Characterization of the Structure and Function of a Novel MAP Kinase Kinase (MKK6)*

(Received for publication, November 6, 1995, and in revised form, December 5, 1995)

Jiuhuai Han†, Jiing-Dwan Lee, Yong J. Jang, Zhuangjie Li, Lili Feng, and Richard J. Ulevitch

From the Department of Immunology, The Scripps Research Institute, La Jolla, California 92037

Mitogen-activated protein (MAP) kinases require dual phosphorylation on threonine and tyrosine residues in order to gain enzymatic activity. This activation is carried out by a family of enzymes known as MAP kinase kinases (MKKs or MEKs). It appears that there are at least four subgroups in this family: MEK1/MEK2 subgroup that activates ERK1/ERK2, MEK5 that activates ERK5/BMK1, MKK3 that activates p38, and MKK4 that activates p38 and J un kinase. Here we describe the characteristics of a new MKK termed MKK6. The clones we isolated encode two splice isoforms of human MKK6 comprised of 278 and 334 amino acids, respectively, and one murine MKK6 with 237 amino acids. Sequence information derived from cDNA cloning indicated that MKK6 is most closely related to MKK3. The functional data revealed from co-transfection assays suggests that MKK6, like MKK3, selectively phosphorylates p38. Unlike the previously described MKks (or MEks), MKK6 exists in a variety of alternatively spliced isoforms with distinct patterns of tissue expression. This suggests novel mechanisms regulating activation and/or function of various forms of MKK6.

The signal transduction pathways that utilize mitogen-activated protein (MAP) kinases have an important role in a variety of cellular responses including growth factor-induced proliferation, gene expression, and compensation for alterations in the extracellular milieu induced by heat shock, UV light, increased extracellular osmolarity etc. (1–4). Four MAP kinase pathways have been defined in yeast (5); these pathways are functionally independent and are regulated by distinct protein kinase cascades. Each pathway results in activation of a separate MAPK by a unique MAP kinase (MKK or MEK). It is now clear that higher eukaryotes also have distinct MAP kinase pathways; such pathways are comprised of kinase cascades leading to the activation of discrete MKks and MAPKs (5–9). Specifically four subgroups of MAPKs have been identified (5): the ERKs (extracellular signal-regulated kinase (10, 11), J un kinases (cJ un amino-terminal kinases (J NK) or stress-activated protein kinase (SAPK)) (12, 13), p38 MAP kinase (14), and BMK1/Erk5 (15, 16). The signal transduction pathway leading to p38 activation is related, in part, to a pathway in yeast leading to activation of a MAP kinase known as Hog1p. To date the activation of this yeast pathway has been shown to occur principally in response to increased extracellular osmolarity (17). Recently Saito and colleagues (18, 19) have defined two distinct pathways leading to Hog1p activation in Saccharomyces cerevisiae.

In mammalian cells p38, the Hog1p homologue is activated by multiple stimuli acting through different receptors. For example Lee et al. (20) showed that p38 is involved in bacterial endotoxin (lipopolysaccharide)-induced cytokine production through the use of pharmacologic inhibitors that are specific for p38 (20). p38 is also activated by other bacterial components, proinflammatory cytokines, and physical-chemical changes in the extracellular milieu (21).

There have been several distinct MKks/MEks identified in mammalian cells; one type, termed MEK1/MEK2, does not phosphorylate or activate p38 or J NK while in contrast is a strong activator of ERK1/ERK2 (7, 9, 22). In contrast, two other MKks known as MKK3 and MKK4 activate p38 but not ERK (6, 7); MKK4 (also known as SEK1/J NKK1) also activates J NK/SAPK (6–8). MKK3 and MKK4 are most closely related to PBS2p, the upstream activator of Hog1p (6, 7).

