Brief Definitive Report

Activation of the p21<sup>ras</sup> Pathway Couples Antigen Receptor Stimulation to Induction of the Primary Response Gene egr-1 in B Lymphocytes
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Summary
The primary response gene egr-1 encodes a sequence-specific transcription factor whose expression is necessary for antigen receptor-stimulated activation of B lymphocytes. The molecular processes involved in linking egr-1 induction to antigen receptor signaling have not been defined. The present study demonstrates that expression of an activated form of p21<sup>ras</sup> results in egr-1 induction similar to that previously shown after antigen receptor cross-linking. In addition, both antigen receptor cross-linking and p21<sup>ras</sup> use the same element in the egr-1 promoter to exert their effects. Using dominant-negative mutants of p21<sup>ras</sup> and raf-1, we demonstrate that induction of egr-1 after antigen receptor cross-linking is mediated by activation of the p21<sup>ras</sup>/mitogen-activated protein kinase signaling pathway. While regulation of the p21<sup>ras</sup> pathway during B cell activation has been intensively studied, this report represents the first description of a biologically relevant event associated with its activation.

Activation and differentiation of B lymphocytes is initiated by an interaction between a multivalent antigen and the antigen receptor (BCR) on the cell surface. As the activation signal enters the cytoplasm, a variety of biochemical changes can be detected. These changes include phosphotidylinositol hydrolysis with subsequent Ca<sup>2+</sup> mobilization and protein kinase C (PKC) activation, and increased tyrosine kinase activity (for review see reference 1). In addition, the low molecular weight G protein p21<sup>ras</sup> is activated after antigen receptor cross-linking on B cells (2–4). These studies were prompted by an early report that found that p21<sup>ras</sup> colocalized with the antigen receptor after cross-linking and aggregation (5).

The p21<sup>ras</sup> signaling pathway has been characterized in a variety of cell types. As a G protein, p21<sup>ras</sup> is active only in the GTP-bound state (6). Two mechanisms for regulating the activation state of p21<sup>ras</sup> have been described. Guanine nucleotide exchange factors (GEFs) augment the exchange of GDP for GTP and are positive activators, while GTPase activating proteins (GAPs) result in inactivation of p21<sup>ras</sup> (6).

Directly downstream of p21<sup>ras</sup> in this signaling pathway is the activation of the serine/threonine kinase raf-1 (7). The mechanism by which p21<sup>ras</sup> activates raf-1 is not entirely understood, but recent reports suggest that it results from recruitment of raf-1 to the cell membrane (8, 9). Activated raf-1 phosphorylates members of the MAPK/ERK kinase (MEK) family of dual specificity kinases (10). After phosphorylation, MEKs activate the mitogen activated protein kinases (MAPKs) by threonine and tyrosine phosphorylation (11). MAPK has been directly linked to the control of transcription due to phosphorylation of certain transcription factors, including c-JUN (12) and a group of ETS family members collectively known as p62TCF (13). While the linearity of the p21<sup>ras</sup>/raf-1/MAPK pathway has been confirmed in many receptor systems, points of divergence have also been described (14).

In B lymphocytes, activation of the p21<sup>ras</sup> pathway after BCR cross-linking has been extensively studied (1). The GEF vav is activated (15, 16) and the GAP factors p120 (3, 17) and neurofibromin (18) inactivated after BCR cross-linking. In addition, recent studies (18a) have shown recruitment of the GEF SOS-1 to the BCR following receptor cross-linking. Activation of raf-1, MEK, and MAPK by BCR cross-linking have all been demonstrated (2). As yet, however, no downstream effectors of the p21<sup>ras</sup>/MAPK pathway have been described in B lymphocytes. This is in contrast to T lymphocytes, where induction of the IL-2 gene, an event critical for antigen receptor–initiated proliferation, appears to be mediated by activation of the p21<sup>ras</sup>/MAPK pathway (19, 20).

Among the earliest genetic events detected after BCR cross-linking is induction of the primary response gene egr-1 (1). Antisense studies have demonstrated that induction of egr-1 is necessary for antigen receptor–mediated activation of both B and T lymphocytes (21, 22). This is presumably accomplished by induction of a second wave of function-related genes whose regulation is controlled by the transcription factor encoded by egr-1.

