A Novel Mechanism of JNK1 Activation
NUCLEAR TRANSLLOCATION AND ACTIVATION OF JNK1 DURING ISCHEMIA AND REPERFUSION

Yoichi Mizukami‡§, Katsuji Yoshioka¶, Sachio Morimoto‖, and Ken-ichi Yoshida‡

From the ¤Department of Legal Medicine, Yamaguchi University School of Medicine, Yamaguchi, the §Department of Molecular Pathology, Cancer Research Institute, Kanazawa University, Ishikawa, and the ‖Department of Clinical Pharmacology, Kyushu University School of Medicine, Fukuoka, Japan

Cytokines and various cellular stresses are known to activate c-Jun NH2-terminal kinase (JNK), which plays a role in conveying signals from the cytosol to the nucleus. Here we investigate the translocation and activation of JNK1 during ischemia and reperfusion in perfused rat heart. Ischemia induces the translocation of JNK1 from the cytosol fraction to the nuclear fraction in a time-dependent manner. Immunohistochemical observation also shows that JNK1 staining in the nucleus is enhanced after ischemia. During reperfusion after ischemia, further nuclear translocation of JNK1 is apparently inhibited. In contrast, JNK1 activity in the nuclear fraction does not increase during ischemia but increases significantly during reperfusion with a peak at 10 min of reperfusion. The activation of JNK1 is confirmed by the phosphorylation of endogenous c-Jun (Ser-63) with similar kinetics. The level of c-jun mRNA also increases during reperfusion but not during ischemia. Based on fractionation and immunohistochemical analyses, an upstream kinase for JNK1, SAPK/ERK kinase 1 (SEK1), is constantly present in both the nucleus and cytoplasm throughout ischemia and reperfusion, whereas an upstream kinase for mitogen-activated protein kinase, MAPK/ERK kinase 1, remains in the cytosol. Furthermore, phosphorylation at Thr-223 of SEK1, necessary for its activation, rapidly increases in the nuclear fraction during postischemic reperfusion. These findings demonstrate that JNK1 translocates to the nucleus during ischemia without activation and is then activated during reperfusion, probably by SEK1 in the nucleus.

In response to ischemia and reperfusion, various pathways in mammalian cells are induced leading to cell death and organ dysfunction. In the heart, prolonged ischemia causes necrosis and contractile dysfunction, but the heart can recover from injury following brief ischemia. Many genes are expressed in the heart during recovery from ischemia (1, 2). In particular, the expression of immediate early genes (e.g. c-fos, c-jun, Egr-1) is rapidly up-regulated during postischemic reperfusion. These gene products are thought to be involved in the determination of cell fate, such as apoptosis (3–5), hypertrophy (6, 7), proliferation (8–10), or cell differentiation (11–13).

Among transcription factors, the expression of c-jun is regulated by a 12-O-tetradecanoylphorbol-13-acetate response element in the c-jun promoter. This element binds with the c-Jun and ATF-2 heterodimer and elevates transcriptional activity. This activity is likely to be positively regulated by the phosphorylation of Ser-63 and Ser-73 in the NH2-terminal region of c-Jun (14–17). Jun NH2-terminal kinases (JNKs)1 also known as stress-activated protein kinases or SAPKs comprise a superfamily of the mitogen-activated protein kinase (MAPK) family of serine/threonine kinases; they are activated by inflammatory cytokines, tumor necrosis factor α, UV irradiation, and heat shock (18–21). JNK1 phosphorylates c-Jun at serine residues in response to cellular stress (18, 19, 22, 23). The induction of c-fos is regulated by a serum response element in the promoter. Transcription factors p67SRF and p62TCF bind to the serum response element, the activity of which is regulated by the phosphorylation of p62TCF by JNK1 (24) or MAPK (25). These transcription factors, substrates for JNK1, are constitutively localized in the nucleus throughout ischemia and reperfusion (26, 27). Therefore, the nuclear translocation of JNK and MAPK is regarded as an important step in the activation of transcription factors.

