-stimulated PC12 cells were prepared according to the method of Seger et al. (14) for analysis of ERK1 and ERK2 phosphorylating activities. PC12 cells and 293 cells were fractionated into cytosolic and nuclear fractions as described by Dignam et al. (19) with modifications described previously (17). ERK3 was highly enriched in the nuclear fraction.

Purification of the Kinase Activity That Phosphorylates ERK3—Extracts were fractionated by chromatography on Q Sepharose, S Sepharose, Mono Q, or Mono S. Protein kinase activities in fractions eluted previously (17). The mutant Y185G ERK2 was made using the Chameleon double-stranded DNA mutagenesis kit (Stratagene, La Jolla, CA).

Subcellular Fractionation—Extracts from rabbit muscle and from NGF-stimulated PC12 cells were prepared according to the method of Seger et al. (14) for analysis of ERK1 and ERK2 phosphorylating activities. PC12 cells and 293 cells were fractionated into cytosolic and nuclear fractions as described by Dignam et al. (19) with modifications described previously (17). ERK3 was highly enriched in the nuclear fraction.

Purification of the Kinase Activity That Phosphorylates ERK3—Extracts were fractionated by chromatography on Q Sepharose, S Sepharose, Mono Q, or Mono S. Protein kinase activities in fractions eluted previously (17). The mutant Y185G ERK2 was made using the Chameleon double-stranded DNA mutagenesis kit (Stratagene, La Jolla, CA).

Subcellular Fractionation—Extracts from rabbit muscle and from NGF-stimulated PC12 cells were prepared according to the method of Seger et al. (14) for analysis of ERK1 and ERK2 phosphorylating activities. PC12 cells and 293 cells were fractionated into cytosolic and nuclear fractions as described by Dignam et al. (19) with modifications described previously (17). ERK3 was highly enriched in the nuclear fraction.

Purification of the Kinase Activity That Phosphorylates ERK3—Extracts were fractionated by chromatography on Q Sepharose, S Sepharose, Mono Q, or Mono S. Protein kinase activities in fractions eluted previously (17). ERK3 was highly enriched in the nuclear fraction. Fractions 20–42, which contained the major peak of activity phosphorylating ERK3, without the C-terminal domain (ERK3ΔCt), and the following ERK3 mutants, S189A ERK3, S189E ERK3, D171A ERK3, and S189T,G191Y ERK3, were expressed and purified as described elsewhere (15, 17). Histidine-tagged ERK2 and K52R ERK2 were expressed as described previously (15). T183S ERK2 and the double mutant T183S,Y185G ERK2 were constructed using methods described earlier (15). The mutant Y185G ERK2 was made using the Chameleon double-stranded DNA mutagenesis kit (Stratagene, La Jolla, CA).
The activities in Q Sepharose fractions 32–40 from rabbit muscle extracts that phosphorylated ERK3 were not further characterized, but may have been due to protein kinase C, because protein kinase C eluted in this region of the gradient and was found to phosphorylate ERK3 in vitro (data not shown).

The ERK3 Kinase Binds Tightly to the ERK3 Catalytic Domain—The ERK3 kinase could be distinguished from other protein kinases based on its tight binding to the catalytic domain of ERK3 (Fig. 2A). GST-ERK3 or GST-ERK3ΔCt bound to glutathione-agarose beads was incubated with the ERK3 kinase activity from rabbit muscle that had first been partially purified on Q Sepharose and S Sepharose. The bound protein kinase activity was measured by its ability to phosphorylate GST-ERK3 or GST-ERK3ΔCt on the beads (Fig. 2A). The ERK3 kinase activity was not eluted with concentrations of NaCl up to 1 M, with 1% Triton X-100 or with 1 M MgCl2. The ERK3 kinase did not bind to GST-ERK2. The tight association of the ERK3 kinase with ERK3 has a parallel in the binding of JNK/SAPK to c-Jun. The binding requires only the kinase domain of ERK3 as deleting the C-terminal domain did not eliminate binding to the ERK3 kinase. Because protein kinase C was able to phosphorylate ERK3 in vitro, we tested its capacity to bind to GST-ERK3. Neither rat brain protein kinase C nor recombinant protein kinase Cα bound to GST-ERK3 (data not shown), indicating that protein kinase C is not the ERK3 kinase.

