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κB Site-dependent Induction of Gene Expression by Diverse Inducers of Nuclear Factor κB Requires Raf-1*

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The transcription factor nuclear factor κB (NF-κB) is sequestered in the cytoplasm of most cell types where it is complexed with its inhibitor (IκB). A large variety of agents, including growth factors, the tumor promoter phorbol 12-myristate 13-acetate, and the cytokine tumor necrosis factor α, initiate signal transduction pathways that converge upon the NF-κB·IκB complex, resulting in the dissociation of IκB and the translocation of NF-κB to the nucleus. It has been demonstrated that the phosphorylation of IκB is associated with NF-κB activation, although the kinase(s) responsible for this process *in vivo* remain unknown. Here we demonstrate that expression of activated forms of the GTP-binding protein Ras or of the serine/threonine kinase Raf-1 results in the activation of transcription specifically through κB sites. This activation appears to be dependent on NF-κB, since co-expression of IκBα eliminates both Ras- and Raf-1-induced transcription. In addition, through the use of a dominant negative form of Raf-1, we show that Raf-1 is a common component utilized by multiple inducers in κB site-driven gene expression. These results illuminate a signal transduction pathway in which NF-κB/Rel family members participate and also implicate a pathway responsible for κB site-dependent gene expression during cell growth and in immune and inflammatory responses.

The proto-oncogene c-*raf* is the cellular homolog of v-*raf*, the acutely transforming gene of the avian sarcoma virus 3611 (1). The c-*raf* gene encodes a ubiquitous serine/threonine kinase, which is activated by a large variety of signals originating at the cell surface (Ref. 2; for review, see Ref. 3). Expression of the Raf-1 kinase activity is believed to play an essential role in mediating the transfer of these signals from the cell surface to the nucleus. In agreement with this view, Raf-1 expression can result in the induction of transcription from both cellular and viral promoters (4–7). A large body of work has recently identified Raf-1 as an essential downstream effector molecule of the membrane localized proto-oncogene product Ras (8–10). Like Raf-1, Ras is thought to be a key signal-transducing molecule involved in the process of cell growth and differentiation and is activated by various extracellular stimuli (for review, see Ref. 11). Two other participants in this pathway, MAP[1] kinase kinase and MAP kinase, lie downstream of Raf-1 and can themselves phosphorylate and activate additional kinases or other proteins, such as transcription factors (for review, see Ref. 12).

Transcription factors that comprise the NF-κB/Rel family are important participants in the regulation of many immune function and inflammation response genes as well as in the regulation of viral gene expression, including that of the human immunodeficiency virus (HIV) (for reviews, see Ref. 13–15). The most well characterized complex containing NF-κB/Rel family members, termed NF-κB, is a heterodimer composed of a 50-kDa (NFKB1) protein, derived from a 105-kDa precursor, and a 65-kDa protein (RelA) (14, 15). Other members of the NF-κB/Rel family include c-Rel, RelB, and p52/p100 (NFKB2) (14, 15). Various homo- and heterodimeric combinations of these family members can be formed *in vitro* and detected *in vivo* and are likely to be unique in at least some aspects of function. In addition, some members of the NF-κB/Rel family can functionally and physically interact with unrelated transcription factors such as C/EBPβ, also known as NF-IL-6 (16, 17).

NF-κB is typically localized in the cytoplasm where it is complexed to its inhibitor, IκB (18). Several forms of IκB have been identified, including IκBα and IκBβ (19). Activation of NF-κB involves dissociation of IκB, allowing active NF-κB to translocate to the nucleus and transcriptionally regulate target genes (18, 20–22). *In vitro* experiments have demonstrated that phosphorylation of IκB eliminates its ability to interact with NF-κB (23, 24). More importantly, it has recently been shown *in vivo* that the activation of NF-κB is accompanied by the phosphorylation (20) and subsequent loss of IκBα (20–22). However, the kinases responsible for this post-translational modification remain unknown. NF-κB, Ras, and Raf-1 are all activated by many of the same processes, including treatment of cells with growth factors (2, 25–27), stimulation with the tumor promoter PMA (2, 28, 29), and activation of the T-cell receptor (28, 30, 31). These observations, in conjunction with the apparent role of phosphorylation in NF-κB activation, prompted an investigation of whether a signal transduction pathway containing Ras and Raf-1 could contribute to the activation of NF-κB.