There have been several studies on the nuclear translocation of MAPK in response to stimulation. It is known that MAPK is first activated in the cytoplasm through the activation of mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) and then translocated to the nucleus (28, 29). MEK is constitutively localized in the cytoplasm (30), which is one of the grounds for the nuclear translocation of MAPK after its activation in the cytoplasm. Recently, Fukuda et al. (31) found a nuclear export signal in the sequence of Xenopus MEK, by which MEK that enters into the nucleus through nuclear pores is exported to the cytosol. We have recently demonstrated that MEK2 is partially present in the nucleus, where it is activated during postischemic reperfusion, although MEK1 exists constitutively in the cytosol (32). MEK2 activation in the nucleus may be involved in MAPK activation during postischemic reperfusion. On the other hand, the mechanism of JNK activation during ischemia and reperfusion is poorly understood, although recent reports show that JNK1 is activated in rat heart and kidney during reperfusion but not during ischemia (33, 34). No nuclear export signal sequence has been identified in SEK1, an upstream kinase for JNK1, and its localization is presently unknown.

The present study is concerned with the nuclear translocation and activation of JNK1 during ischemia and reperfusion.

This work was supported in part by grants from the Ministry of Education, Science, and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: JNK(s), c-Jun NH2-terminal kinase(s); MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; SEK1, stress-activated protein kinase/extracellular signal-regulated kinase 1; GST, glutathione S-transferase.
JNK1 Activation during Ischemia and Reperfusion

**EXPERIMENTAL PROCEDURES**

**Materials**—Anti-JNK1 antibody (C-17), anti-SEK1 antibody, and protein A-agarose were bought from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-histone H1 antibody was from Leinco Technologies Inc. Anti-phospho-Jun antibody, anti-phospho-SEK1 antibody, c-Jun, and phospho-c-Jun were from New England Biolabs, Inc. (Beverly, MA). Anti-MEK1 antibody was from Upsate Biotechnology Inc. (Lake Placid, NY). Anti-c-Jun/AP-1 antibody was from Transduction Laboratories (Lexington, KY). The enhanced chemiluminescence reaction kit assay and anti-rabbit immunoglobulin G antibodies coupled to peroxidase were obtained from Amersham, and the Vectastatin kit was from Vector Laboratories (Burlingame, CA). The biotin blocking system and anti-rabbit immunoglobulin G antibodies coupled to peroxidase were obtained from Amersham, and the Vectastatin kit was from Vector Laboratories (Lexington, KY). The enhanced chemiluminescence reaction kit and anti-c-Jun/AP-1 antibody was from Transduction Laboratories (Lake Placid, NY). Anti-c-Jun/AP-1 antibody was from Transduction Laboratories (Lake Placid, NY). Anti-c-Jun/AP-1 antibody was from Transduction Laboratories (Lake Placid, NY).

**Perfusion Protocol**—Male Wistar rats weighing about 200 g were anesthetized with sodium pentobarbital. The aortas were cannulated and attached to a Langendorff apparatus. The hearts were perfused and then subjected to global ischemia for 0–40 min and reperfusion for 0–30 min after ischemia for 20 min at 37 °C, as described previously (35, 36). The hearts were quickly frozen in liquid nitrogen and stored at −70 °C for later biochemical analyses.

**Subcellular Fractionation**—Subcellular fractionation was performed essentially as described previously (36, 37) with minor modifications.

**Results**

**Nuclear Translocation of JNK1 during Ischemia and Reperfusion**—To investigate the subcellular localization of JNK1 during ischemia, we first performed the fractionation of the perfused rat hearts. Electron microscopic observation showed that the 1,000 × g pellet (nuclear fraction) contained the nuclei and myofibrils and that the 100,000 × g pellet (membrane fraction) included membrane vesicles and mitochondria (data not shown). Histone H1, a nuclear protein, was localized mainly in the nuclear fraction (80.1 ± 5.2%, n = 8, Fig. 1B), and the localization of the SEK1, an upstream kinase for JNK1, is also investigated in ischemic heart. Several lines of evidence obtained here demonstrate that JNK1 translocates to the nucleus during ischemia where it is then activated during reperfusion, probably by nuclear SEK1.

