Activation of a C-terminal Transcriptional Activation Domain of ERK5 by Autophosphorylation

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ERK5 plays a crucial role in many biological processes by regulating transcription. ERK5 has a large C-terminal-half that contains a transcriptional activation domain. However, it has remained unclear how its transcriptional activation activity is regulated. Here, we show that the activated kinase activity of ERK5 is required for the C-terminal-half to enhance the AP-1 activity, and that the activated ERK5 undergoes autophosphorylation on its most C-terminal region. Changing these phosphorylatable threonine and serine residues to unphosphorylatable alanines significantly reduces the transcriptional activation activity of ERK5. Moreover, phosphomimetic mutants of the C-terminal-half of ERK5 without an N-terminal kinase domain are shown to be able to enhance the AP-1 activity in fibroblastic cells. These results reveal the role of the stimulus-induced ERK5 autophosphorylation in regulation of gene expression.

The mitogen-activated protein kinase (MAPK)2 cascades play an essential role in transducing extracellular signals to cytoplasmic and nuclear effectors, and regulate a variety of cellular functions, including cell proliferation, differentiation, and stress responses. The MAPK cascades are composed of three classes of protein kinases: MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). Each MAPK is activated by specific members of MAPKK, which are activated by MAPKKK (1–13). Four subfamily members of the MAPK family have been relatively well studied; extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38, and ERK5. ERK5 is activated by oxidative stress, hyperosmolarity, and several growth factors including epidermal growth factor and nerve growth factor (14–18). MEK5 is the upstream MAPKK that specifically phosphorylates and activates ERK5 (16, 19). It has been shown that ERK5 directly phosphorylates and activates several transcription factors including c-Myc, Sap1a, c-Fos, Fra-1, and MEF2 family members (15, 17, 20–22). ERK5 knock-out mice die in embryonic stages with angiogenic failure and cardiovascular defects, and in the adult stages, ERK5 is required for endothelial cell survival and maintenance of blood vascular integrity (23–26). The targeted deletion of MEK5 also causes early embryonic death with cardiovascular defects (27). Moreover, knockdown studies in Xenopus have shown that ERK5 and MEK5 play an essential role in neural differentiation (28).

ERK5 has a large, unique C-terminal-half not found in other MAPK family members (19, 29). Because of this unique 400-amino acid extension, ERK5 is also called big MAP kinase 1 (BMEK1). The C-terminal-half of ERK5 has two proline-rich domains and a bipartite NLS, and shares no high homology with other proteins (19, 30, 31). Kasler et al. (32) demonstrated that the C-terminal-half of ERK5 has a potent transcriptional activation domain. It was subsequently shown that the ERK5 C-terminal-half is essential for transactivation of MEF2C (30). Moreover, a recent study has shown that ERK5 has two transactivation domains in its C-terminal region (33). However, the molecular mechanisms regulating the transcriptional activation activity of the ERK5 C-terminal region have remained elucidated.

Here we show that the activated kinase activity of ERK5 is required for the C-terminal-half to enhance the transactivation activity of AP-1, and that the activated ERK5 undergoes autophosphorylation on its C-terminal-half. Furthermore, our results show that phosphorylation of the C-terminal-half of ERK5 is necessary for the enhancement of the transactivation activity of AP-1. These results reveal the importance of the ERK5 autophosphorylation in ERK5 signaling, and thus identify a novel role for the C-terminal-half of ERK5 in MAPK signal transduction mechanisms.

EXPERIMENTAL PROCEDURES

Plasmids—pcDNA3 HA1, pcDNA Myc1, and pcDNA3 FLAG1 have been described previously (22, 31). ERK5 AEF, ERK5 KM, MEK5 WT, and MEK5D have been described previously (15, 17, 31). The mutagenesis of ERK5 was performed using the QuikChange site-directed mutagenesis kit (Stratagene) as follows: ERK5C S421A (Ser421 with Ala), ERK5C S433A (Ser433 with Ala), ERK5C S496A (Ser496 with Ala), ERK5C S697A (Ser697 with Ala), ERK5C S721A (Ser721 with Ala), ERK5C T723A (Thr723 with Ala), ERK5C S793A (Ser793 with Ala), ERK5C S412A (Ser412 with Ala), and ERK5C S723A (Ser723 with Ala).