Our recent results indicate that egr-1 induction after BCR cross-linking requires a serum response element (SRE) and adjacent ETS motifs in the egr-1 promoter (McMahon, S.B.,...
and J.G. Monroe, manuscript submitted for publication). A similar combination of SRE/ETS motifs mediates c-fos induction in fibroblasts in response to serum growth factors (for review see reference 13). The c-fos SRE is occupied by a ternary complex composed of a homodimer of serum response factor (SRF) and a single molecule of p62TCF, which binds the adjacent ETS motif (13). Transcription of c-fos results from phosphorylation of p62TCF by MAPK (13). Because of the similarity in expression pattern and regulatory elements between c-fos and egr-1, this study was undertaken to determine whether the p21\textsuperscript{TM}/MAPK pathway was responsible for egr-1 induction in B cells. Using dominant-negative mutants of p21\textsuperscript{TM} and raf-1, we demonstrate that in B lymphocytes, induction of egr-1 depends on activation of the p21\textsuperscript{TM} pathway. This study represents the first demonstration of any functional consequence of p21\textsuperscript{TM} activation after BCR cross-linking.

Materials and Methods

B Lymphocyte Isolation and Culture. The spleens of 8- to 12-wk BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were removed aseptically from animals killed by cervical dislocation. Spleens were minced and T lymphocytes removed by treatment with anti-Thy-1.2 (HO13.4) antisera and rabbit complement (Pel-Freeze Biologicals, Rogers, AZ). Red blood cells were removed by hypotonic lysis and the remaining cells spun over a 75% Percoll cushion. Cells were placed in culture for 72 h in B cell assay media (RPMI 1640 with 10% FCS, 2 mM L-glutamine, nonessential amino acids, 100 \mu g/ml each of penicillin and streptomycin, and 5 x 10\textsuperscript{-5} M 2-ME) with LPS (Salmonella Typhosa; Sigma Chemical Co., St. Louis, MO) at 50 \mu g/ml. After 72 h of culture, B cell blasts were transiently transfected with vectors, as described below.

Plasmid Construction. The reporter plasmid pBL395 contains egr-1 promoter sequences from −395 to +65 linked to the gene encoding chloramphenicol acetyltransferase (CAT). The plasmid containing point mutations in SRE #4 was generated by the PCR method with Vent (Exo-) polymerase (New England Biolabs, Beverly, MA). The expression vector pEJ encodes a constitutively active form of c-Ha-ras (25) and was provided by Dr. R. Muschel (University of Pennsylvania, Philadelphia, PA). The expression vector pASN17 encodes a p21\textsuperscript{TM} molecule, which is constitutively active due to a substitution at amino acid 12 (glycine → valine), resulting in decreased GTP hydrolysis and accumulation of p21\textsuperscript{TM} molecules in the active, GTP-bound form (25).

Results

Expression of a Constitutively Active Form of p21\textsuperscript{TM} Activates the egr-1 Promoter in B Lymphocytes.Previous studies have demonstrated that promoter elements including an SRE and two adjacent ETS motifs mediate egr-1 induction in response to BCR cross-linking (McMahon, S.B., and J.G. Monroe, manuscript submitted for publication). Because of recent studies demonstrating that activation of the p21\textsuperscript{TM}/MAPK pathway is responsible for SRE-dependent expression of c-fos in fibroblasts (13), the participation of this pathway in induction of egr-1 in B lymphocytes was evaluated. B cell blasts were transiently transfected with a combination of a construct (pBL395) containing the egr-1 promoter upstream of the CAT reporter gene and the p21\textsuperscript{TM} expression vector pEJ (25). pBL395 contains egr-1 promoter sequences from −395 to +65 which includes four SREs, a cAMP response element, and a binding site for the EGR-1 protein, as well as potential binding sites for other transcription factors (23). The pEJ vector encodes a p21\textsuperscript{TM} molecule, which is constitutively active. As is evident in Fig. 1, cotransfection of pEJ resulted in a dose-dependent increase in transcription from the egr-1 promoter as assessed by CAT activity. Cotransfection of the empty vector (pBR322) had no detectable effect on egr-1 promoter activity.

The Promoter Element Mediating egr-1 Induction by Activated p21\textsuperscript{TM} Colocalizes with the Element Mediating Induction by BCR Cross-linking. While the results presented in Fig. 1 indicate that activated p21\textsuperscript{TM} causes an increase in the activity of a construct containing a large region of the egr-1 promoter, these data fail to address whether p21\textsuperscript{TM} and BCR cross-linking use the same cis-regulatory elements. Our previous studies demonstrated that induction of egr-1 after BCR cross-linking in B lymphocytes was dependent on the presence of an SRE at −360 (SRE #4) (McMahon, S.B., and J.G. Monroe, manuscript submitted for publication). To determine whether expression of activated p21\textsuperscript{TM} mimics BCR cross-linking in this regard, an egr-1 promoter containing a mutated version of SRE #4 was tested for activity in the presence of activated p21\textsuperscript{TM}. Like pBL395, this CAT reporter construct contains egr-1 promoter sequences from −395 to +65. However, four point mutations were introduced into the core of SRE #4. These mutations have previously been shown to inhibit SRF binding to the c-fos SRE and consequently abolish transcription mediated by this element (29).
A key intermediate in the MAPK pathway is the serine/threonine kinase raf-1 (10). To determine whether p21ras activation was necessary for antigen receptor-mediated induction, a dominant-negative version of raf-1 (NAraf) was used (27). NAraf contains only the NH2-terminal regulatory region of Raf-1, lacks the COOH-terminal kinase domain, and efficiently blocks Raf-1-mediated events (10). To determine whether p21ras activation after antigen receptor cross-linking was functioning through this pathway, a dominant-negative version of Raf-1 (NAraf) was used (27). NAraf contains only the NH2-terminal regulatory region of Raf-1, lacks the COOH-terminal kinase domain, and efficiently blocks Raf-1-mediated events (27). When an expression vector (pmt-NAraf) encoding the dominant-negative mutant was cotransfected into B cells along with pBL395, induction of egr-1 promoter activity was substantially decreased (Fig. 4). As with the p21ras dominant-negative vector, this decrease was evident whether the