**Discussion**

FIG. 1. Subcellular localization of JNK1 in ischemic heart. Subcellular fractions were prepared from hearts after ischemia for 0–40 min, and the fractions were subjected to immunoblotting with anti-JNK1 antibody, anti-histone H1 antibody, and anti-MEK1 antibody. N, nuclear; M, membrane; C, cytosol. Panel A shows representative immunoblots of three independent experiments, and panel B shows the amounts of JNK1 (% of sum of nuclear (□), membrane (●), and cytosol (△) fractions of control heart, n = 3) determined by densitometry from immunoblots obtained with anti-JNK1 antibody (p < 0.05 versus control, mean ± S.E.).

The frozen hearts were minced and homogenized in 2 volumes of STE buffer (0.32 M sucrose, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM NaCl, 50 mM β-mercaptoethanol, 20 μM leupeptin, 0.15 μM pepstatin A, 0.2 μM phenylmethanesulfonyl fluoride, 50 mM NaF, 1 mM sodium orthovanadate, 0.4 mM microcystin) in a Polytron homogenizer. The homogenates were mixed with 2 volumes of STE buffer and centrifuged (1,000 × g, 10 min) to obtain pellets. The pellets were washed once and suspended in STE buffer (nuclear fraction). The supernatant was centrifuged (100,000 × g, 60 min) to obtain the cytosol fraction and the pellet, which was then suspended in STE buffer (membrane fraction). The nuclear fraction was solubilized in Triton buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.2 mM sodium orthovanadate, 20 μM leupeptin A, 0.2 μM phenylmethanesulfonyl fluoride, 50 mM NaF, 0.4 mM microcystin LR). The fraction was centrifuged (15,000 × g, 30 min, 4 °C), and the supernatant (nuclear extract) was stored at −70 °C.

Electrophoresis and Immunoblotting—The subcellular fractions and molecular mass standards were electrophoresed on 10% polyacrylamide gels in the presence of SDS and transferred to nitrocellulose membranes (0.45 μm). The blots were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween 20 and incubated with antibody. After washing, the antigens were visualized by enhanced chemiluminescent detection reagents.

**JNK1 Activity Assay**—Nuclear extracts were prepared as described above and immunoprecipitated with anti-JNK1 monoclonal antibody (G151-333.8; Pharmingen). Immunocomplexes were subjected to kinase assays using 2 μg of GST-c-Jun (1–79) as a substrate.

**Northern Blotting Analysis**—Total RNA was prepared and analyzed as described previously (38, 39).

**Immunohistochemistry**—Rat hearts were immersed in OCT compound (Miles) and rapidly frozen in liquid nitrogen. The specimens were fixed on glasses with PLP solution (0.02M NaIO4, 0.075 M phosphate buffer, 2% paraformaldehyde) for 10 min at 4 °C and then incubated with 1:25 dilutions of antibodies in 1% bovine serum albumin in phosphate-buffered saline for 1 h and then incubated with biotinylated secondary antibodies for 1 h. The sections were subsequently incubated with a Vectastain kit. The peroxidase label was visualized by exposing the sections to diaminobenzidine.