The abbreviations used are: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; WT, wild type; HA, hemagglutinin; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; MEKK, MEK kinase.
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(Thr223 and Ser760 with Ala), ERK5C T723A/S766A (Thr723 and Ser766 with Ala), ERK5C T723A/S784A (Thr723 and Ser766, Ser784 with Ala), ERK5C T723A/S766A (Thr723, Ser726, Ser760, and Ser766 with Ala), ERK5C 3A (Ser760, Ser764, and Ser766 with Ala). ERK5 1A (Thr723 with Ala), ERK5 4A (Thr723, Ser760, and Ser766 with Ala), ERK5C 1D (Thr723 with Asp), ERK5C 4D (Thr723, Ser760, Ser764, and Ser766 with Ala). ERK5 N and MEK5D were transfected in COS7 cells. After 24 h, the cells were harvested with lysis buffer B (20 mM Tris-Cl (pH 7.5), 12.5 mM β-glycerophosphate, 150 mM NaCl, 2 mM EGTA, 10 mM NaF, 1.5 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 17 µg/ml aprotinin, 1 mM vanadate, 1% Triton-100). The supernatants of the cell lysates were incubated with the anti-FLAG M2 affinity gel (Sigma) for 2 h at 4 °C. Then the resin was washed twice with wash buffer (50 mM Tris-Cl, 150 mM NaCl (pH 7.4)). The resin was incubated with five volumes of a solution containing 100 mg/ml FLAG peptide in elution buffer (20 mM HEPES (pH 7.5)) for 30 min at 4 °C. The bound FLAG fusion protein was eluted by competitive elution.

Phosphatase Treatment—Cells were lysed in phosphatase buffer (50 mM Tris-HCl (pH 7.0), 0.1 mM EDTA, 5 mM dithiothreitol, 0.01% Brij 35, 2 mM MnCl2) and incubated with 40 units of purified λ-protein phosphatase (New England Biolabs) for 45 min at 30 °C. Then samples were treated with Laemmli’s sample buffer, separated by SDS-PAGE, and then analyzed by immunoblotting.

RESULTS AND DISCUSSION

Both the N-terminal- and the C-terminal-halves of ERK5 Are Required for the Activation of AP-1 Activity—To investigate a role of the unique C-terminal-half of ERK5 in its signaling mechanism, we made two deletion constructs of ERK5: ERK5N (residues 1–407) and ERK5C (residues 401–806) (Fig. 1A). ERK5N has a kinase catalytic domain and a Thr–Glu–Tyr (TEY) activation motif, whereas ERK5C has an NLS. We previously reported that activation of the ERK5 pathway is able to activate the transactivation activity of AP-1 and that the N-terminal-half of ERK5 alone is not enough for maximal activation of the transactivation activity of AP-1 (22). Thus we examined whether the C-terminal-half of ERK5 is required for maximal activation of AP-1 activity. We expressed full-length ERK5 or ERK5N or ERK5C with or without MEK5D, a constitutively active form of MEK5 (17), in NIH 3T3 cells, and performed a reporter assay measuring the transcriptional activity of AP-1. Expression of ERK5 or MEK5D alone had negligible effects, but co-expression of ERK5 and MEK5D enhanced the AP-1 activity by 21-fold over the control (Fig. 1B). Co-expression of ERK5N and MEK5D enhanced it by 6-fold over the control, and co-expression of ERK5C and MEK5D by 2-fold (Fig. 1B). However, co-expression of ERK5N and ERK5C with MEK5D increased the AP-1 activity by 20-fold, the extent being nearly the same as that in the case of co-expression of full-length ERK5 and MEK5D (Fig. 1B). These results suggest that both the N-terminal- and C-terminal-halves of ERK5 are required for maximal activation of AP-1 activity and that co-expression of ERK5N and ERK5C is able to mimic full-length ERK5 expression in this transcriptional activation.