Because it was difficult to elute the ERK3 kinase from the GST-ERK3 on glutathione-agarose beads, we ascertained if the activity that was bound to GST-ERK3 on beads would phosphorylate exogenously added ERK3. As shown in Fig. 2B, the ERK3 kinase bound to GST-ERK3ΔCt on beads phosphorylated not only bound GST-ERK3ΔCt but also added His10-ERK3ΔCt, which is different in size from GST-ERK3ΔCt. It seemed unlikely that the ERK3 kinase was ERK3 itself because ERK3 autophosphorylation is intramolecular not intermolecular (17). However, it was possible that the protein bound to ERK3 was not an ERK3 kinase but an activator that accelerated ERK3 autophosphorylation. To demonstrate that the ERK3 kinase was not ERK3 or an activator of ERK3 autophosphorylation, a catalytically defective mutant, D171A ERK3, that neither autophosphorylates nor is phosphorylated by wild type ERK3 in vitro (17) was tested as a substrate for the ERK3 kinase. The ERK3 kinase bound tightly to GST-D171A ERK3ΔCt and it phosphorylated GST-D171A ERK3ΔCt or added His10×D171A ERK3ΔCt as well as the wild type protein (Fig. 2, A and B), indicating that the protein bound to GST-ERK3 is an ERK3 protein kinase. Phosphoamino acid analysis showed that the ERK3 kinase phosphorylated ERK3 on serine (Fig. 2C).

Subcellular Localization of the Kinase That Phosphorylates ERK3—Because ERK3 is primarily in the nucleus (17), the subcellular distribution of the ERK3 kinase was examined. Cytosolic and nuclear extracts from multiple cell types were tested for the ERK3 kinase activity by binding to GST-ERK3ΔCt on beads and assay of the bound material by phosphorylation of GST-ERK3ΔCt. Activity that bound tightly to ERK3ΔCt and phosphorylated it was found in both cytosolic and nuclear extracts (Fig. 2).
and nuclear extracts of PC12 and 293 cells (Fig. 3), and in extracts of other cell lines such as NIH3T3, Cos, and Jurkat T cells (data not shown). The distribution of the activity between cytosolic and nuclear fractions was not changed by extracellular stimuli including epidermal growth factor, NGF, or phorbol ester. These agents activate ERK1 and ERK2 and cause their translocation to the nucleus (27), but may not be physiological regulators of ERK3.

**Regulation of the ERK3 Kinase**—To test the possibility that the ERK3 kinase, like MEK1 and MEK2, is regulated by phosphorylation, its activity was measured before and after treatment with PP2A. The initial rate of ERK3 phosphorylation by the ERK3 kinase was reduced 85–90% by a 45-min treatment with PP2A (Fig. 4). Preincubation of PP2A with okadaic acid blocked the inactivation of the ERK3 kinase, demonstrating that loss of activity is due to dephosphorylation of the ERK3 kinase preparation.