MATERIALS AND METHODS

Cells and Transfections—Balb/c 3T3 cells, obtained from American Type Culture Collection, were maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, penicillin, and streptomycin. DNA transfections were performed by the calcium phosphate precipitation method. Cells were seeded 24 h prior to transfection at 1 × 10^6 cells/100-mm plate. A precipitate was left on the cells for approximately 16 h, after which the cells were washed with phosphate-buffered saline; subsequently, fresh medium, containing 0.5% serum, was added. The plasmid pGEM was used to equalize the amount of DNA transfected in each experiment to 15 μg. In all experiments cells were incubated for an additional 48 h, then harvested, and the CAT activity

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[1] The abbreviations used are: MAP, mitogen-activated protein; PMA, phorbol 12-myristate 13-acetate; TNFα, tumor necrosis factor α; CAT, chloramphenicol acetyltransferase; HIV, human immunodeficiency virus; LTR, long terminal repeat; C/EBP, CCAAT/enhancer-binding protein.

determined. In indicated experiments, either TNFα, PMA, or serum was added 24 h prior to harvest. TNFα (obtained from Promega) was added to a final concentration of 10 ng/ml, PMA (obtained from Sigma) was added to a final concentration of 100 ng/ml, and for experiments involving induction by serum, the medium containing 0.5% serum was removed and fresh medium containing 10% serum added. In all cases, 1 unit of relative activity represents the CAT activity obtained after transfection of the reporter gene alone. All experiments were performed at least three times with similar results.

Plasmids—Plasmids encoding RafBXB, Raf301, v-H-Ras, and the B4X reporter were a kind gift of U. R. Rapp and are described elsewhere (32). The 3X-κB-CAT and 3X-mutκB-CAT (26), the HIV-LTR-CAT and HIV-ΔκB-CAT (31), and the IκBα expression vector (33) have been described previously. Multiple preparations of each plasmid were used in the experiments.

CAT Assay—Cells were washed, scraped in phosphate-buffered saline, pelleted in a centrifuge, and resuspended in 0.25 M Tris, pH 7.6. Cells were then lysed by freeze-thaw and the supernatant collected after pelleting to remove cellular debris. Equal amounts of protein, as determined by the Bradford assay (Bio-Rad), were then analyzed for chloramphenicol acetyltransferase activity using the fluor diffusion assay (34).

RESULTS

Ras and Raf-1 Activate Transcription through NF-κB Binding Sites—It was first determined whether the constitutively activated forms of Ras and Raf-1, v-H-Ras and RafBXB, respectively, could activate transcription through NF-κB binding sites in transient transfection assays. RafBXB lacks the amino-terminal domain, which functions to negatively regulate Raf-1 kinase activity. This deletion results in a constitutively active form of the protein (3, 32). These initial experiments utilized the reporter 3X-κB-CAT, which contains three copies of the class I major histocompatibility complex κB site. A similar construct, 3X-mutκB-CAT, containing three mutated κB sites, was also used. The mutated κB sites have been shown previously to be unresponsive to NF-κB (26). The results shown in Fig. 1*A* indicate that co-transfection into Balb/c 3T3 cells of vectors encoding v-H-Ras and the reporter plasmid 3X-κB-CAT led to a greater than 4-fold induction of gene expression. Since Raf-1 has been shown to lie downstream of Ras in other signal transduction pathways, it was of interest to determine if it too could activate transcription through κB sites. Co-transfection of RafBXB with 3X-κB-CAT resulted in a 12-fold activation of transcription (Fig. 1*A*), indicating that this serine/threonine kinase can activate gene expression through a κB site. To investigate whether these results could be demonstrated for κB sites in their native DNA context, we utilized a reporter plasmid containing the HIV-LTR attached upstream of the CAT gene (HIV-LTR-CAT). Previous studies have demonstrated that the κB sites found within the HIV-LTR bind NF-κB and significantly contribute to its transcriptional induction (31). Co-transfection of either RafBXB or v-H-Ras expression vectors with HIV-LTR-CAT resulted in 8- and 4-fold stimulation of gene expression, respectively (Fig. 1*B*). Neither the 3X-mutκB-CAT reporter nor a reporter containing mutated κB sites within the HIV-LTR linked to CAT (HIV-ΔκB-CAT) were activated when co-transfected with either RafBXB or v-H-Ras, demonstrating that the observed activation occurred specifically through functional κB sites (Fig. 1, *A* and *B*). Moreover, the vector in which RafBXB is inserted was not responsible for the κB site-dependent induction of gene expression since it did not activate transcription when containing an insert encoding a kinase-deficient form of Raf-1 (data not shown). These results confirm previous work indicating that the HIV-LTR κB sites are responsive to Ras (35) and further demonstrate that the serine/threonine kinase Raf-1 can mediate transcriptional induction specifically through NF-κB binding sites.