It has been shown that the kinase activity of ERK5 is necessary for the activation of AP-1 activity induced by full-length ERK5 (22). We then examined whether the kinase activity of ERK5N was required for the enhancement of AP-1 activity by co-expression of ERK5N and ERK5C. We made two mutants of ERK5N, ERK5N AEF, and ERK5N KM. ERK5N AEF is an unphosphorylatable mutant, in which threonine and tyrosine residues at the activating phosphorylation sites (TEY) are replaced by alanine and phenylalanine, respectively. ERK5 KM
is a kinase-deficient mutant, in which Lys\textsuperscript{84} is replaced by methionine. The reporter assay showed that ERK5N AEF or ERK5N KM did not stimulate the AP-1 activity when co-expressed with ERK5C and MEK5D (Fig. 1C). These results indicate clearly that the kinase activity of ERK5N is required for the enhancement of AP-1 activity.

The N-terminal-half of ERK5 Phosphorylates the C-terminal-half of ERK5—We found that several mobility-shifted bands (slowly migrating bands) of ERK5C in SDS-polyacrylamide gel electrophoresis appeared when co-expressed with ERK5N and MEK5D (Fig. 2A). All the shifted bands disappeared when incubated with \( \beta \)-protein phosphatase \textit{in vitro} (Fig. 2B), indicating that the shifted bands of ERK5C are caused by phosphorylation of ERK5C. It is likely that there are multiple phosphorylation states in ERK5C. It has previously been shown that ERK5 is able to undergo autophosphorylation on its own C-terminal domain at several sites (35, 36). It is therefore likely that activated ERK5N phosphorylates ERK5C in our assay system. In fact, the shifted bands of ERK5C did not appear when co-expressed with ERK5N AEF or ERK5N KM in the presence of MEK5D or co-expressed with ERK5N WT in the absence of MEK5D (Fig. 2A). Then, we examined the substrate specificity of ERK5N against ERK5C in comparison with that of ERK5N against c-Fos. Under conditions of low amounts of ERK5N and MEK5D (15–0.5 ng), ERK5C, but not c-Fos, was phosphorylated by ERK5N (Fig. 2C). This result suggests that ERK5N phosphorylates ERK5C more efficiently than c-Fos. These results together with the results shown in Fig. 1 suggest the possibility that activated ERK5N-mediated phosphorylation of ERK5C is required for the maximal activity of ERK5C to enhance AP-1 activity.

**FIGURE 1.** Both the N-terminal- and C-terminal-halves of ERK5 are required for the activation of AP-1 activity. A, mouse ERK5 and ERK5 deletion mutants used in these experiments. B and C, indicated expression plasmids were transfected with pAP1-Luc, the reporter plasmid, in NIH 3T3 cells. After 24 h, the cells were harvested and then lysates were subjected to the luciferase assay.