p38 and J NK are often activated in parallel (21) but independent activation of p38 also has been observed (23). Simultaneous activation of ERK and p38 also occurs when cells are exposed to a stimulus such as lipopolysaccharide or increased extracellular osmolarity (14, 24). Unlike yeast MAP kinase pathways which appear to operate independently, cross-talk seems to occur between the mammalian MAP kinase pathways (6, 7, 25, 26). Emerging data suggest that the MAPK signal transduction pathways in mammalian cells are much more complex than homologous systems in yeast. One level of such complexity might exist at the level of the MAPK kinases. Indeed multiple activators of p38 or J NK can be detected by fractionation of the fibroblasts activated by hyperosmolar media (27). Thus there is a need to identify all of the p38 upstream activators in order to fully understand the regulation of p38. To isolate additional MKks which function as activators of p38, we have employed a polymerase chain reaction (PCR)-based strategy with degenerate oligonucleotides based on conserved kinase domains present in the known members of the MKK family. This approach resulted in the isolation of two human cDNAs and one murine cDNA encoding closely related proteins of the MKK family. These clones are the different splice forms of one gene which we have designated MKK6, which is selectively expressed in different tissues. Amino acid sequence comparison revealed that the proteins encoded by these cDNAs are most closely related to MKK3, and co-transfection studies show that MKK6, like MKK3, activates p38 but fails to activate...
either ERK or JNK. In this article we also present data showing that unlike MKK4, MKK3 and MKK6 are not regulated by Rac or Cdc42.

**EXPERIMENTAL PROCEDURES**

cDNA Cloning—Degenerate oligonucleotides (AARYNTGAYGTYGNGNT and ATNCKYTCGNGGCATRANGG) were designed using published information from the conserved kinase subdomain of known MKKs (7, 8, 22); these oligonucleotides were employed as PCR primers to isolate fragments of MKK-related cDNAs from a human placenta cDNA library. Comparison of the sequence of 48 clones with the degenerate primers revealed that one fragment was specific to the MKK family of enzymes. In the overlapping regions MKK6 is nearly identical to human MKK6 except in the 5' end fragment (37-1560) that have nearly identical sequence. These clones are human fetal brain cDNAs that were sequenced by the Washington University-Merck EST project. The clone R15387 was requested and completely sequenced as noted above. The sequence data indicated these two clones are identical and contained a complete open reading frame. One positive clone was isolated from the murine jejunum library by screening a ZAPII murine jejunum library (Stratagene, La Jolla, CA) and a ZAPALI murine jejunum library (provided by Dr. Z. Chen, The Scripps Research Institute, La Jolla, CA). Two positive clones were obtained from the human library after screening 2 x 10^8 phage. DNA sequencing of both strands of each clone was performed by the Microchemistry Core Facility at The Scripps Research Institute using a model 373A automated sequencer (Applied Biosystems, Foster City, CA). The sequence indicated these two clones are identical and both contain a complete open reading frame. One positive clone was isolated from the murine library after screening 3 x 10^9 plaque-forming units. The sequencing of this clone was done as noted above. The murine clone shows strong homology to the human clones except at the 5' end. Nucleotide sequence comparison of the 5' end sequence of the murine clone with the GenBank Data base reveals four sets of nucleotide sequences (GenBank accession number H80816, H06765, T66783, and R15387) that have nearly identical sequence. These clones are human fetal brain cDNAs that were sequenced by the Washington University-Merck EST project. The clone R15387 was requested and completely sequenced as noted above.

Northern Blot Analysis—An RNA tissue blot was purchased from Clontech (San Francisco, CA). The blot contains 2 μg of poly(A) RNA isolated from different human tissues, fractionated by denaturing agarose gel and transferred onto a nylon membrane. The blot was hybridized to a probe that was prepared by labeling the 5' end fragment (−404 to −199 bp) of MKK6 with 32P dATP by random priming. The blot was stripped by incubation for 2 min at 100°C in water and reprobed with a probe corresponding to the 5' end sequence (−150 to −199 bp) of MKK6b/6c. The blot was stripped again and reprobed with a probe containing the coding region and 3' untranslated region (−87 to −550) of MKK6. Hybridization was performed overnight at 50°C using 50% formamide, 5 x SSPE (1 x SSPE, 150 mM NaCl, 15 mM sodium citrate, pH 7.2), 5 x Denhard'ts, 1% SDS, and 200 μg/ml single-stranded fish sperm. The blot was washed twice with 1 x SSC (1 x SSC, 150 mM NaCl, 10 mM NaH2PO4, 1 mM EDTA, pH 7.2), 0.1% SDS, and 1 mM EDTA, and finally with 0.1 x SSC, 0.1% SDS, 1 mM EDTA at 65°C prior to autoradiography.

