Oncogenic Ras Enhances NF-κB Transcriptional Activity through Raf-dependent and Raf-independent Mitogen-activated Protein Kinase Signaling Pathways*

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The small GTP-binding protein Ras functions as an inducer of intracellular signaling pathways that are responsible for regulating cellular functions including proliferation (1, 2). Mutations in Ras alleles are found in approximately 30% of human cancers, leading to chronic GTP binding and constitutive activation of signal transduction cascades. Thus, it has been shown that oncogenic Ras stimulates mitogen-activated protein (MAP) kinase signaling cascades and that these and other signal transduction pathways are critical for Ras to initiate transformed NIH-3T3 and Rat-1 fibroblasts. Both dominant negative and constitutively active components of signaling pathways have been tested for their ability to regulate NF-κB. These experiments show that Ras utilizes Raf-dependent and Raf-independent pathways to activate NF-κB transcriptional activity, both of which require the stress-activated kinase p38 or a related kinase. In the case of Raf, activation of NF-κB by an auto-crine factor stimulates κB-dependent transcriptional activity.

The mammalian transcription factor NF-κB is involved in regulating the expression of genes required for inflammatory responses, for suppression of apoptosis, and for controlling cell growth (14, 15). NF-κB is a member of the Rel family of transcription factors, which consists of five members, c-Rel, p50 (NF-κB1), p65 (RELA), p52 (NF-κB2), and RELB. The different family members can form homodimers or heterodimers, and different subunit combinations have different functions in regulating transcription (14). The classic NF-κB activator of transcription is a heterodimer composed of a p50 and p65 subunit. This heterodimer is a potent activator of gene expression from κB sites due to the presence of at least two transactivation domains in the C-terminal region of p65 (16, 17). The carboxy-terminal 30 amino acids comprise a transactivation domain (TA1) that belongs to the class of acidic activators and can activate transcription from GAL4 binding sites when fused to the GAL4 DNA-binding domain.

NF-κB activity is regulated, at least in part, by its subcellular localization. NF-κB is held in the cytoplasm, where it is inactive, through an interaction of the p65 or c-Rel subunits with the inhibitor protein, IκB (14). A variety of extracellular stimuli activate signal transduction pathways that target the NF-κB/IκB complex for disruption (15). These pathways target IκB for degradation by the proteasome by leading to the phosphorylation and ubiquitination of IκB (14, 15). Degradation of IκB results in the disruption of the NF-κB/IκB complex, allowing NF-κB to be transported into the nucleus, where it can activate gene expression (14, 15). Serine residues 32 and 36 are required for the inducible phosphorylation of IκB through the recently identified IκB kinases (18–22).

Several lines of evidence suggest that NF-κB plays an important role in cellular transformation. NF-κB is activated by a variety of cellular oncogenes including Her2/Neu (23), and two members of the NF-κB family, v-rel and p52/lyt-10, and the IκB family member Bcl-3 are potentially oncogenic (14). Transient transfection experiments have shown that oncogenic forms of H-Ras and Raf-1 can activate a reporter driven by NF-κB binding sites (24, 25). Importantly, expression of a modified, superrepressor form of IκBα blocked the ability of oncogenic
Ras alleles to induce focus formation in 3T3 cells (26). The requirement of NF-κB for Ras transformation is based partly on the ability of NF-κB to suppress transformation-associated apoptosis (27). Interestingly, it was observed that oncogenic Ras activates NF-κB through the ability to stimulate transcriptional function dependent on the transactivation domains of the p65/RelA subunit (26). We show here that oncogenic Ras activates NF-κB transcripational activity largely through a Raf-independent mechanism that utilizes a SEK- and p38-dependent pathway. The ability of Raf-1 to activate NF-κB is MEK/ERK-dependent but appears to require an autocrine pathway that ultimately utilizes the same SEK/p38-dependent mechanisms as oncogenic Ras.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—Parental Ras-transformed, and Raf-transformed NIH-3T3 cells were maintained in Dulbecco’s modified Eagle’s medium (high glucose) supplemented with 10% fetal calf serum, penicillin, and streptomycin. For experiments using conditioned medium, conditioned medium was made by taking medium that had been on parental Ras-transformed, or Raf-transformed cells for 48 h and adding fresh medium at a 3:1 ratio. Conditioned medium was added to cells 16–24 h after they were transfected (see below), and cells were harvested 24 h after the addition of conditioned medium. The PD98059, SB202190, and SB203580 compounds were obtained from Calbiochem and were used at a final concentration of 4 μM, 700 nM, and 1.2 μM, respectively. The compounds were added to cells at the indicated concentrations in fresh medium 16–24 h after the cells had been transfected (see below), and cells were harvested 24 h after the addition of the compounds.