The results show that JNK1 activation occurs during ischemia and reperfusion. The nuclear fraction (70 μg of protein) was prepared from hearts after ischemia for 0–40 min (C) or after reperfusion for 0–30 min following ischemia for 20 min ( ● ) and subjected to immunoblotting with anti-JNK1 antibody. The figure shows the amounts of JNK1 in the nuclear fraction ( □ , % of the fractions in control heart, n = 3) determined by densitometry from immunoblots with anti-JNK1 antibody (p < 0.05 versus control, mean ± S.E.).
the recovery remained almost constant throughout ischemia and reperfusion (data not shown). The fractions were analyzed by immunoblotting using an antibody against JNK1. In untreated hearts, JNK1 predominated in the cytosol fraction (85 ± 2.7%, n = 3), whereas the nuclear fraction contained only 5.6 ± 1.9%. The amount of JNK1 in the cytosol fraction was decreased significantly after 20 min of ischemia (51.3 ± 14.9%) with a concomitant increase in the nuclear fraction (40.4 ± 13.3%). After ischemia for 40 min, the JNK1 level reached a maximum in the nuclear fraction and became undetectable in the cytosol fraction. JNK1 in the membrane fraction remained at control levels throughout ischemia (0–40 min) and reperfusion (0–30 min) (Fig. 1A and B). The sum of JNK1 in the three fractions remained almost unchanged during ischemia (Fig. 1B). Immunohistochemical observation using anti-JNK1 antibody also showed that JNK1 was localized mainly in the cytosol under control conditions, and the nuclear staining was enhanced after 40 min of ischemia (see Fig. 3, A and B). The staining was inhibited by preincubating the antibody with the peptide or by only secondary antibody (data not shown). These observations, taken together with the immunoblotting and immunohistochemical analyses, show that ischemia induces the translocation of JNK1 from the cytosol to the nucleus. In contrast, MEK-1 remains strictly in the cytosol fraction during ischemia (0–40 min, Fig. 1A) and reperfusion (see Fig. 7B), indicating that the ischemia-induced translocation of JNK1 is not because of nonspecific translocation of protein kinases.

Next, hearts were subjected to reperfusion for 0–30 min after global ischemia for 20 min; reperfusion following ischemia for 40 min could not restore myocardial contraction and relaxation. Fractionation and immunoblot analyses showed that the increase in nuclear-JNK1 during ischemia was blocked by reperfusion and that the amount of JNK1 in the nuclear fraction remained almost constant during reperfusion (Fig. 2). Immunohistochemical observation also gave similar results (Fig. 3).
It is known that c-Jun is most likely phosphorylated at Ser-63 and Ser-73 by activated JNK1 and that it positively regulates the induction of the c-jun mRNA by binding to the c-jun promoter. As shown in Fig. 6, the level of c-jun mRNA did not increase during 40 min of ischemia but increased rapidly during reperfusion (Fig. 6A). c-fos mRNA has also been reported to be induced through JNK1 activation in response to such stresses as UV irradiation. Northern blot analysis indicates that postischemic reperfusion induces c-fos expression as well as c-jun expression (Fig. 6B). These observations indicate that activated JNK1 may participate in the induction of c-jun and c-fos in the nucleus during reperfusion but not during ischemia.

Localization and Activation of SEK1, an Upstream Kinase for JNK1, during Ischemia and Reperfusion—The subcellular localization of SEK1, an upstream kinase for JNK1, is presently unknown. We used fractionation and immunoblotting techniques to examine the distribution of SEK1 during ischemia.
and reperfusion. As shown in Fig. 7, anti-SEK1 antibody recognized only one band corresponding to SEK1 with a molecular mass of 45 kDa, and the antibody reaction is inhibited by preincubation with the immunizing peptide or only secondary antibody. Under control conditions, SEK1 is localized mainly in the cytosol fraction and partially in the nuclear fraction. During ischemia and reperfusion, SEK1 was constantly observed in the nuclear fraction (Fig. 7). Next, we examined the distribution of SEK1 under control conditions by immunohistochemistry using the anti-SEK1 antibody. Whereas MEK1 was essentially found in the cytoplasm, SEK1 was present in both the cytoplasm and nuclei in the heart (Fig. 8). The staining was blocked by preincubation of the antibody with the immunizing peptide or by only secondary antibody as well as in immunoblotting analysis. SEK1 has been reported to be activated by MEK kinase 1 through phosphorylation at Thr-223 (40, 41). Fractionation and immunoblotting analyses using anti-phospho-SEK1 antibody revealed that the phosphorylation of SEK1 in the nuclear fraction increased rapidly during reperfusion (Fig. 9), suggesting that after translocation from the cytosol to the nucleus during ischemia, JNK1 may be activated by SEK1 in the nucleus during reperfusion.

**DISCUSSION**

It is generally thought that the nuclear translocation of MAPK family members is associated with their activation (42). However, we demonstrated that ischemia induces the translocation of JNK1 from the cytosol to the nucleus without its activation and that the translocated JNK1 is then activated during reperfusion, probably by nuclear SEK1, an upstream kinase for JNK1.