Sites Phosphorylated on ERK3 by the ERK3 Kinase—We determined previously that ERK3 autophosphorylated in vitro and was phosphorylated in intact cells on Ser\textsuperscript{189} (17), the residue comparable to Thr\textsuperscript{183}, one of the two activating phosphorylation sites in ERK2 (Fig. 5A). The stoichiometry of phosphorylation of ERK3 by the ERK3 kinase was 0.7 mol phosphate/mol ERK3, consistent with a single site of phosphorylation. In comparison, incorporation due to autophosphorylation was never greater than 0.04 mol of phosphate/mol of ERK3 even after overnight incubation. To determine if Ser\textsuperscript{189} was the site phosphorylated by the ERK3 kinase, this residue was mutated to alanine (S189A ERK3) or glutamic acid (S189E ERK3) (Fig. 5B). The ERK3 kinase bound to GST-S189A ERK3 and GST-S189E ERK3 on beads as determined by its ability to phosphorylate GST-ERK3\textsuperscript{ΔCt} and mutants are as described in Fig. 2. Top, an autoradiogram showing 32P incorporation into GST-ERK3\textsuperscript{ΔCt} and GST-S189A ERK3 and GST-S189E ERK3\textsuperscript{ΔCt} bound to glutathione-agarose beads were incubated with Mono S fractions containing the ERK3 kinase activity from rabbit muscle. After the beads were washed as described under Material and Methods, the bound ERK3 kinase activity was measured by its ability to phosphorylate GST-ERK3\textsuperscript{ΔCt} and mutants are as described in Fig. 2. Top, an autoradiogram showing 32P incorporation into GST-ERK3\textsuperscript{ΔCt} and mutants. Bottom, Coomassie Blue stain of GST-ERK3\textsuperscript{ΔCt} and mutants. C, phosphoamino acid analysis of phosphorylated GST-ERK3\textsuperscript{ΔCt} and GST-S189A ERK3\textsuperscript{ΔCt}. Spots close to the origin were partially hydrolyzed phosphorylated products. The positions of the phosphoamino acid standards are indicated.
support the conclusion that Ser\textsuperscript{189} is the site phosphorylated by the ERK3 kinase. To confirm that the ERK3 kinase phosphorylated the same site on ERK3 that was phosphorylated in intact cells, tryptic phosphopeptide maps of ERK3 and ERK3\textsubscript{Dc} phosphorylated by the ERK3 kinase were compared to a map of ERK3 phosphorylated in intact cells. Each map revealed a major phosphopeptide (Fig. 6\textsuperscript{A–C}) that migrated as a single spot if tryptic phosphopeptides from ERK3 phosphorylated \textit{in vitro} were mixed with those from ERK3 phosphorylated in intact cells (Fig. 6\textsuperscript{D}). This major phosphopeptide was absent from S189A ERK3 phosphorylated by the ERK3 kinase (Fig. 6\textsuperscript{E}). The incorporation into S189A ERK3 phosphorylated by the ERK3 kinase (Fig. 6\textsuperscript{E}). The incorporation into S189A ERK3 was about 1–2\% of that incorporated into wild type ERK3. The addition of tryptic phosphopeptides from phosphorylated wild type ERK3 to those from phosphorylated S189A ERK3 restored the major phosphopeptide (Fig. 6\textsuperscript{F}). These data indicate that Ser\textsuperscript{189} of ERK3, the site phosphorylated in intact cells, is the major site phosphorylated by the ERK3 kinase.

Specificity of the ERK3 Kinase—The specificity of the ERK3 kinase was characterized. For these experiments, the ERK3 kinase was partially purified from rabbit muscle and then affinity purified on GST-ERK3\textsubscript{Dc} bound to glutathione-agarose beads. Similar results were obtained with the ERK3 kinase prior to binding to GST-ERK3\textsubscript{Dc} on beads. His\textsubscript{10}\textsubscript{ERK3\textsubscript{Dc}} was phosphorylated by the ERK3 kinase but less efficiently than bound GST-ERK3\textsubscript{Dc} (Fig. 7\textsuperscript{A}). In ERK2, Thr\textsuperscript{183} and Tyr\textsuperscript{185} are the activating phosphorylation sites. These residues were interchanged with Ser\textsuperscript{189} and Gly\textsuperscript{191}, the comparable residues in ERK3. The double mutant His\textsubscript{10}S189T, G191Y ERK3\textsubscript{Dc} was phosphorylated by the ERK3 kinase primarily on threonine and to a lesser extent on serine but not on tyrosine (Fig. 7, A and B). Thus, unlike MEK1 and MEK2, the ERK3 kinase did not phosphorylate tyrosine in the phosphorylation site at a position equivalent to Tyr\textsuperscript{185} of ERK2. Further, the ERK3 kinase did not phosphorylate His\textsubscript{6}K52R ERK2 or the ERK3-like mutants T183S ERK2, Y185G ERK2, and T183S,Y185G ERK2 (Fig. 7A).