Plasmid Constructs—The 3XκB-luc, Gal4-p65, Gal5-luc, Ras12V, RasBBD, and Raf130 plasmids have been described previously (24, 26). pKA290R was provided by Jonathan Chernoff, SEK(RR) was provided by Dennis Templeton. Ras12V37G, Ras12V40C, Ras12V35S, and Rac115 were provided by Channing Der.

Cell Transfections and Luciferase Assays—Cells were transfeected by the calcium phosphate precipitation method essentially as described previously (24). Briefly, semiconfluent cells were transfected with 10.0–15.0 μg of DNA. Unless otherwise indicated, the DNA precipitate contained 1.0 μg of luciferase reporter construct, 1.0 μg of CMV-lacZ plasmid as an internal control for transfection efficiency, and 5.0–7.0 μg of expression vector. For Gal4-p65, 0.4 μg of the lacZ plasmid was used. The pGEM plasmid was used as carrier DNA to bring the final DNA concentration to 10.0–15.0 μg. The DNA precipitate was added to cells, and 16 h later the cells were washed and fresh medium was added. Cells were harvested approximately 24 h later, washed twice with phosphate-buffered saline, and resuspended in 0.25 M Tris (pH 7.8). Cell lysates were made by freeze-thawing three times. Protein concentrations were determined, and 100 μg of protein was assayed for luciferase activity as described previously (28). β-Galactosidase assays were also performed to correct for variations in transfection efficiency. All transfections were performed in duplicate, and all were repeated at least three times.

RESULTS

Blocking the Raf/MEK/ERK Pathway Does Not Inhibit Ras Activation of NF-κB—Since previous work had shown that NF-κB transcripational activity was enhanced by expression of either oncogenic Ras or Raf (24–26), we assumed that the Ras/Raf/MEK/ERK signaling pathway was responsible for NF-κB activation in response to oncogenic Ras. To determine if oncogenic Ras required activation of Raf to activate NF-κB, we used a Raf construct that expresses only the first 150 aminoterminal amino acids of Ras or Raf, Raf130. This construct expresses the regulatory but not the catalytic domain of Raf and functions as a dominant negative by blocking signaling through Raf (25). Parental NIH-3T3 cells were transiently cotransfected with an activated Ha-Ras expression vector, HRas12V, as described previously (24), and the Raf130 expression vector. NF-κB activity was measured in two ways. First, NF-κB-dependent gene expression was measured by using a luciferase reporter plasmid, 3XκB-luc, where luciferase gene expression is under control of a promoter that contains three NF-κB DNA binding sites. Second, enhancement of NF-κB transcripational activity was measured by using a Gal4-p65 fusion expression vector. This fusion protein contains the carboxyl-terminal 30 amino acids of p65/RelA (TA1) fused to the DNA binding domain of the yeast transcription factor Gal4 (26). For cotransfections that contained Gal4-p65, a reporter plasmid, Gal5-luc, that puts luciferase gene expression under control of a promoter that contains five Gal4 DNA-binding sites was included. The results of these transfections are shown in Fig. 1A. These experiments show that blocking Raf does not have a significant effect on the ability of oncogenic Ras to enhance NF-κB-dependent gene expression or transcripational activity. Possibly, Raf130 cannot compete for an endogenous effector that activates NF-κB, or the region required for NF-κB activation is found outside of the Ras effector domain. To show that the dominant negative Raf construct was producing a functional protein, cells were transfected with HRas12V, Raf130, and a Gal4-Elk1 construct. Elk1 is a transcription factor that is activated in response to activation of MAP kinases (29, 30). The Gal4-Elk1 construct contains

![Fig. 1. Blocking either Raf or MEK does not inhibit oncogenic Ras activation of NF-κB.](image-url)
the Gal4 DNA binding domain fused to the carboxyl-terminal transactivation domain of Elk1. Raf130 was able to inhibit the Ras activation of Gal4-Elk1 (see Fig. 1A), demonstrating that functional Raf130 was produced in these cells.