IκBα Specifically Inhibits Transcriptional Activation by Ras and Raf-1—IκBα appears to play a significant role in the regulation of the NF-κB/Rel family of transcription factors (19, 20–22). IκBα inhibits NF-κB activity by sequestering it in the cytoplasm and by inhibiting its DNA binding capacity (33, 36). Both of these properties of IκBα are specific for members of the NF-κB/Rel family of proteins (36). If NF-κB/Rel family members are mediating the induction of transcription observed by Ras and Raf-1, it may be possible to inhibit this activation by the introduction of IκBα. The co-transfection of vectors encoding v-H-Ras or RafBXB with 3X-κB-CAT resulted in a strong induction of transcription, as shown above and in Fig. 2*A*. Co-transfection of IκBα with RafBXB or v-H-Ras and 3X-κB-CAT resulted in a complete inhibition of activation. Essentially identical results were obtained using the HIV-LTR-CAT reporter plasmid (data not shown). The parent vector in which IκBα is inserted, psoCmin, had only a minor effect on activation by Ras and Raf-1 (data not shown). To determine if the inhibition by IκBα was specific for κB sites, the ability of IκBα to inhibit Ras and Raf-1 transcriptional activation through an AP-1/Ets site was tested. Previous studies have shown that Ras and Raf-1 induce expression from the AP-1/Ets site found within the enhancer of the polyomavirus (4, 32, 37). Co-transfection of vectors encoding v-H-Ras or RafBXB with B4X, a reporter plasmid containing four copies of the AP-1/Ets site upstream of CAT, resulted in an 8- or 17-fold activation, respectively (Fig. 2*B*). When IκBα was co-transfected with RafBXB or v-H-Ras and B4X, absolutely no inhibition was observed, demonstrating that the inhibitory effect of IκBα was specific for κB sites and also that IκBα did not alter the expression or directly inhibit the activity of v-H-Ras or RafBXB. Analysis of RafBXB protein levels by Western blot confirmed that its expression was not inhibited in the presence of IκBα (data not shown). The ability

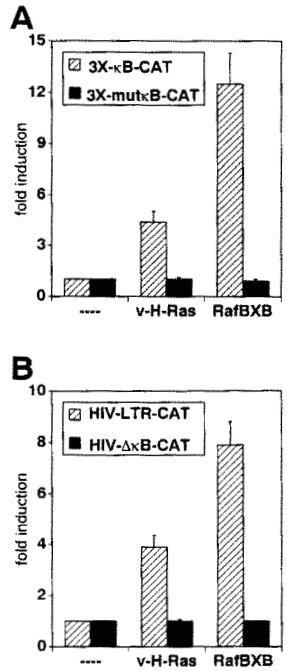

FIG. 1. **Ras and Raf-1 activate gene expression specifically through NF-κB binding sites.** Balb/c 3T3 cells were transfected with 5 μg of reporter plasmid either alone or together with 5 μg of plasmid encoding v-H-Ras or RafBXB by the calcium phosphate procedure as described under "Materials and Methods." *A*, 3X-κB-CAT or 3X-mutκB-CAT were transfected alone or together with v-H-Ras or RafBXB. *B*, HIV-LTR-CAT or HIV-ΔκB-CAT were transfected alone or with v-H-Ras or RafBXB. Results obtained using reporters containing functional κB sites 3X-κB-CAT or HIV-LTR-CAT are indicated with *striped bars*, and those using mutated κB sites 3X-mutκB-CAT or HIV-ΔκB-CAT are indicated with *solid bars*. The presence of v-H-Ras or RafBXB is indicated at the *bottom* of the histogram. The average and standard error of three separate experiments is shown.