**FIG. 6.** Tryptic phosphopeptide mapping of phosphorylated ERK3. Autoradiograms of tryptic phosphopeptide maps of A, ERK3 phosphorylated by the ERK3 kinase; B, ERK3\textsubscript{Dc} phosphorylated by the ERK3 kinase; C, ERK3 phosphorylated in intact cells; D, mixture of ERK3 phosphorylated by the ERK3 kinase and in intact cells; E, S189A ERK3 phosphorylated by the ERK3 kinase; F, mixture of ERK3 and S189A ERK3 phosphorylated by the ERK3 kinase. Equal counts/min were loaded onto each plate for mapping.

**FIG. 7.** Specificity of the ERK3 kinase. A, the ERK3 kinase bound to GST-ERK3\textsubscript{Dc} on glutathione-agarose beads, phosphorylated both GST-ERK3\textsubscript{Dc} and added His\textsubscript{6}ERK3\textsubscript{Dc} or His\textsubscript{6}S189T, G191Y ERK3\textsubscript{Dc}, but not added His\textsubscript{6}ERK2 mutants (all at 30 \(\mu\)g/ml). An autoradiogram is shown. The mobilities of GST-ERK3\textsubscript{Dc}, His\textsubscript{6} ERK3\textsubscript{Dc} and His\textsubscript{6}ERK2 are indicated. Added His\textsubscript{6}ERK2 mutants T183S ERK2, Y185G ERK2, and T183T,Y185G ERK2 displayed auto-phosphorylation rates higher than ERK3 and different from each other. K52R ERK2 lacked the ability to autophosphorylate. B, phosphoamino acid analysis of phosphorylated S189T,G191Y ERK3\textsubscript{Dc}. Spots near the origin were partially hydrolyzed phosphorylated products. The phosphoamino acid standards are indicated.

**DISCUSSION**

A concept that has developed from studies in yeast and mammalian cells is that of the MAP kinase module (1, 9, 28, 29). A MAP kinase module is a three-kinase cascade including a MAP kinase or ERK, a MEK, and an activator of MEK, MEK kinase or MEKK. Thus far, studies indicate that the MEK component has the greatest substrate specificity of enzymes in the cascade (1, 14, 18). The known MEK family members se-
known MEKs, the ERK3 kinase will not phosphorylate tyrosine when tyrosine is introduced into the appropriate position of the phosphorylation lip of ERK3. Importantly, this ERK3 kinase phosphorylates Ser189 of ERK3, the site phosphorylated in intact cells. Thus, the ERK3 kinase identified here may be the upstream regulator of ERK3. From a primarily cytosolic location when inactive, ERK1 and ERK2 are translocated in part to the nucleus upon activation, while the activating MEKs are believed to remain cytosolic (27, 34). In contrast, ERK3 is found primarily in the nucleus and the ERK3 kinase is present in both cytosolic and nuclear extracts. This suggests a regulatory mechanism in which the ERK3 kinase may receive signals from membrane bound or cytoplasmic cues and shuttle into the nucleus to phosphorylate ERK3 (Fig. 8).

Acknowledgments—We thank David Robbins (University of California, San Francisco) for his input during the early stages of this work, Clark García and Peiqun Wu for preparation of some of the bacterial proteins and cell extracts, Alphonsus Dang for MEK1 and MEK2 immunoprecipitation, and J. O. Hicks for preparation of the manuscript.