In addition to using dominant negative Raf to block MEK/ERK signaling, we have also used a commercially available MEK/ERK-inhibitory compound, PD98059, which inhibits MEK activation. To test the effect of this compound in our system, cells were cotransfected with the constructs shown in Fig. 1B. The medium on these cells was changed 16–24 h after transfection, and fresh medium containing 4 μM PD98059 was added to the cells. The cells were harvested 24 h later, and luciferase assays were performed. These results, shown in Fig. 1B, demonstrate that blocking the Raf/MEK/ERK pathway does not inhibit Ras activation of NF-κB-dependent gene expression or transcriptional activity. As a control, PD98059 was found to block Ras activation of Gal4-Elk1 (see Fig. 1B). In addition, PD98059 inhibited the activation of NF-κB by an activated Raf construct (RafBXB), showing that Raf activation of MEK/ERK is required for Raf to activate NF-κB. These data indicate that oncogenic Ras can activate NF-κB transcriptional activity independently of Raf.

**Ras Does Not Require an Interaction with Raf to Activate NF-κB**—Ras has been shown to interact with several different effectors in addition to Raf (31, 32). Selected mutations within the Ras effector domain (amino acids 32–40) result in a Ras protein that retains the ability to interact with some effectors while losing the ability to interact with others. For example, Ras40C and Ras37G interact with PI-3 kinase and Ras-GDS, respectively, but they no longer interact with Raf (7–9). An additional effector mutant, Ras35S, can interact with Raf, but it no longer interacts with PI-3 kinase or Ras-GDS (9). We have tested each of these Ras effector mutants for their ability to activate NF-κB-dependent gene expression. Each of the Ras effector mutants is a double mutant that contains the activating 12V mutation in addition to the effector loop mutation (9). NIH-3T3 cells were cotransfected with the effector mutants and the 3XκB-luc reporter plasmid, and the results are shown in Fig. 2. Each effector mutant activates NF-κB, showing that Ras utilizes effectors other than Raf to activate NF-κB. Similar results were obtained for activation of Gal4-p65 (data not shown).

**Raf Activates NF-κB through an Autocrine Feedback Loop**—Since oncogenic Ras does not require activation of Raf to activate NF-κB, we wanted to determine how oncogenic Ras is able to activate NF-κB. Activation of Raf has been shown to result in JNK activation in addition to ERK activation. However, activation of JNK occurs within 16–24 h of Raf activation, while ERK activation occurs after a few minutes (31). Experiments using conditioned medium from Raf-transformed cells have suggested that the delayed JNK activation is due to the release of autocrine factor. Since our results suggested that Ras does not require the activation of the Raf/MEK/ERK pathway to activate NF-κB, we wanted to determine if activation of NF-κB by Raf could be due to its delayed ability to activate JNK or a related kinase through the release of an autocrine factor.

First, we wanted to determine if there was a difference in the time it takes for Ras and Raf to activate NK-κB. NIH-3T3 cells were cotransfected with either HRas12V or RafBXB and 3XκB-luc. The cells were harvested at different time points after transfection as shown in Fig. 3A. These transient transfections show that Ras can activate NF-κB as early as 12 h after transfection, while Raf takes 24 h to give the same level of NF-κB activity. The same results are obtained when cells are cotransfected with Gal4-p65 (data not shown), indicating that
the effect on NF-κB activity occurs at the transcriptional activity level. This result suggested, but did not prove, that the ability of oncogenic Raf to activate NF-κB required an autocrine function.

To determine if the delayed activation of NF-κB by Raf could be due to the production of an autocrine factor, two different approaches were used. First, the medium on cells that had been transfected with HRas12V or RafBXB was changed every 8–10 h for 48 h after transfection. Changing the medium on RafBXB-transfected cells reduced NF-κB activity by approximately 3–4-fold, while changing the medium had no significant effect on the ability of oncogenic Ras to activate NF-κB (Fig. 3B). Next, medium from Ras- or Raf-transformed NIH-3T3 cells was collected and added to parental NIH-3T3 cells that had been transfected with 3X-B-luc (data not shown) or Gal4-p65. The cells were harvested 24 h later, and luciferase assays were performed. The cells that received medium from parental NIH-3T3 cells had little Gal4-p65 activity, while those that received medium from Ras- or Raf-transformed cells had approximately 6-fold higher Gal4-p65 activity (Fig. 3C). These results suggest that Raf does not activate NF-κB directly but that activation of Raf results in the production of an autocrine factor that can function to activate Ras or a signaling pathway utilized by Ras.