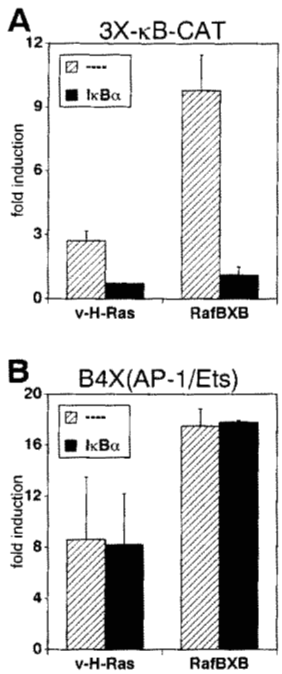

FIG. 2. **IκBα inhibits Ras- and Raf-1-induced gene expression mediated through NF-κB binding sites but not AP-1/Ets sites.** *A*, Balb/c 3T3 cells were transfected either with 5 μg of 3X-κB-CAT alone, together with 5 μg of v-H-Ras or RafBXB encoding plasmid, or with 5 μg of the plasmids encoding v-H-Ras or RafBXB as well as 0.25 μg of the IκBα-expressing plasmid. *B*, cells were transfected with the same combinations of plasmids as described in *A* except that 1 μg of B4X reporter, which contains four copies of the AP-1/Ets binding site fused upstream of CAT, was used in place of 3X-κB-CAT. The *striped bars* indicate the results obtained in the absence of IκBα, and the *solid bars* indicate the results in its presence. The presence of v-H-Ras or RafBXB is indicated *below* the histograms, and the reporter used is indicated *above* each figure. The average and standard error of three separate experiments is shown. Transfections were performed as described under "Materials and Methods."

of IκBα to inhibit Ras- and Raf-1-induced gene expression from κB site-driven reporters may indicate that this inhibitor is not a direct target of this pathway. Alternatively, the high level expression of IκBα following transfection may "mask" direct inactivation through the Ras/Raf pathway by providing a large pool of unmodified proteins able to inhibit NF-κB activity. In any case, these results suggest that members of the NF-κB/Rel family of transcription factors are targets of a signal transduction pathway containing Ras and Raf-1 that results in activation through κB sites.

Serum-, PMA-, and TNFα-induced Expression from a κB Site-driven Promoter Utilizes Raf-1—The transcription factor NF-κB and the signal-transducing molecules Ras and Raf-1 are activated by many of the same agents, including serum and the tumor promoter PMA. This observation, in addition to the above results, prompted an investigation into whether Raf-1 is a component of the signal transduction pathways initiated by serum or PMA that results in gene expression mediated through κB sites. Moreover, it was of interest to determine if other inducers of NF-κB, such as TNFα (38), also made use of Raf-1 in the activation of gene expression from κB sites. A dominant negative form of Raf-1 (Raf301) was utilized in these experiments. The lysine comprising the ATP binding site of Raf-1 was converted to a tryptophan in Raf301 (8, 32), eliminating its kinase activity and, more importantly, creating a protein with dominant negative properties specific for endogenous Raf-1 activity (32). The ability of Raf301 to inhibit Ras-induced gene expression through κB sites (data not shown) demonstrates that Raf-1 is downstream of Ras in this pathway and, furthermore, suggests that Raf301 should effectively block serum-, PMA-, and, TNFα-induced gene expression through a κB site if they also utilize Raf-1 in this process. The addition of either serum, PMA, or TNFα to quiescent Balb/c 3T3 cells subsequent to transfection with the 3X-κB-CAT reporter resulted in 6-, 2-, or 12-fold activation respectively, as shown in Fig. 3. The participation of members of the NF-κB/Rel family was demonstrated by the ability of co-transfected IκBα to inhibit stimulation of 3X-κB-CAT by all three inducers (Fig. 3). When the vector encoding the dominant negative form of Raf-1 (Raf301) was co-transfected with 3X-κB-CAT and the cells then stimulated with PMA, serum, or TNFα as before, a significant reduction in the inducibility of 3X-κB-CAT was observed (Fig. 3). Similar results were observed with the HIV-LTR-CAT reporter (data not shown). The inability of Raf301 to completely suppress activation by the inducers tested (including Ras) may reflect inefficiencies in its inhibitory function. This view is supported by the observation that other dominant negative forms of Raf-1 more effectively inhibited wild-type Raf-1 activity (32) and by the inability of other investigators to see complete inhibition by Raf301 (32, 39, 40). Alternatively, additional κB site-specific pathways downstream of Ras that do not include Raf-1 may exist. Neither of the reporters containing mutated κB sites, 3X-mutκB-CAT or HIV-ΔκB-CAT, were induced by serum, PMA, or TNFα, verifying that the observed results occurred specifically through NF-κB binding sites (data not shown). Since the dominant negative form of Raf-1 specifically inhibits endogenous Raf-1 activity (32), it can be concluded that Raf-1 is a necessary component in serum-, PMA-, and TNFα-induced transcription from κB site-dependent promoters.