REFERENCES
1. Cobb, M. H., and Goldsmith, E. (1995) J. Biol. Chem. 270, 14843–14846
2. Blumer, K. J., and Johnson, G. L. (1994) Trends Biochem. Sci. 19, 236–239
3. Davis, R. J. (1993) J. Biol. Chem. 268, 14553–14556
4. Robbins, D. J., Zhen, E., Cheng, M., Xu, S., Ebert, D., and Cobb, M. H. (1994) Adv Cancer Res 63, 93–116
5. Sontag, E., Fedorov, S., Kamibayashi, C., Robbins, D., Cobb, M., and Mummy, M. (1993) Cell 75, 187–197
6. Ahn, N. G., Seger, R., Bratlien, R. L., Diltz, C. D., Tonks, N. K., and Krebs, E. G. (1991) J. Biol. Chem. 266, 4202–4227
7. Crews, C. L., Alessandrini, A., and Erikson, R. (1992) Science 258, 478–480
8. Zheng, C.-F., and Guan, K.-L. (1994) Adv Cancer Res 63, 299–307
9. Boulton, T. G., Yancopoulos, G. D., Gregory, J. S., Slaughter, C., Moomaw, C., and Cobb, M. H. (1993) J. Biol. Chem. 268, 5097–5106
10. Boulton, T. G., Nye, S. H., Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H., and Yancopoulos, G. D. (1993) Cell 65, 663–673
11. Zheng, C.-F., and Guan, K.-L. (1993) Science 260, 701–705
12. Knighton, D. R., Xuong, N.-H., Hsu, J., and Cobb, M. H. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 1021–1025
13. Kikkawa, U., Minakuchi, R., Takai, Y., and Nishizuka, Y. (1983) J. Biol. Chem. 258, 14373–14381
14. Seger, R., Ahn, N. G., Posada, J., Munar, E. S., Jensen, A. M., Cooper, J. A., Robbins, D. J., and Cobb, M. H. (1994) Cell 79, 299–308
15. Robbins, D. J., and Cobb, M. H. (1992) J. Biol. Chem. 267, 14373–14381
16. Robbins, D. J., Zhen, E., Owaki, H., Vanderbilt, C., Ebert, D., Geppert, T. D., and Cobb, M. H. (1993) J. Biol. Chem. 268, 5097–5106
17. Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H., and Yancopoulos, G. D. (1993) Cell 65, 663–673
18. Zheng, C.-F., and Guan, K.-L. (1994) Adv Cancer Res 63, 299–307
19. Boulton, T. G., Yancopoulos, G. D., Gregory, J. S., Slaughter, C., Moomaw, C., Hsu, J., and Cobb, M. H. (1990) Science 249, 64–67
20. Boulton, T. G., Yancopoulos, G. D., Gregory, J. S., Slaughter, C., Moomaw, C., Hsu, J., and Cobb, M. H. (1990) Science 249, 64–67
21. Boulton, T. G., Vanderbilt, C., Ahn, N. G., Posada, J., Munar, E. S., Jensen, A. M., Cooper, J. A., Robbins, D. J., and Cobb, M. H. (1994) Cell 79, 299–308
22. Robbins, D. J., and Cobb, M. H. (1992) J. Biol. Chem. 267, 14373–14381
23. Kikkawa, U., Minakuchi, R., Takai, Y., and Nishizuka, Y. (1983) J. Biol. Chem. 258, 14373–14381
24. Kikkawa, U., Minakuchi, R., Takai, Y., and Nishizuka, Y. (1983) J. Biol. Chem. 258, 14373–14381
25. Wu, J., Michel, H., Rossomando, A., Posada, J., Munar, E. S., Jensen, A. M., Cooper, J. A., Robbins, D. J., and Cobb, M. H. (1994) Cell 79, 299–308
26. Wu, J., Harrison, J. K., Ten Eyck, L. F., Ten Eyck, L. F., Ashford, V. A., Xuong, N.-H., Hsu, J., and Cobb, M. H. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 1021–1025
27. Knighton, D. R., Xuong, N.-H., Hsu, J., and Cobb, M. H. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 1021–1025