The nuclear translocation of JNK1 appears to involve an active transport system rather than diffusion in the cell according to a concentration gradient, since the nuclear transport of JNK1 is stronger than those of MAPK and protein kinase C isoforms during ischemia as reported previously (32, 43, 44), and cytosolic JNK1 becomes concentrated in the nucleus after 40 min of ischemia. Hypoxia and glucose depletion during ischemia should inhibit oxidative phosphorylation in the mitochondria and lead to a decrease in intracellular ATP. The decrease in ATP, in turn, will result in the inhibition of H⁺-ATPase activity in the cell, which rapidly lowers the intracellular pH (45). The decrease in intracellular pH and ATP concentration may inhibit the enzyme activities of JNK1 and upstream kinases. Reperfusion will produce a rapid recovery in the intracellular pH to normal levels and increase the ATP level so that JNK1 could be activated during reperfusion. The idea that JNK1 is translocated to the nucleus during ischemia and activated in the nucleus during reperfusion is supported by the following observations. First, the activity of JNK1 increases in the nucleus during reperfusion, whereas the amount of JNK1 in the nucleus remains unchanged. Second, SEK1, an upstream kinase for JNK, is also present in the nucleus and is phosphorylated at Thr-223, which is required for its activation, in response to reperfusion. Third, the time course of SEK1 phosphorylation is almost identical to that of JNK1 activation. Finally, c-Jun is phosphorylated rapidly after reperfusion, and the mRNA for c-jun is also expressed early; there seems to be no time delay due to the nuclear translocation of JNK1 after reperfusion. This appears to be a common phenomenon in various tissues since the same translocation and activation of JNK1 are also observed in ischemic mouse liver.²

However, we could not completely exclude the possibility that cytosolic JNK1 is also activated and then translocated to the nucleus since the activated form of SEK1 was detected in the cytosol as well as in the nucleus during reperfusion. In recent reports, UV irradiation and osmotic stress have been shown to initiate the clustering of cell surface receptors, and this subsequently induces the activation and nuclear entry of JNK (24, 46). However, it is also reported that γ-radiation results in the activation of nuclear JNK1 without nuclear translocation (47). Kharbanda et al. (48) have shown that JNK1 is activated through nuclear c-Abl activation in response to DNA-damaging agents, suggesting that a pathway for JNK1 activation may exist in the nucleus. Thus, there must be several pathways for JNK1 activation in the cell for various forms of stress as described previously by Adler et al. (49). In this report, SEK1, an upstream kinase for JNK1, was found to be partially present in the nucleus even in unstimulated cells. SEK1 does not possess the three leucine residues critical for nuclear export activity. In addition, SEK1, with a molecular mass of ~45 kDa, is small enough to pass through nuclear pores. Thus, SEK1 could be localized in various cellular compartments that may participate in different JNK1 activation pathways induced by distinct forms of stress.

It is well known that cytosolic Ca²⁺ levels increase during ischemia and reperfusion. Cytosolic and nuclear Ca²⁺ concentrations equilibrate rapidly in the cell since the nuclear envelope is very permeable to Ca²⁺ (50). The rise in cellular Ca²⁺ is known to trigger the cellular responses through the activation of various enzymes such as protein kinase C (51–53), calmodulin-dependent protein kinase II (54), calpain (55), and nitric oxide synthase (56). JNK1 activation is reported to be inhibited by reperfusion with low [Ca²⁺]² buffer (34), suggesting that unknown Ca²⁺-dependent kinase(s) may be involved in the activation of JNK1 as an upstream kinase(s).