Expression of a Dominant-negative Mutant of p21ras Inhibits BCR-mediated Induction of egr-1 in B Lymphocytes. The demonstration that egr-1 promoter activity is induced by a constitutively active form of p21ras fails to directly address the role of the endogenous p21ras molecule in mediating egr-1 induction during BCR-mediated activation of B lymphocytes. To determine whether activation of p21ras plays a role in this process during normal BCR signaling, a construct encoding a dominant-negative mutant of p21ras (pASN17) was transfected into B lymphocytes (26). The dominant-negative p21ras mutant contains a substitution at position 17 (serine → asparagine) (26). Position 17 is adjacent to the nucleotide binding pocket and, by analogy to other G proteins (26), may be critical for interacting with the γ phosphate of GTP. ASN17 is consequently 40-fold less efficient at binding GTP in vitro (26) and presumably inhibits the function of normal p21ras molecules by sequestering the cellular pool of GEF into inactive complexes (6). Cotransfection studies were performed with pASN17 (or the empty vector, pMSG) together with the egr-1 promoter construct pBL395. As demonstrated in Fig. 3 A, the induction of egr-1 promoter activity after BCR cross-linking is decreased by ~75% by cotransfection of pASN17. Induction of egr-1 was inhibited at two different ratios of the dominant-negative/reporter vectors (1:20 and 1:2). Somewhat unexpectedly, induction of egr-1 promoter activity in response to treatment with the phorbol ester PMA was also inhibited by the expression of ASN17 (Fig. 3 B). Like the inhibition of antigen receptor-mediated induction, this effect was evident at two different ratios of the dominant-negative/reporter vectors. Inhibition of PMA-mediated egr-1 induction by ASN17 suggests that at least some of the effects of PMA in B cells occur upstream of p21ras activation.

Expression of a Dominant-negative Mutant of Raf-1 Inhibits Antigen Receptor-mediated Induction of egr-1. Activation of p21ras by growth-promoting agents in all cell types that have been examined results in activation of MAPK. As discussed above, a key intermediate in this cascade is the serine/threonine kinase Raf-1 (10). To determine whether p21ras activation after antigen receptor cross-linking was functioning through this pathway, a dominant-negative version of Raf-1 (NAraf) was used (27). NAraf contains only the NH2-terminal regulatory region of Raf-1, lacks the COOH-terminal kinase domain, and efficiently blocks Raf-1-mediated events (27). When an expression vector (pmt-NAraf) encoding the dominant-negative Raf-1 mutant was cotransfected into B cells along with pBL395, induction of egr-1 promoter activity was substantially decreased (Fig. 4). As with the p21ras dominant-negative vector, this decrease was evident whether the

The wild-type or mutated versions of pBL395 were cotransfected into B cell blasts with the p21ras expression vector pEJ. As shown in Fig. 2, expression of activated p21ras strongly induced CAT activity from the wild-type egr-1 promoter (3.94% conversion). Mutation of SRE #4 resulted in a marked decrease in CAT activity (0.15% conversion). These values reflect a decrease from 131-fold induction with the wild-type promoter to 5-fold with the mutant SRE promoter (relative to levels obtained with the empty cassette pBR322), indicating that SRE #4 is necessary for the induction of egr-1 promoter activity by p21ras.

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Figure 3. Expression of a dominant-negative form of p21\textsuperscript{TM} inhibits egr-1 induction in B cells. LPS-induced B cell blasts were cotransfected with 20 \( \mu \)g of the egr-1 promoter/CAT construct pBL395 and 1 \( \mu \)g (1) or 10 \( \mu \)g (10) of the p21\textsuperscript{TM} dominant-negative expression vector pASN-17 (or the empty cassette pMSG). Transfected cells were stimulated with either goat anti-\( \mu \) antibodies at 20 \( \mu \)g/ml (A) or PMA at 10 ng/ml (B), as described in Materials and Methods. Results of the CAT assay are expressed as fold induction.