DISCUSSION

The simplest interpretation of the data presented, when considered together with the apparent role of phosphorylation in NF-κB activation (20, 23, 24), is that Raf-1 itself or a kinase downstream of Raf-1 directly phosphorylates the NF-κB/IκB complex, resulting in the dissociation of IκB and the translocation of active NF-κB to the nucleus. However, other mechanisms could also explain the results presented. For example, it is conceivable that neither NF-κB nor IκB are post-translationally modified by this pathway but instead the immediate target is an unrelated molecule that can interact with NF-κB. Members of the C/EBP and AP-1 transcription factor families both

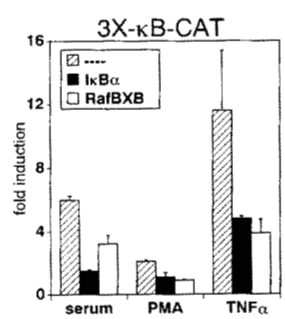

FIG. 3. **Multiple inducers of NF-κB utilize Raf-1 in κB site-dependent gene expression.** 5 μg of 3X-κB-CAT reporter plasmid was transfected either alone or together with 0.25 or 5 μg of plasmid expressing IκBα or Raf301 respectively. For the indicated experiments, cells were stimulated with either serum (final concentration = 10%), TNFα (final concentration = 10 ng/ml), or PMA (final concentration = 100 ng/ml) 24 h prior to harvest. *Striped bars* are results obtained in the absence of Raf301 or IκBα, *solid bars* are in the presence of IκBα, and *open bars* are in the presence of Raf301. The inducers are indicated *below* the histogram and the reporter used is shown *above* the figure. The average and standard error of three separate experiments is shown. Transfections and the addition of inducers were as described under "Materials and Methods."

physically and functionally interact with NF-κB subunits (16, 17).[2] Furthermore, their transcriptional activity is enhanced by direct phosphorylation mediated by a pathway containing Ras (42, 43). In this scenario, AP-1 and/or C/EBP family members would be directly phosphorylated by this pathway, modifying their interaction with NF-κB, and resulting in a complex with both κB site-specific binding activity and enhanced transcriptional inducibility. Although NF-κB would not be directly targeted, its transactivation ability would be enhanced by the altered interaction with phosphorylated AP-1 and/or C/EBP members.

Although Ras and Raf-1 have been generally investigated and characterized in terms of their role in cell growth and differentiation, another function of these proteins is in the transduction of signals during immune and inflammation responses. For example, engagement of the T-cell receptor or interaction of the lymphokine interleukin-2 with its receptor results in the activation of Ras, Raf-1, and likewise NF-κB (28, 30, 31, 44, 45). The evidence presented here indicates that the inflammatory cytokine TNFα also utilizes Raf-1 in κB site-dependent activation of transcription, an observation that is supported by preliminary data demonstrating that TNFα treatment of Balb/c 3T3 cells results in a modified form of the Raf-1 protein.[3] The presence of this slower migrating form of Raf-1 on a SDS-PAGE gel has been shown to correlate with the induction of Raf-1 kinase activity (3). It is conceivable that additional agents that activate NF-κB during immune and inflammation responses do so through a signal transduction pathway containing Ras and Raf-1.