**FIGURE 2.** The N-terminal-half of ERK5 phosphorylates the C-terminal-half of ERK5. A, COS7 cells were transfected with the indicated expression plasmids. After 24 h, the cells were harvested with lysis buffer A. Then the cell lysates were separated by SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting with anti-Myc antibody. B, COS7 cells were transfected with the indicated expression plasmids. After 24 h, the cells were harvested with lysis buffer and treated with or without \( \alpha \)-protein phosphatase (400 units) for 45 min at 30 °C. Then the cell lysates were treated with Laemmli’s sample buffer, separated by SDS-PAGE and analyzed by immunoblotting with anti-Myc antibody. C, COS7 cells were transfected with the indicated expression plasmids. After 24 h, the cells were harvested with lysis buffer A, and then the cell lysates were separated by SDS-PAGE and analyzed by immunoblotting with anti-HA and anti-FLAG antibody. The solid arrowhead indicates a nonspecific band recognized by the anti-HA antibody.
C-terminal-half of ERK5, we investigated the phosphorylation sites in ERK5C, which are responsible for the mobility shifts. There are five putative MAPK phosphorylation sites (S/TP sites) in the C-terminal region; Ser$^{421}$, Ser$^{433}$, Ser$^{697}$, Thr$^{723}$, and Ser$^{793}$. Moreover, activated human recombinant ERK5 is shown to undergo autophosphorylation on several residues in its C-terminal region in vitro (35), among which there are two non-proline-directed serines; Ser$^{496}$ and Ser$^{721}$. Then we gen-
FIGURE 3. Phosphorylatable sites in the C-terminal-half of ERK5. A, schematic representation of a mouse C-terminal-half of ERK5 and its mutants, in which serine or threonine residues were replaced by alanine in these experiments (upper). COS7 cells were transfected with the indicated expression plasmids. After 24 h, the cells were harvested with lysis buffer A, and then all lysates were separated by SDS-PAGE and analyzed by immunoblotting with anti-Myc antibody (middle, lower). B, schematic representation of ERK5 C-terminal-half constructs used in these experiments (left). COS7 cells were transfected with the indicated expression plasmids. After 24 h, the cells were harvested with lysis buffer A, and then the all lysates were separated by SDS-PAGE and analyzed by immunoblotting with anti-Myc antibody (right). C, schematic representation of the C-terminal-half of ERK5 and its sequence alignment (human, mouse, and Xenopus) (upper). COS7 cells were transfected with the indicated expression plasmids. After 24 h, the cells were harvested with lysis buffer A, and then all lysates were separated by SDS-PAGE and analyzed by immunoblotting with anti-Myc antibody (lower). D, activated ERK5N protein was purified as described under “Experimental Procedures.” COS7 cells were co-expressed with ERK5N and MEK5D (Fig. 3B, right), suggesting that there may be one or several cooperative phosphorylation sites in the C-terminal region, residues 740–806. In this region, there are five serine residues that are not proline-directed but are conserved among Xenopus, mouse and human ERK5 (Fig. 3C, upper). Therefore, we made several mutant forms of ERK5 in which, in addition to Thr<sup>723</sup>, one or several of these conserved serine residues were replaced by alanines. As shown in Fig. 3C, when consecutive three serines (Ser<sup>760</sup>, Ser<sup>764</sup>, and Ser<sup>766</sup>) and Thr<sup>723</sup> were mutated, the mobility shift did not take place, suggesting that Thr<sup>723</sup>, Ser<sup>760</sup>, Ser<sup>764</sup>, and Ser<sup>766</sup> are the sites responsible for the ERK5C mobility shifts induced by ERK5N-mediated phosphorylation.

Next, we performed in vitro kinase assay using separately purified ERK5N and ERK5C proteins.
The obtained result showed that ERK5C protein was phosphorylated in the presence of ERK5N protein, but not in the absence of ERK5N protein (Fig. 3D). Moreover, the mutant ERK5C, ERK5C 4A (T723, S760, S764, S766A) was much less phosphorylated than wild-type ERK5C (Fig. 3D). These results suggest that ERK5N directly phosphorylates ERK5C.