There is much evidence that nuclear JNK1 is involved in transcriptional regulation. JNK1 can phosphorylate c-Jun and enhance its transcriptional activity. JNK1 in the nucleus can phosphorylate the transcription factor p62CRF at the c-fos promoter, leading to c-fos induction. As shown in this study, during posts ischemic reperfusion, JNK1 is activated, and the expression of mRNA for c-jun and c-fos is strongly enhanced. Therefore, we speculate that c-Jun and c-Fos synergistically activate the function of the AP-1 complex (57, 58) during reperfusion, and this may induce the expression of genes for contractile proteins (e.g. skeletal α-actin, myosin heavy chain) which have an AP-1 element in their promoter regions (59, 60). Such gene expression may have relevance to tissue repair or disease development in the ischemic heart. Furthermore, tumor necrosis factor activates sphinogomelysin, generating ceramide (61, 62). Tumor necrosis factor and ceramide induce apoptosis and DNA fragmentation and activate JNK1 (63–65). Since ischemia-reperfusion has been shown to induce apoptotic cell death in the heart (66), JNK1 activation might be involved in the signaling pathway leading to apoptosis in the ischemic heart.

**Acknowledgments**—We thank Dr. M. Hibi (Osaka University) for providing the GST-c-Jun expression vector plasmid and T. Hirata (undergraduate student, School of Medicine, Yamaguchi University) for technical assistance with the immunohistochemistry.

**REFERENCES**

1. Ouellette, A. J., Malt, R. A., Sukhatme, V. P., and Bonventre, J. V. (1990) *J. Clin. Invest.* 85, 766–771
2. Webster, K. A., Ducher, D. J., and Bishopric, N. H. (1994) *Circ. Res.* 74, 679–686
3. Estus, S., Zakis, W. J., Freeman, R. S., Gruda, M., Bravo, R., and Johnson, E., Jr. (1994) *J. Cell Biol.* 127, 1717–1727
4. Xu, Z., Dickens, M., Raineyaud, J., Davis, R. J., and Greenberg, M. E. (1995) *Science* 270, 1326–1331
5. Roffler-Tarlov, S., Brown, J. J., Tarlov, E., Stolarov, J., Chapman, D. L., Alexiou, M., and Papaioannou, V. E. (1996) *Development* 123, 1–9
6. Wheldaw, P. F., and Hesketh, J. E. (1992) *Biochem. J.* 281, 143–147
7. Rothman, A., Wiler, B., Button, D., and Taylor, P. (1994) *J. Biol. Chem.* 269, 6399–6404
8. Chen, B., Polunovsky, V., White, J., Blazar, B., Nakhleb, R., Jessurun, J.,
JNK1 Activation during Ischemia and Reperfusion

Peterson, M., and Bitterman, P. (1992) *J. Clin. Invest.* **90**, 1778–1785

9. Busam, K. J., Roberts, A. B., and Sporn, M. B. (1992) *J. Biol. Chem.* **267**, 18974–18979

10. Kovary, K., and Bravo, R. (1992) *Mol. Biol. Cell.* **12**, 5015–5023

11. Bengal, E., Ransone, L., Scharffman, R., Dwarki, V. J., Tapscott, S. J., Wentraub, H., and Verma, I. M. (1992) *Cell* **68**, 507–519

12. Bohmann, D., Ellis, M. C., Staszewski, L. M., and Mlodzik, M. (1994) *Cell* **78**, 973–986

13. Treier, M., Bohmann, D., and Mlodzik, M. (1995) *Cell* **83**, 753–766

14. Binetruy, B., Smeal, T., and Karin, M. (1994) *Nature* **351**, 122–127

15. Pulverer, B., Kyriakis, J. M., Avruch, J., Nikolakaki, E., and Woodgett, J. R. (1991) *Nature* **353**, 670–674

16. Karin, M. (1995) *J. Biol. Chem.* **270**, 16483–16486

17. Hunter, T., and Karin, M. (1992) *Cell* **70**, 375–387

18. Hibi, M., Lin, A., Smeal, T., Minden, A., and Karin, M. (1993) *Genes Dev.* **7**, 2133–2148

19. Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994) *Nature* **369**, 156–160