**Activation of the Rac Pathway Enhances NF-κB Activity**

The preceding results suggest that JNK or a JNK-related kinase may be required for Ras to activate NF-κB. Ras activates the JNK signaling pathway by activating the small GTP-binding proteins Rac and Cdc42. Activation of Rac leads to the activation of both JNK and p38 MAP kinases (11–13). To determine if activated Rac could activate NF-κB, NIH-3T3 cells were cotransfected with a constitutively active form of Rac, Rac115I, and 3X-B-luc. Fig. 4 shows that Rac115I can enhance NF-κB activation by approximately 2.0 μg of Rac115I, 0.4 μg of Gal4-p65, 1.0 μg of Gal4-Etk1, 2.0 μg of HRas12V, 2.0 μg of RafBXB, 3.0 μg SEK(KR). Cells were harvested 48 h after transfection, and luciferase activity was determined as described under “Experimental Procedures.” Fold luciferase activity was presented relative to the activity obtained from the transfection containing empty expression vector, whose value was placed at 1.0. Bars represent S.D. values obtained from at least three independent transfections performed in duplicate.

**Blocking JNK/p38 Signaling Inhibits Ras Activation of NF-κB**

A, NIH-3T3 cells were cotransfected with the indicated expression vectors at the following concentrations: 1.0 μg of 3X-B-luc, 1.0 μg of Gal5-luc, 0.4 μg of Gal4-p65, 1.0 μg of Gal4-Etk1, 2.0 μg of HRas12V, 2.0 μg of RafBXB, 3.0 μg SEK(KR). Cells were harvested 48 h after transfection, and luciferase activity was determined as described under “Experimental Procedures.” Fold luciferase activity is presented relative to the activity obtained from the transfection containing empty expression vector, whose value was placed at 1.0. Bars represent S.D. values obtained from at least three independent transfections performed in duplicate. B, cells were transfected as described for A except that 3.0 μg of PAK299R was used instead of SEK(KR).
that blocking the upstream JNK and p38 signaling pathways at multiple control points inhibits Ras activation of NF-κB.

Blocking p38 Activity Inhibits Ras Activation of NF-κB—The preceding results demonstrated that oncogenic Ras utilizes signaling components that can activate both JNK and p38 MAK kinases to activate NF-κB, but they have not shown whether JNK or p38 or both are required for Ras to activate NF-κB. p38 is phosphorylated, indicating that it is activated, in Ras-transformed cells; therefore, we wanted to determine if p38 was required for NF-κB activation in these cells. To do this, we used the commercially available p38 inhibitor compounds SB202190 and SB203580. These compounds were added to Ras-transformed cells that had been transfected with 3XκB-luc or Gal4-p65 and Gal5-luc. The results of these transfections are shown in Fig. 6. These experiments show that blocking p38 inhibits Ras activation of NF-κB-dependent gene expression and transcriptional activity. Ras-transformed cells were also transfected with Gal4-Elk1, which we have shown to be activated by the Raf/MEK/ERK pathway in our system. The addition of the p38 inhibitors had no effect on activation of Gal4-Elk1 (data not shown), while the addition of the MEK inhibitor PD98059 blocked Gal4-Elk1 activity in these cells. Additionally, dominant negative forms of JNK did not inhibit the ability of oncogenic Ras to activate κB-dependent gene expression (data not shown). These results demonstrate that p38 or a related kinase is involved in the activation of the transcription function of the p65/RelA NF-κB subunit in response to signals initiated by oncogenic Ras.

DISCUSSION

We have presented evidence, summarized in Fig. 7, that oncogenic Ras enhances NF-κB transcriptional activity through a signaling pathway that utilizes the p38 MAP kinase. Ras activates a MAP kinase signaling cascade that most likely includes the small GTP-binding protein Rac and the MEKK, PAK, and MKK4/SEK1 kinases. MKK4/SEK1 can activate both the JNK kinases and p38. However, none of the dominant negative JNK constructs we tested blocked Ras activation of NF-κB, while compounds that blocked p38 blocked Ras activation of NF-κB. Therefore, we conclude that p38 or a closely related kinase and/or the downstream kinases it activates are responsible for oncogenic Ras activation of NF-κB.