We made an additional mutant, ERK5C S760, S764, S766A, and termed it ERK5C 3A. ERK5C T723A and ERK5C T723, S760, S764, S766A were also termed ERK5C 1A and ERK5C 4A, respectively (Fig. 4A). We also made phosphomimetic mutants of ERK5C, ERK5C T723D and ERK5C T723, S760, S764, S766D and termed them ERK5C 1D and ERK5C 4D, respectively (Fig. 4A). We then expressed each of them with ERK5N in the absence or presence of MEK5D. As already shown in Fig. 3C, ERK5C 4A did not show mobility shift, whereas ERK5C 1A (T723A) and 3A (S760, S764, S766A) did show mobility-shifted bands except for the most slowly migrating band when co-expressed with ERK5N and MEK5D (Fig. 4B, left). In contrast, both ERK5C 1D (T723D) and ERK5C 4D (T723, S760, S764, S766D) migrated more slowly than ERK5C WT in the absence of MEK5D (Fig. 4B, right). In the case of ERK5C 4D, co-expression of MEK5D did not further shift its migrating position (Fig. 4B, right). These results also support our idea that phosphorylation of these four sites is responsible for the ERK5C mobility shifts. Next, we made four mutant forms of full length ERK5, in which one (T723) or all of the four sites were mutated to alanines or aspartic acids (ERK5 1A, ERK5 4A, ERK5 1D and ERK5 4D), and expressed each of them with or without MEK5D. Their behaviors in electrophoresis with or without MEK5D were almost identical to those of the corresponding ERK5C mutants (Fig. 4C, see Fig. 4B), except that ERK5 4A and ERK5 4D still showed a small mobility shift when co-expressed with MEK5D. As the mobility-shifted band of ERK5 4A is similar to that of ERK5 KM, which does not undergo autophosphorylation, this mobility shift could be caused by MEK5D-dependent phosphorylation of ERK5 on the TEY site in the N-terminal-half of ERK5. All these results are consistent with our idea that the four sites are ERK5 autophosphorylation sites.

FIGURE 5. Phosphorylation of the C-terminal-half of ERK5 is necessary for the maximal enhancement of its transcriptional activation activity. A and B, NIH 3T3 cells were transfected with the indicated expression plasmids and pAP-1-Luc. After 24 h, the cells were harvested, and then lysates were subjected to the luciferase assay. C, NIH 3T3 cells were transfected with the indicated expression plasmids and MEF2-Luc. After 24 h, the cells were harvested, and then lysates were subjected to the luciferase assay. D, hypothetical model illustrating two roles of the activated kinase activity of ERK5: phosphorylation of transcription factors and autophosphorylation on the C-terminal region to activate its transcriptional activation domain. ERK5 1A (T723A), ERK5 3A (S760, S764, S766A), ERK5 4A (T723, S760, S764, S766A), ERK5 1D (T723D), and ERK5 4D (T723, S760, S764, S766D).
Next, we examined when and how the C-terminal region of endogenous ERK5 is phosphorylated upon biological stimulation. The obtained results showed that endogenous ERK5 exhibited several mobility-shifted bands upon stimulation with EGF or H$_2$O$_2$ and the most slowly migrating band appeared at 10 min after EGF treatment (30 µM) or at 2 min after H$_2$O$_2$ treatment (100 mM) (Fig. 4D, upper and lower). An exogenously expressed ERK5 WT with MEK5D exhibited the mobility-shifted band that corresponded to the most slowly migrating band of endogenous ERK5, whereas an exogenously expressed ERK5 KM with MEK5D did not exhibit this band (Fig. 4C). Therefore, the most slowly migrating band of endogenous ERK5 results from the phosphorylation of the C-terminal region of ERK5. These results taken together suggest that the C-terminal region of ERK5 is phosphorylated in response to biological stimulation.

We then examined the kinase activity of each full-length mutant form of ERK5. ERK5 1A, 4A, 1D, and 4D were all phosphorylated on the TEY activation sites by MEK5D, and their activated form exhibited nearly the same kinase activity toward myelin basic protein as compared with wild-type ERK5 (Fig. 4E).