cDNA Constructs—The A, E, or M mutants of MKK6 were created by the substitution of Ser153 and Thr155 with Ala or Glu and Lys with Met using a PCR-based procedure as described (28). The MKK6 cDNA and the mutants were cloned into the expression vector pCDNA3 (Invitrogen, San Diego, CA) with the HA epitope tag added so that the protein contained an HA-tag in the amino-terminal region. The cloning sites were HindII and KpnI. The HA-tag sequence is YPYDVPDYA(Y/P)DVPDYASGPFV(P)DVAAYAA. The tag was added by a PCR recombination as described (28). The sequence of all constructs was confirmed by DNA sequencing in the The Scripps Research Institute Sequencing Center (courtesy of S. Drayna or J. K. 1CDNA encoding a FLAG-epitope tag were prepared using a pCDNA3 vector (29). The constructs of Rac1(661L), Cdc42(661L), RhoA(636L), Ras(636L), Raf(222W), and Mhc-tagged Erk1 have been described (29). The truncated form of MKK1, here termed MKK1Δ, is in pCMV5 (25). The HA-tagged MKK3, MKK4(5EK/1J)NKK1 were prepared as described (30). The recombinant GST-ATF2 was made by replacing the amino-terminal fragment from the bacterial expression vector pGEX-2T vector (Pharmacia, Uppsala, Sweden). The recombinant GST-ATF2 protein contains 1-109 amino-terminal amino acids of ATF2.

Proteins—GST-ATF2 was purified by affinity chromatography on GSH-agarose (Sigma) as described (30). His-tagged p38 was purified by the nickel-chelate column as described (31). Myelin basic protein (MBP) was from Sigma.

**Transient Expression of Various cDNAs—**COS-7 cells were main-
about 80% identical to MKK3 and 40% to MKK4. We believe that the cDNAs for MKK6 and MKK6b contain full-coding regions because both clones contain in-frame stop codons 5’ upstream of the first ATG sequence. The first in-frame ATG of MKK6 has been designated as position 1. Since it is the first ATG codon and the typical Kozak sequence, it should be the authentic start site for MKK6. The first ATG found in MKK6b is at position –168. Although not a typical Kozak sequence, translation of MKK6b mRNA most likely begins at this position. The murine clone (GenBank U93066, Fig. 1D) which is closely related to MKK6b has the latter ATG sequence but not an equivalent ATG at position 1. Thus we predict that MKK6 contains 278 amino acids while MKK6b contains 334 amino acid residues. Of interest are differences between MKK6 and MKK6b in DNA sequence before position –154; this may be due to differential splicing. A dendrogram created by progressive pairwise alignment comparison of the MKK family is shown in Fig. 1C.

When we compared the sequence of the murine cDNA clone we isolated with the sequence of human MKK6 or MKK6b we noted that the murine clone has a 62-base pair deletion covering the region from residues 16 to 77. Due to this deletion, the ATG at position 122 is most likely to be the starting codon. We predict that this murine clone contains 237 amino acids. Although at the DNA level, this murine clone is most closely related to human MKK6b, it has a completely different 5’ sequence beginning with position –219 and a unique 3’ sequence starting from position 1056 when compared with MKK6b. This may result from differential splicing, although additional studies are required to firmly establish this. On the basis of these differences we term this murine cDNA, murine MKK6c. The sequence of murine MKK6c is shown in Fig. 1D. Further investigation is required to determine whether a comparable form exists in human. Amino acid sequence comparison reveals that the murine MKK6c is 97.6% identical to human MKK6b in the overlapping region. Conservation of the amino acid sequence between species has been found in other MKKs and may indicate the importance of this family proteins (9, 22).