An increasing amount of evidence suggests that NF-κB is required during cell proliferation. For instance, NF-κB activation has been observed to occur specifically during the G_0-G_1 phase in 3T3 fibroblasts (26), a time when various immediate early genes are activated, including the proto-oncogene c-*myc* and the melanoma stimulatory factor-encoding gene, *gro* (46, 47). Interestingly, the promoter regions of both these genes contain κB sites, and, more importantly, these sites are required for their induction in response to various stimuli (48, 49). Further evidence implicating NF-κB in the regulation of cell growth is the discovery that truncated (v-*Rel*), overexpressed (Bcl-3), differentially spliced (p65Δ), and rearranged (Lyt-10) members of the NF-κB or IκB family are potentially oncogenic (14, 50). Finally, it has been demonstrated that at least one type of transformed cell, fibroblasts transformed by the human T cell leukemia virus-1 Tax protein, can be reverted to the non-transformed state through the inhibition of NF-κB activity (41). These observations, when considered together with the ubiquitous nature of NF-κB and its activation by a diverse group of agents, strongly suggest a more general role for this transcription factor in the regulation of cell growth.

The activation of NF-κB/Rel family members by growth stimulatory proteins such as Ras and Raf-1 is consistent with the known function of these proteins during cellular proliferation and in immune and inflammation responses. Although it is presently unclear whether members of the NF-κB/Rel family are directly activated by the signal transduction pathway defined here, it is apparent that the transcription factors that comprise this family are important in the conversion of signals carried by Ras and Raf-1 in the cytoplasm to changes in gene expression within the nucleus.

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[2] B. Stein, A. S. Baldwin, Jr., D. Ballard, W. C. Greene, P. Angel, and P. Herrlich, *EMBO J.*, in press.

[3] T. S. Finco and A. S. Baldwin, Jr., unpublished observation.