**Phosphorylation of the C-terminal-half of ERK5 Is Necessary for the Maximal Enhancement of Its Transcriptional Activation Activity**—To investigate the role of the phosphorylation of the C-terminal-half of ERK5 in its transcriptional activation activity, we examined the effect of expression of several ERK5 or ERK5C mutants on the transactivation activity of AP-1. In the reporter assay, expression of ERK5 1A or ERK5 4A with MEK5D enhanced the AP-1 activity less than wild-type ERK5; ERK5 4A was less potent than ERK5 1A (Fig. 5A, left). As no significant differences were found in the kinase activity among ERK5 WT, ERK5 1A, and ERK5 4A (Fig. 4D), the lower enhancement of AP-1 activity with ERK5 1A or ERK5 4A would result from the partial or complete lack of the autophosphorylation on the C-terminal region. In the reporter assay in which each ERK5C mutant was co-expressed with ERK5N and MEK5D, ERK5C 1A, ERK5C 3A, or ERK5C 4A enhanced the AP-1 activity less than wild-type ERK5C; ERK5C 4A was much less potent than others (Fig. 5A, right), suggesting also the importance of the phosphorylation of these sites. In addition, expression of ERK5C 4D alone without ERK5N and MEK5D enhanced the AP-1 activity by 4-fold over the control (Fig. 5B). These unexpected findings suggest that the phosphorylated C-terminal-half of ERK5 by itself without kinase activity is able to perform part of ERK5 function. However, our observation that ERK5 4A or ERK5C 4A still had the activity to enhance the AP-1 activity also suggests that the C-terminal region of ERK5 should have a basal transcriptional activation activity, which could be enhanced by its phosphorylation.

ERK5 is also known to enhance the transcriptional activity of MEF2. We then investigated whether autophosphorylation of ERK5 is involved in the enhancement of MEF2 activity. Co-expression of wild-type ERK5 and MEK5D enhanced the MEF2 activity by 3-fold over the control, whereas co-expression of ERK5 4A and MEK5D enhanced the MEF2 activity to lesser extent (Fig. 5C, left). Moreover, expression of ERK5C 4D enhanced the MEF2 activity by 1.7-fold over the control (Fig. 5C, right). Therefore, these results suggest that autophosphorylation of the C-terminal-half of ERK5 is also important for the activation of MEF2 activity.

Our results show firstly that the activated kinase activity of ERK5 is required for the C-terminal-half to maximally enhance the AP-1 activity and that the activated ERK5 undergoes autophosphorylation on its most C-terminal region. Subsequent analysis demonstrates that the C-terminal region phosphorylation is required for the maximal activation of the transcriptional activation activity of the ERK5 C-terminal region. Based on these results, we propose a hypothetical model, in which the activated kinase activity of ERK5 has two roles in gene expression; one role is to activate transcription factors by direct phosphorylation, and another is to activate the transcriptional activation activity of the C-terminal-half by autophosphorylation, presumably, intramolecular autophosphorylation (Fig. 5D). Two regions in the C-terminal region of ERK5 are autophosphorylated; T227 and a region containing S760, S764, and S766. These two important phosphorylation regions may additively enhance transcription and seem equally important for the transcriptional activation activity (see Fig. 5A). How autophosphorylation enhances the transcriptional activation activity of the C-terminal-half should be elucidated in future studies.