Tissue Distribution of MKK6—Because of the observed differences in nucleotide sequences in the 5’- and 3’- region of the MKK6 cDNA clones we wondered if there is tissue-specific expression of alternatively spliced forms. To do this we made specific probes based upon unique sequences in the 5’- region of MKK6 and MKK6b, and probed blots containing poly(A)+ RNA (Fig. 2). This experiment revealed a high level of expression of MKK6 mRNA with size of 1.7 kilobase pairs in skeletal muscle (Fig. 2A). The probe encompassing the specific 5’- end region of MKK6b detected multiple mRNA bands with the size of 1.8, 2.4, 4.5, and 11 kilobase pairs. The 11-kilobase pair band only was noted in skeletal muscle and pancreas. We suspect that the multiple bands represent additional differential splicing forms of MKK6. The MKK6 mRNA containing the sequence we used in this probe are enriched in heart, skeletal muscle, pancreas, and liver and detectable in brain, placenta, and lung (Fig. 2B). These data suggest that tissue-specific splicing occurs and may play a role in the control of MKK6 expression. Northern blot analysis of tissues for expression of splicing occurs and may play a role in the control of MKK6 muscle, pancreas, and liver and detectable in brain, placenta, 11-kilobase pairs. The 11-kilobase pair band only was noted in skeletal muscle and pancreas. We predict that MKK6 contains 278 amino acids while MKK6b contains 334 amino acid residues. Of interest are differences between MKK6 and MKK6b in DNA sequence before position –154; this may be due to differential splicing. A dendrogram created by progressive pairwise alignment comparison of the MKK family is shown in Fig. 1C.

When we compared the sequence of the murine cDNA clone we isolated with the sequence of human MKK6 or MKK6b we noted that the murine clone has a 62-base pair deletion covering the region from residues 16 to 77. Due to this deletion, the ATG at position 122 is most likely to be the starting codon. We predict that this murine clone contains 237 amino acids. Although at the DNA level, this murine clone is most closely related to human MKK6b, it has a completely different 5’ sequence beginning with position –219 and a unique 3’ sequence starting from position 1056 when compared with MKK6b. This may result from differential splicing, although additional studies are required to firmly establish this. On the basis of these differences we term this murine cDNA, murine MKK6c. The sequence of murine MKK6c is shown in Fig. 1D. Further investigation is required to determine whether a comparable form exists in human. Amino acid sequence comparison reveals that the murine MKK6c is 97.6% identical to human MKK6b in the overlapping region. Conservation of the amino acid sequence between species has been found in other MKKs and may indicate the importance of this family proteins (9, 22).

Tissue Distribution of MKK6—Because of the observed differences in nucleotide sequences in the 5’- and 3’- region of the MKK6 cDNA clones we wondered if there is tissue-specific expression of alternatively spliced forms. To do this we made specific probes based upon unique sequences in the 5’- region of MKK6 and MKK6b, and probed blots containing poly(A)+ RNA (Fig. 2). This experiment revealed a high level of expression of MKK6 mRNA with size of 1.7 kilobase pairs in skeletal muscle (Fig. 2A). The probe encompassing the specific 5’-end region of MKK6b detected multiple mRNA bands with the size of 1.8, 2.4, 4.5, and 11 kilobase pairs. The 11-kilobase pair band only was noted in skeletal muscle and pancreas. We suspect that the multiple bands represent additional differential splicing forms of MKK6. The MKK6 mRNA containing the sequence we used in this probe are enriched in heart, skeletal muscle, pancreas, and liver and detectable in brain, placenta, and lung (Fig. 2B). These data suggest that tissue-specific splicing occurs and may play a role in the control of MKK6 expression. Northern blot analysis of tissues for expression of MKK6s with a probe encompassing the coding region which is most similar in all MKK6 clones is shown in Fig. 2C. No additional bands were noted with this probe; transcripts of MKK6 gene are most abundant in the skeletal muscle.