20. Derijard, B., Hibi, M., Wu, J. H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994) *Cell* **76**, 1925–1937

21. Force, T., Pombo, C. M., Avruch, J. A., Bonventre, J. V., and Kyriakis, J. M. (1996) *Circ. Res.* **78**, 947–953

22. Smeal, T., Binetruy, B., Mercola, D., Birrer, M., and Karin, M. (1991) *Nature* **354**, 494–496

23. Devary, Y., Gottlieb, R. A., Smeal, T., and Karin, M. (1992) *Cell* **71**, 1081–1091

24. Cavigelli, M., Dolfi, F., Clare, F.-X., and Karin, M. (1995) *EMBO J.* **14**, 5957–5964

25. Gille, H., Sharrocks, A. D., and Shaw, P. E. (1992) *Nature* **358**, 414–417

26. Webster, K. A., Discher, D. J., and Bishop, N. H. (1993) *J. Biol. Chem.* **268**, 16070–16077

27. Brand, T., Sharma, S., Fleischmann, K. E., Duncker, D. J., McFalls, E. O., Verdouw, D. P., and Schaper, W. (1992) *Circ. Res.* **71**, 1351–1360

28. Lenormand, P., Sardet, C., Pages, G., L’Allemand, G., Brunet, A., and Pouysségur, J. (1993) *J. Cell Biol.* **122**, 1077–1088

29. Gonzalez, F. A., Seth, A., Raden, D. L., Bowman, D. S., Feller, S. M., Kharbanda, S., Ren, R., Pandey, P., Shafman, T. D., Feller, S. M., Weihselbaum, R. B., and Kufe, D. W. (1995) *Nature* **376**, 758–788

30. Adler, V., Schaffer, A., Kim, J., Dolan, L., and Ronai, Z. (1995) *J. Biol. Chem.* **270**, 20671–20677

31. Mizukami, Y., and Yoshida, K. (1997) *Biochem. Biophys. Acta* **1317**, 36–44

32. Yoshida, Y., Hirata, T., and Yoshida, K. (1997) *FEBS Lett.* **401**, 247–251

33. Bak, M. I., and Ingwall, J. S. (1994) *J. Clin. Invest.* **93**, 40–49

34. Rosette, C., and Karin, M. (1996) *Science* **274**, 1194–1197

35. Chen, Y.-R., Meyer, C. F., and Tan, T. H. (1996) *J. Biol. Chem.* **271**, 1079–1088

36. Kharbanda, S., Ren, R., Pandey, P., Shafman, T. D., Feller, S. M., Weihselbaum, R. B., and Kufe, D. W. (1995) *Nature* **376**, 758–788

37. Chiu, R., Boyle, W. J., Meek, J., Smeal, T., Hunter, T., and Karin, M. (1988) *Cell* **54**, 514–552

38. Michalopoulos, N. H., Jayasena, V., and Webster, K. A. (1992) *J. Biol. Chem.* **267**, 18977–18984

39. Nathan, C., and Xie, Q. (1994) *J. Biol. Chem.* **269**, 13725–13728

40. Halazonetis, T. D., Georgeopolous, K., Greenberg, M. E., and Leder, P. (1988) *Cell* **55**, 917–924

41. Chiu, R., Boyle, W. J., Meek, J., Smeal, T., Hunter, T., and Karin, M. (1988) *Cell* **54**, 514–552

42. Bishopric, N. H., and Karin, M. (1992) *J. Biol. Chem.* **267**, 18977–18984

43. Gillespie-Brown, J., Fuller, S. J., Bogoyevitch, M. A., Cowley, S., and Sugden, P. H. (1995) *J. Biol. Chem.* **270**, 28092–28096

44. Kim, Y.-K., Yim, C., and Hannun, Y. A. (1991) *J. Biol. Chem.* **266**, 484–489

45. Hannun, Y. A. (1994) *J. Biol. Chem.* **269**, 3125–3128

46. Sluss, H. K., Barrett, T., Derijard, B., and Davis, R. J. (1994) *Mol. Cell. Biol.* **14**, 3876–3884

47. Westwick, J. K., Weitzel, C., Minden, A., Karin, M., and Brenner, D. A. (1994) *J. Biol. Chem.* **269**, 26396–26401

48. Westwick, J. K., Bilal, A., B创伤, D. A., and Brenner, D. A. (1994) *J. Biol. Chem.* **270**, 22689–22692

49. Gettlib, R. A., Burlinson, K. O., Klein, R. A., Babior, B. M., and Engler, R. L. (1994) *J. Clin. Invest.* **94**, 1621–1628