We have also shown that oncogenic Raf enhances NF-κB transcriptional activity apparently through the same pathway as Ras although by a different initiating mechanism. Raf activation of NF-κB most likely occurs through an autocrine feedback loop, since conditioned medium from Raf-transformed cells activated NF-κB transcriptional activity when added to parental cells. Raf has been shown to induce the expression of a epidermal growth factor and transforming growth factor-α, and Raf’s ability to activate the SAPKs, to which JNK and p38 belong, is assumed to require the production of these or related factors (31). We do not know the identity of the autocrine factor that Raf utilizes to activate NF-κB in our system, but it is possible that it is a epidermal growth factor or transforming growth factor-α. Recent work has shown that oncogenic Raf activates NF-κB in HEK 293 cells through an autocrine loop that activates the SAPKs (39). In that study, blocking the epidermal growth factor receptor, which binds both a epidermal growth factor and transforming growth factor-α, inhibited the ability of Raf to activate NF-κB-dependent gene expression. Future studies will address the identity of this factor in our system.

The p38 family of MAP kinases consists of four members, p38α, p38β, p38γ, and p38δ (e.g. see Ref. 40). These kinases are activated both by stress-inducing signals including osmotic shock and by UV irradiation and inflammatory cytokines like interleukin-1 and tumor necrosis factor-α (11–13). NF-κB is activated by many of same stimuli that activate p38, and recent evidence indicates that p38 plays a role in the ability of certain stimuli to activate NF-κB transcriptional activity (e.g. see Ref. 41). The exact mechanism that p38 uses to activate NF-κB is not understood. For example, we do not know if p38 phosphorylates NF-κB itself or if one of the p38-regulated kinases can phosphorylate NF-κB. Phosphorylation of the p65 NF-κB subunit has been shown to stimulate NF-κB transcriptional function (42, 43). Another possibility is that NF-κB is not the direct target of one of these kinases but that p38 targets a transcriptional coactivator such as CBP to stimulate transcriptional function. Additional studies will be required to determine if p38 acts directly on NF-κB or if it targets other components of the transcription machinery.

Ras utilizes multiple signaling components to transform cells. These include Raf, PAK, Rac, and PI3-K (1–3, 7, 38, 44),

\[ \text{J. L. Norris and A. S. Baldwin, Jr., unpublished results.} \]
and our data demonstrate that the transcription factor NF-κB is activated by Raf (Refs. 24 and 26; Fig. 1B), Rac (Fig. 4), and phosphatidylinositol 3-kinase and is inhibited by blocking PAK (Fig. 5B). Based on our data with oncogenic Ras and Raf, the p38 stress-activated kinase pathway appears to control this response. Since NF-κB has been found to be required for the ability of Ras to transform cells (26, 27), it will be important to determine whether the p38 pathway is a necessary component for providing the dominant negative SEK/MKK4 construct.