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REFERENCES
1. Sturgill, T. W., and Wu, J. (1991) *Biochim. Biophys. Acta* 1092, 350–357
2. Ahi, N. G., Seger, R., and Krebs, E. G. (1992) *Curr. Opin. Cell Biol.* 4, 992–999
3. Nishida, E., and Gotoh, Y. (1993) *Trends Biochem. Sci.* 18, 128–131
4. Marshall, C. J. (1995) *Cell* 80, 179–185
5. Krebs, M. (1996) *Curr. Opin. Cell Biol.* 8, 205–215
6. Robinson, M. J., and Cobb, M. H. (1997) *Curr. Opin. Cell Biol.* 9, 180–186
7. Schaeffer, H. J., and Weber, M. J. (1999) *Mol. Cell. Biol.* 19, 2435–2444
8. Davis, R. I. (2000) *Cell* 103, 239–252
9. Chang, L., and Karin, M. (2001) *Nature* 410, 37–40
10. Chen, Z., Gibson, T. B., Robinson, F., Silvestro, L., Pearson, G., Xu, B., Wright, A., Vanderbilt, C., and Cobb, M. H. (2001) *Chem. Rev.* 101, 2449–2476
11. Kyriakis, J. M., and Avruch, J. (2001) *Physiol. Rev.* 81, 807–869
12. Johnson, G. L., and Lapadat, R. (2002) *Science* 298, 1911–1912
13. Pouyssegur, J., Volmat, V., and Lenormand, P. (2002) *Biochem. Pharmacol.* 64, 755–763
14. Abe, J., Kusuhara, M., Ulevitch, R. J., Berk, B. C., and Lee, J. D. (1996) *J. Biol. Chem.* 271, 16586–16590
15. Kato, Y., Kivrachen, Y. V., Tapping, R. I., Han, J., Ulevitch, R. J., and Lee, J. D. (1997) *EMBO J.* 16, 7054–7066
16. Kato, Y., Tapping, R. I., Huang, S., Watson, M. H., Ulevitch, R. J., and Lee, J. D. (1998) *Nature* 395, 713–716
17. Kamakura, S., Moriguchi, T., and Nishida, E. (1999) *J. Biol. Chem.* 274, 26563–26571
18. Fukuwara, S., Marinissen, M. J., Chiariello, M., and Gutkind, J. S. (2000) *J. Biol. Chem.* 275, 21730–21736
19. Zhou, G., Bao, Z. Q., and Dixon, J. E. (1995) *J. Biol. Chem.* 270, 12665–12669
20. English, J. M., Pearson, G., Baer, R., and Cobb, M. H. (1998) *J. Biol. Chem.* 273, 3854–3860
21. Yang, C. C., Ornatsky, O. I., McDermott, J. C., Cruz, T. F., and Prody, C. A. (1998) *Nucleic Acids Res.* 26, 4771–4777
22. Terasawa, K., Okazaki, K., and Nishida, E. (2003) *Genes Cells* 8, 263–273
23. Regan, C. P., Li, W., Boucher, D. M., Spatz, S., Su, M. S., and Kuida, K.
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(2002) Proc. Natl. Acad. Sci. U. S. A. 99, 9248–9253
24. Sohn, S. J., Sarvis, B. K., Cado, D., and Winoto, A. (2002) J. Biol. Chem. 277, 43344–43351
25. Yan, L., Carr, J., Ashby, P. R., Murry-Tait, V., Thompson, C., and Arthur, J. S. (2003) BMC. Dev. Biol. 3, 11
26. Hayashi, M., Kim, S. W., Imanaka-Yoshida, K., Yoshida, T., Abel, E. D., Elce, R., Yang, Y., Ulevitch, R. J., and Lee, J. D. (2004) J. Clin. Investig. 113, 1138–1148
27. Wang, X., Merritt, A. J., Seyfried, J., Guo, C., Papadakis, E. S., Finegan, K. G., Kayahara, M., Dixon, J., Boot-Handford, R. P., Cartwright, E. J., Mayer, U., and Tournier, C. (2005) Mol. Cell. Biol. 25, 336–345
28. Nishimoto, S., Kusakabe, M., and Nishida, E. (2005) EMBO Rep. 6, 2754–2759
29. Lee, J. D., Ulevitch, R. J., and Han, J. (1995) Biochem. Biophys. Res. Commun. 213, 715–724
30. Yan, C., Luo, H., Lee, J. D., Abe, J., and Berk, B. C. (2001) J. Biol. Chem. 276, 10870–10878
31. Kondoh, K., Terasawa, K., Morimoto, H., and Nishida, E. (2006) Mol. Cell. Biol. 26, 1679–1690
32. Kasler, H. G., Victoria, J., Duramad, O., and Winoto, A. (2000) Mol. Cell. Biol. 20, 382–838
33. Akaike, M., Che, W., Marmarosh, N. L., Ohta, S., Osawa, M., Ding, B., Berk, B. C., Yan, C., and Abe, J. (2004) Mol. Cell. Biol. 24, 8691–8704
34. Woronicz, J. D., Lina, A., Calnan, B. J., Szychowski, S., Cheng, L., and Winoto, A. (1995) Mol. Cell. Biol. 15, 6364–6376
35. Mody, N., Campbell, D. G., Morrice, N., Peggie, M., and Cohen, P. (2003) Biochem. J. 372, 567–575
36. Buschbeck, M., and Ullrich, A. (2005) J. Biol. Chem. 280, 2659–2667