REFERENCES

1. Rapp, U. R., Goldborough, M. D., Mark, G. E., Bonner, T. I., Groffen, J., Reynolds, F. H., Jr., and Stephenson, F. R. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 4218–4222
2. Morrison, D. K., Kaplan, D. R., Rapp, U. R., and Roberts, T. M. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 8855–8859
3. Rapp, U. R. (1991) *Oncogene* **6,** 495–500
4. Wasylyk, C., Wasylyk, B., Heidecker, G., Huleihel, M., and Rapp, U. R. (1989) *Mol. Cell. Biol.* **9,** 2247–2250
5. Kaibuchi, K., Fukumoto, Y., Oku, N., Hori, Y., Yamamoto, T., Toyoshima, K., and Takai, Y. (1989) *J. Biol. Chem.* **264,** 20855–20858
6. Qureshi, S. A., Rim, M., Bruder, J., Kolch, W., Rapp, U. R., Sukhatme, V. P., and Foster, D. A. (1991) *J. Biol. Chem.* **266,** 20594–20597
7. Jamal, S., and Ziff, E. (1990) *Nature* **344,** 463–466
8. Kolch, W., Heidecker, G., Lloyd, P., and Rapp, U. R. (1991) *Nature* **349,** 426–428
9. Wood, K. W., Sarnecki, C., Roberts, T. M., and Blenis, J. (1992) *Cell* **68,** 1041–1050
10. Williams, N. G., Roberts, T. M., and Li, P. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 6924–6928
11. Satoh, T., Nakafuku, M., and Kaziro, Y. (1992) *J. Biol. Chem.* **267,** 24149–24152
12. Leevers, S. J., and Marshall, C. J. (1992) *Trends Cell Biol.* **2,** 283–286
13. Baeuerle, P. A., and Baltimore, D. (1991) in *Hormonal Control Regulation of Gene Expression* (Cohen, P., and Foulkes, J. G., eds) pp. 409–432, Elsevier/North Holland Biomedical Press, Amsterdam
14. Grilli, M., Chiu, J. S., and Lenardo, M. J. (1993) *Int. Rev. Cytol.* **143,** 1–62
15. Nolan, G. P., and Baltimore, D. (1992) *Curr. Opin. Genet. Dev.* **2,** 211–224
16. LeClair, K. P., Blanar, M. A., and Sharp, P. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 8145–8149
17. Stein, B., Cogswell, P. C., and Baldwin, A. S., Jr. (1993) *Mol. Cell. Biol.* **13,** 3964–3974
18. Baeuerle, P. A., and Baltimore, D. (1988) *Science* **242,** 540–546
19. Zabel, U., and Baeuerle, P., (1990) *Cell* **61,** 255–265
20. Beg, A. A., Finco, T. S., Nantermet, P. V., and Baldwin, A. S. (1993) *Mol. Cell. Biol.* **13,** 3301–3310
21. Brown, K., Park, S., Kanno, T., Franzoso, G., and Siebenlist, U. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 2532–2536
22. Sun, S.-C., Ganchi, P. A., Ballard, D. W., and Greene, W. C. (1993) *Science* **259,** 1912–1915
23. Ghosh, S., and Baltimore, D. (1990) *Nature* **344,** 678–682
24. Shirakawa, F., and Mizel, S. B. (1989) *Mol. Cell. Biol.* **9,** 2424–2430
25. Olashaw, N. E., Kowalik, T. F., Huang, E-S., and Pledger, W. J. (1992) *Mol. Biol. Cell* **3,** 1131–1139
26. Baldwin, A. S., Jr., Azizkhan, J. C., Jensen, D. E., Beg, A. A., and Coodly, L. R. (1991) *Mol. Cell. Biol.* **11,** 4943–4951
27. Mulcahy, L. S., Smith, M. R., and Stacey, D. W. (1985) *Nature* **313,** 241–243
28. Downward, J., Graves, J. D., Warne, P. H., Rayter, S., and Cantrell, D. A. (1990) *Nature* **346,** 719–723
29. Sen, R., and Baltimore, D. (1986) *Cell* **47,** 921–928
30. Siegel, J. N., Klausner, R. D., Rapp, U. R., and Samelson, L. E. (1990) *J. Biol. Chem.* **265,** 18472–18480
31. Nabel, G., and Baltimore, D. (1987) *Nature* **326,** 711–713
32. Bruder, J. T., Heidecker, G., and Rapp, U. R. (1992) *Genes & Dev.* **6,** 545–556
33. Beg, A. A., Ruben, S. M., Scheinman, R. I., Haskill, S., Rosen, C. A., and Baldwin, A. S., Jr. (1992) *Genes & Dev.* **6,** 1899–1913
34. Neuman, J. R., Morency, C. A., and Russian, K. O. (1987) *BioTechniques* **5,** 444–448
35. Arenzana-Seisdedos, F., Israel, N., Bachelerie, F., Hazan, U., Alcami, J., Dautry, F., and Virelizier, J.-L. (1989) *Oncogene* **4,** 1359–1362
36. Haskill, S., Beg, A. A., Tompkins, S. M., Morris, J. S., Yurocko, A. D., Sampson-Johannes, A., Mondal, K., Ralph, P., and Baldwin, A. S., Jr., (1991) *Cell* **65,** 1281–1289
37. Imler, J. L., Schatz, C., Wasylyk, C., Chatton, B., and Wasylyk, B. (1988) *Nature* **332,** 275–278
38. Osborn, L., Kunkel, S., and Nabel, G. J. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 2336–2340
39. Qureshi, S. A., Rim, M., Bruder, J., Kolch, W., Rapp, U., Sukhatme, V. P., and Foster, D. A. (1991) *J Biol. Chem.* **266,** 20594–20597
40. Alexandropoulos, K., Qureshi, S. A., Bruder, J. T., Rapp, U., and Foster, D. A. (1992) *Cell Growth Diff.* **3,** 731–737
41. Kitajima, I., Shinohara, T., Bilakovics J., Brown, D. A., Xu, X., and Nerenberg, M. (1992) *Science* **258,** 1792–1795
42. Nakajima, T., Kinoshita, S., Sasagawa, T., Sasaki, K., Naruto, M., Kishimoto, T., and Akira, S., (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 2207–2211
43. Binteruy, B., Smeal, T., and Karin, M. (1991) *Nature* **351,** 122–127
44. Turner, B., Rapp, U., App, H., Greene, M., Dobashi, K., and Reed, J (1991) *Proc. Natl. Acad. Sci.* **88,** 1227–1231
45. Arima, N., Kuziel, W. A., Grdina, T. A., and Greene, W. C. (1992) *J. Immunol.* **149,** 83–91
46. Greenberg, M., and Ziff, E., (1984) *Nature* **311,** 433–438
47. Anisowicz, A., Bardwell, L., and Sager, R. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 7188–92
48. Duyao, M., Buckler, A., and Sonnenshein, G. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 4727–4732
49. Anisowicz, A., Messineo, M., Lee, S. W., and Sager, R. (1991) *J. Immunol.* **147,** 520–527
50. Narayanan, R., Klement, J. F., Ruben, S. M., Higgins, K. A., and Rosen, C. A. (1992) *Science* **256,** 367–370