Enzymatic Activity of MAPK Kinases—We next investigated one aspect of the substrate specificity of MKK6 by asking if members of the MAP kinase family were activated by this enzyme. To do this we coexpressed MKK6 or MKK6b in COS-7 cells by transient transfection together with epitope-tagged forms of p38, JNK1, or ERK1. At the same time we compared the activities of MKK3 and MKK4 with MKK6. When specificities toward ERK1 were examined we used MBP as a substrate for ERK1 or p38 (Fig. 3A). In contrast, ATF2 was used as a substrate for JNK1 and p38 in a parallel set of studies (Fig. 3B). In this latter set of studies cotransfection with MKK6b was not performed (Fig. 3B). The enzyme activity of p38, JNK1, or ERK1 were determined by immunokinase assay after immunoprecipitation with the anti-epitope antibody. The overexpression of MKK6b in COS-7 cells caused increased activity of p38 (Fig. 3, A and B). In contrast, the co-expression of MKK6 does not enhance JNK1 (Fig. 3B) or ERK1 activity (Fig. 3A). The activation of p38 by MKK6 was similar to that of MKK3. The similar substrate specificity of MKK3 and MKK6b is consistent with the high homology between these two enzymes. In these studies we noticed that the migration of JNK1-phosphorylated GST-ATF2 is slightly slower than p38-phosphorylated GST-ATF2. This suggests differences in ATF2 phosphorylation by JNK1 and p38. Further studies are underway to verify this. Sequence comparison of MKK6 with other MKKs suggested that Ser151 and Thr155 may be phosphorylation sites required for enzymatic activity. To investigate this we modified the MKK6 cDNA by replacing Ser151 and Thr155 with Ala (A mutant) or Glu (E mutant). An additional mutant was made by changing Lys25 to Met to delete the ATP binding site (M mutant). Epitope-tagged versions of these mutants were made by adding an HA-tag to the amino-terminal of MKK6; the proteins encoded by these cDNAs were expressed in COS-7 cells. HA-tagged proteins were immunoprecipitated using anti-HA monoclonal antibody 12CA5. The immunoprecipitates were used in a coupled MKK assay to determine the activity of MKK6 or its
mutants by including recombinant His-p38 and MBP in the kinase reaction mixture (Fig. 3C). All three mutants fail to activate p38; previous studies with MEK where A and M mutants were created also resulted in a loss of activity (33). Here the E mutant also failed to activate p38 while in contrast, the analogous structural change in MEK produced an active enzyme (33).

Regulation of MAPK Kinase Activation—We and others recently reported that the low molecular weight GTP-binding proteins (29, 34) Rac1 and Cdc42, possibly via activation of p21-activated kinase (PAK) (29, 35), regulate the activation of p38 and JNK. Other data suggest that a MKK kinase, MEKK1, may lie downstream of these regulators and thus function as an activator of the MKKs (34). To examine whether MKK6 is regulated in a similar manner, we co-expressed dominant active forms of Rac1, Cdc42, or MEKK1 with HA-tagged MKK6, MKK4, or MKK3 in COS-7 cells. The activity of MKKs were determined by immunokine assay. Overexpression of the active-form Rac1, Cdc42, or MEKK1 led to activation of MKK4 only; we failed to observe activation of MKK6 or MKK3 as detected by our assay system (Fig. 4). This observation confirms the previous report that MKK4 (JNK1) is downstream of Rac1, Cdc42, and MEKK1 (34) and further suggests that other regulatory steps lead to activation MKK6 or MKK3.