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REFERENCES
1. Boguski, M. S., and McCormick, F. (1993) Nature 366, 643–654
2. Khorasani, F., and Der, C. J. (1994) Cancer Metastasis Rev. 13, 67–89
3. Marshall, C. J. (1995) Cell 80, 179–195
4. Karin, M. (1996) J. Biol. Chem. 270, 16483–16486
5. Derijard, B., Hibi, M., Wu, I.-H., Barret, T., Su, B., Dent, T., Karin, M., and Davis, R. (1994) Cell 76, 1025–1037
6. Olsen, M., Ashworth, A., and Hall, A. (1995) Science 269, 1270–1272
7. Rodriguez-Viciana, P., Warner, P., Khwaja, A., Marte, B., Dappa, D., Das, P., Waterfield, M., Ridley, A., and Downward, J. (1997) Cell 80, 357–467
8. White, M., Nicolette, C., Minden, A., Polverino, A., Van Aelst, L., Karin, M., and Wigler, M. (1995) Cell 80, 533–541
9. Khorasani, F., White, M., Westwick, J., Solski, P., Chrzanowska-Wodnicka, M., Van Aelst, L., Wigler, M., and Der, C. J. (1996) Mol. Cell. Biol. 16, 3923–3933
10. Marshall, C. J. (1996) Curr. Opin. Cell Biol. 8, 197–204
11. Fanger, G. R., Gerwins, P., Wildmann, C., Jarpe, M. B., and Johnson, G. L. (1997) Curr. Opin. Genet. Dev. 7, 67–74
12. Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 24313–24316
13. Karin, M. (1996) Annu. N. Y. Acad. Sci. 851, 139–146
14. Baldwin, A. (1996) Annu. Rev. Immunol. 14, 649–681
15. Ghosh, S., May, M. J., and Kopp, E. B. (1998) Annu. Rev. Immunol. 16, 225–260
16. Schmitz, M. L., and Baeuerle, P. (1991) EMBO J. 10, 3805–3817
17. Ruben, S. M., Narayanan, R., Kleinert, J. F., Chen, C.-H., and Rosen, C. A. (1992) Mol. Cell. Biol. 12, 444–454
18. Regnier, C. H., Song, H. Y., Gao, X., Goeddel, D. V., Cao, Z., and Roth, M. (1997) Cell 80, 373–383
19. DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E., and Karin, M. (1997) Nature 388, 548–554
20. Zandi, E., Rothwarf, D. M., Delhase, M., Hayakawa, M., and Karin, M. (1997) Cell 91, 243–252
21. Mercurio, F., Zuh, H., Murray, B. W., Shevchenko, A., Bennett, B. L., Li, J., Young, D. B., Barba, M., Mann, M., Manning, A., and Rao, A. (1997) Science 280, 860–866
22. Woronicz, J. D., Gao, X., Cao, Z., Roth, M., and Goeddel, D. V. (1997) Science 280, 860–866
23. Galan, C., Garcia-Ramirez, J., Solski, P., Westwick, J., Der, C. J., Neznanov, N., Oshima, R., and Hauser, C. (1996) J. Biol. Chem. 271, 7992–7998
24. Finco, T., and Baldwin, A. S. Jr. (1993) J. Biol. Chem. 268, 17676–17679
25. Bruder, J. T., Heidecker, G., and Sapp, U. B. (1995) Genes Dev. 6, 545–556
26. Finco, T. S., Westwick, J. K., Norris, J. L., Beg, A. A., Der, C. J., and Baldwin, A. S. Jr. (1997) J. Biol. Chem. 272, 24113–24116
27. Maye, M. W., Wang, C.-Y., Cogswell, P. C., Rogers-Graham, K. S., Lowe, S. W., Der, C. J., and Baldwin, A. S. (1997) Science 278, 1812–1815
28. Cogswell, P. C., Mayo, M. W., and Baldwin, A. S. (1997) J. Exp. Med. 185, 491–497
29. Janknecht, R., Krust, W. H., Pingo, V. and Nordheim, A. (1993) EMBO J. 12, 5097–5104
30. Treisman, R. (1996) Curr. Opin. Cell Biol. 8, 205–215
31. McCarthy, S. A., Samuels, M. L., Pritchard, C. A., Abraham, J. A., and McMahon, M. (1994) Trends Cell Biol. 4, 24113–24116
32. Samuels, M. L., and McMahon, M. (1995) Mol. Cell. Biol. 14, 7855–7866
33. Perona, R., Montaner, S., Saniger, L., Sanchez-Perez, I., Bravo, R., and Lacal, J. (1997) Genes Dev. 11, 463–475
34. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M., and Zon. L. I. (1994) Nature 372, 794–798
35. Derijard, B., Rajagopal, J., Barrett, T., Wu, I. H., Han, J., Ulltewich, R. J., and David, R. J. (1995) Science 267, 682–685
36. Lin, A., Minden, A., Martinetti, H., Claret, F. X., Langer-Carter, C., Mercurio, F., Johnson, G. L., and Karin, M. (1995) Science 268, 286–290
37. Sellis, M. A., and Chernoff, J. (1997) Trends Cell Biol. 7, 162–167
38. Yang, Y., Chen, Z., Ambrose, D., Liu, J., Gibbs, J. B., Chernoff, J., and Field, J. (1997) Mol. Cell. Biol. 17, 445–446
39. Troppmair, J., Hartkamp, J., and Rapp, U. (1998) Oncogene 17, 685–690
40. Goodert, M., Cuenda, A., Craxton, M., Jakes, R., and Cohen, P. (1997) EMBO J. 16, 3663–3671
41. Vanden Berghe, W., Plaisance, S., Bois, E., De Bosscher, K., Schmitz, M. L., Fiers, W., and Haegeman, G. (1998) J. Biol. Chem. 273, 3285–3290
42. Zhong, H., SuYang, H., Erdjument-Bromage, H., Tempst, P., and Ghosh, S. (1997) Cell 89, 413–424
43. Wang, D., and Baldwin, A. S. (1998) J. Biol. Chem. 273, 29411–29416
44. Qiu, R. G., Chen, J., Kirn, D., McCormick, F., and Symons, M. (1995) Nature 374, 457–459