DISCUSSION

Herein we describe the properties of a newly identified member of a family of enzymes known as MAP kinase kinases (MKK). Nucleotide sequence data indicates that the human cDNA clones we isolated encode two proteins generated from one gene; the distinct mRNA species are produced through alternative splicing from a single gene encoding a MAP kinase kinase, here termed MKK6. There is tissue-specific expression of the isoforms of MKK6. Functional studies indicate that MKK6 can activate p38 but not ERK or JNK. In contrast to the previously described members of this family, which are expressed in almost all tissues with a single transcript at the mRNA level (7–9, 22), numerous splice variants of this gene exist. One splice form, MKK6, appears restricted to expression in skeletal muscle. In contrast, MKK6 is present in various forms in multiple tissues. Thus this represents the first report suggesting there are tissue-specific isoforms of the MKK family. Unique functions for tissue-specific splice forms of MKK6 is not provided by our work. However, demonstration of the presence of such forms suggests the possibility of regulation that is tissue and/or cell specific.

Here we also determined that Ser$^{151}$ and Thr$^{155}$ are likely to be important phosphorylation sites since mutation of these residues to alanine prevented activation of MKK6. This region

FIG. 4. Regulation of the activation of MKK3, MKK4, and MKK6. COS-7 cells were transfected with epitope-tagged MKK6, MKK4, or MKK3 together with empty expression vector or an expression vector encoding active-form MEKK1, Rac1, Cdc42, Ras, RhoA, and Raf. Some of the cell cultures were exposed to UV radiation (50 J/m$^2$). The MKK6, MKK4, or MKK3 was isolated by immunoprecipitation with the use of anti-epitope antibody. The kinase activity was measured in the immunocomplex with $[\gamma$-32P]ATP and recombinant p38 as substrate. The product of the phosphorylation reactions was visualized after SDS-PAGE by autoradiography.

FIG. 5. Proposed signaling pathways for activation of p38.
is analogous to one established to be important by others for MEK (33). We also attempted to replace Ser151 and Thr185 with Glu to simulate the negative charge resulting from phosphorylation. This approach did result in a gain-of-function mutation for MEK (33). Here we failed to observe a similar effect. This may be due to the fact that the phosphorylation sites of MEK are both Ser while in MKK6 the site is determined by a Thr and Ser. The Glu may not mimic phosphothreonine in the same way that it does phosphoserine.

Insofar as the MAP kinase family is concerned it was not surprising that MKK6 and MKK3 are quite similar in substrate specificity. MKK6 and MKK3 can be classified into a subgroup distinct from others members of the MKK family. This is justified based on sequence homologies and ability to activate p38 without activating ERK or JNK. Moreover here we showed that regulators of MKK4, namely Rac1 and Cdc42, apparently do not control activation of MKK6. Thus there may be two distinct pathways leading to p38 activation; one via MKK6/3 and another involving MKK4. Interestingly the studies of Saito and colleagues (18, 19) with S. cerevisiae revealed two distinct pathways leading to Hog1p activation. One involves a two-component histidine kinase system leading to activation of the MKK encoded by the PBS2 gene (18). In contrast, an alternative pathway leading to PBS2p activation was discovered involving a membrane protein termed Sho1 that directly activates PBS2 through SH3 interactions (19).

Given the distinct expression patterns of MKK6, it is likely that this MKK acts as a regulator of p38 activation depending on expression in a given tissue and/or cell type. A future challenge will be to define the specific function of individual p38 activators and how these proteins interact with other components of the signal machinery to transduce extracellular information into cellular responses. Gene targeting of individual MKKs in cultured cells and mice should shed light on this issue.

We suggest that multiple signaling pathways can control activation of p38. A model which takes into account the data presented here as well as the findings of others (7, 8, 26, 34, 35) is provided in Fig. 5. Investigations that may lead to the elucidation of alternative pathways for p38 activation via MKK3 and/or MKK6 are currently underway.

Acknowledgments—We thank Dr. R. J. Davis for the MKK3 and MKK4 cDNA, Dr. M. Karin for the JNK1(MKK4) construct, Dr. G. L. Johnson for the MEKK1 construct, and Dr. G. M. Bakoh for many essential reagents. We also thank Dr. J. C. Mathison for helpful discussion and Betty Chastain for excellent secretarial assistance.

REFERENCES

1. Blenis, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5889–5892
2. Blumer, K. J., and Johnson, G. L. (1994) Trends Biochem. Sci. 19, 236–240
3. Cano, E., and Mahadevan, L. C. (1995) Trends Biochem. Sci. 20, 117–122
4. Seger, R., and Krebs, E. G. (1995) Trends Biochem. Sci. 20, 735–738
5. Davis, R. J. (1994) Trends Biochem. Sci. 19, 470–473
6. Lin, A., Minden, A., Martinetto, H., Clarlet, F.-X., Lange-Carter, C., Mercurio, F., Johnson, G. L., and Karin, M. (1995) Science 268, 286–290
7. Derijard, B., Raingeaud, J., Barrett, T., Wu, I., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) Science 267, 682–685
8. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M., and Zon, L. I. (1994) Nature 369, 156–160
9. Crews, C. M., Alessandri, A. A., and Erickson, R. L. (1992) Science 258, 478–480
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 Yanopoulos, G. D. (1991) Cell 65, 663–675
11. Seger, R., Ahn, N. G., Boulton, T. G., Yanopoulos, G. D., Panayotatos, N., Radziejewska, E., Ericsson, L., Bratlien, R. L., Cobb, M. H., and Krebs, E. G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6142–6146
12. Kyriakis, J. M., Banerjee, P., Nikidakakis, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994) Nature 369, 156–160
13. Derijard, B., Hibi, M., Wu, I., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994) Cell 76, 1025–1037
14. Han, J., Lee, J.-D., Bibbs, L., and Ulevitch, R. J. (1994) Science 265, 808–811
15. Lee, J.-D., Ulevitch, R. J., and Han, J. (1995) Biochem. Biophys. Res. Commun. 213, 715–724
16. Zhou, G., Bao, Z. O., and Dixon, J. E. (1995) J. Biol. Chem. 270, 12664–12669
17. Brexter, J. L., de Valoir, T., Dyer, N. D., Winter, E., and Gustin, M. C. (1993) Science 259, 1760–1763
18. Maeda, T., Wurgler-Murphy, S. M., and Saito, H. (1994) Nature 369, 242–245
19. Lee, J.-D., Tai, T., Takakawa, M., and Saito, H. (1995) J. Biol. Chem. 270, 554–558
20. Lee, J.-D., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heyes, R. J., Landwater, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I., Fisher, S., Livi, G. P., and Lee, J.-D. (1995) Nature 373, 739–746
21. Raingeaud, J., Gupta, S., Rogers, J. S., Dickens, M. H., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) J. Biol. Chem. 270, 7420–7426
22. Zhu, G., Young, P. R., White, J. R., Adams, J. L., and Young, P. R. (1994) Science 268, 554–558
23. Kramer, R. M., Roberts, E. F., Striffler, B. A., and Johnstone, E. M. (1995) J. Biol. Chem. 270, 27395–27398
24. Weinstein, S. L., Sanghera, J. S., Lemke, K., Defranaco, A. L., and Pelech, S. L. (1992) J. Biol. Chem. 267, 14955–14962
25. Lange-Carter, C. A., Pieman, C. M., Gardner, A. M., Blumer, K. J., and Johnson, G. L. (1993) Science 260, 315–319
26. Moriguchi, T., Kawasaki, H., Matsuda, S., Gotoh, Y., and Nishida, E. (1995) J. Biol. Chem. 270, 12969–12972
27. Innis, M. A., Gefand, D. H., Shinsky, J. J., and White, T. J. (1990) PCR Protocols, Academic Press, New York
28. Zhang, S., Han, J., Jels, M. A., Chernoff, J., Kraus, U. G., Ulevitch, R. J., and Bakoh, G. M. (1995) J. Biol. Chem. 270, 12934–12936
29. Guan, K., and Dixon, J. E. (1991) Anal. Biochem. 192, 267
30. Lin, A., Minden, A., Martinetto, H., Clarlet, F.-X., Lange-Carter, C., Mercurio, F., Johnson, G. L., and Karin, M. (1995) Cell 81, 1147–1157
31. Mander, E., Leung, T., Salihuddin, H., Zhao, Z., and Lim, L. (1994) Nature 367, 40–46