Discussion

The results presented here demonstrate that activation of the p21\textsuperscript{TM} pathway couples antigen receptor cross-linking to induction of egr-1 in B lymphocytes. Transfection of a constitutively active form of p21\textsuperscript{TM} resulted in a dose-dependent increase in egr-1 promoter activity. Previous studies (McMahon, S. B., and J. G. Monroe, manuscript submitted for publication) demonstrated that induction of egr-1 after antigen receptor cross-linking requires the presence of a specific SRE, that is, SRE #4. In this study, we demonstrate that SRE #4 is also necessary for egr-1 induction in response to activated p21\textsuperscript{TM}. Finally, using dominant-negative mutants of p21\textsuperscript{TM} and raf-1, we demonstrate that induction of egr-1 promoter activity after BCR cross-linking involves activation of the p21\textsuperscript{TM}/raf-1/MAPK pathway. By using transient transfection of primary B cells, we have been able to directly implicate this pathway in activation of the egr-1 promoter.

In addition to stimulation of B lymphocytes by antigen receptor cross-linking, we examined the effects of PMA treatment. PMA treatment directly activates PKC. As such, the finding that the dominant-negative p21\textsuperscript{TM} mutant inhibited the PMA effect was somewhat unexpected and suggests that the effect of PMA on egr-1 transcription is mediated through the p21\textsuperscript{TM} pathway. When these studies were initiated, PKC activation had not been shown to effect any of the early events in the p21\textsuperscript{TM} pathway. Recent reports have suggested a mechanism for the inhibition seen in Fig. 3 by demonstrating that in addition to activating PKC, PMA can directly bind and activate the GEF \\textit{sra} (30). This potential effect of PMA on an early regulatory component in the p21\textsuperscript{TM} pathway may explain the results obtained here, which show that inhibitory mutants of p21\textsuperscript{TM} and raf-1 block induction of egr-1 by PMA.

As already noted, in B lymphocytes, egr-1 induction after BCR cross-linking or by expression of a constitutively active form of p21\textsuperscript{TM} (shown here) requires a specific SRE (SRE #4). This is noteworthy since the egr-1 gene contains five SREs, each of which are capable of binding SRF and regulating transcription in various cell types. In myeloid cells, for example, a variety of stimuli, including PMA, preferentially require SRE #5 for induction (31), while in PC12 cells, SREs #1 and #2 are necessary for nerve growth factor-induced expression (32). Similar to the studies presented here, expression of the v-raf oncogene in fibroblasts induces egr-1 promoter activity with a preference for SRE #4 (33). These results suggest a requirement for SRE #4 by receptors and stimuli that activate the p21\textsuperscript{TM} signaling pathway.

A potential explanation for preferential utilization of SRE #4 by stimuli that activate the p21\textsuperscript{TM} pathway may be provided by recent studies. Induction of c-fos by growth factors in fibroblasts results from phosphorylation of p62TCF by MAPK (13). Furthermore, p62TCF binds to a subset of ETS motifs that are adjacent to SREs (13). In the egr-1 promoter,
motifs that are adjacent to SREs (13). In the egr-1 promoter, high affinity p62TCF binding sites occur at only three locations, two of which are adjacent to SRE #4 (13, 23). We have recently shown that the two p62TCF binding motifs adjacent to SRE #4 are necessary for egr-1 promoter activity in response to BCR cross-linking or PMA treatment ( McMahon, S. B., and J. G. Monroe, manuscript submitted for publication). Therefore, the presence of these motifs adjacent to SRE #4 is likely to mediate its preferential utilization in response to antigen receptor activation of the p21TM pathway in B lymphocytes by allowing efficient ternary complex formation by p62TCF and SRF.

Involvement of the p21TM pathway in BCR-generated signal transduction in B lymphocytes has been the subject of intensive study. Consequently, a significant amount of information is available regarding the proteins that regulate p21TM in B cells (4, 5, 15-17). In addition, several studies have identified the kinases that are active in this pathway (1, 2). However, to date, no functional consequence of p21TM activation in B cells has been demonstrated. In this report, we show that induction of egr-1, a gene whose expression is necessary for lymphocyte activation, is mediated by the p21TM pathway after cross-linking of the B cell antigen receptor. These findings assign a crucial role to p21TM in B cell activation.

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References

1. Cambier, J.C., C.M. Pleiman, and M.R. Clark. 1994. Signal transduction by the B cell antigen receptor and its coreceptors. Annu. Rev. Immunol. 12:457-486.
2. Tordai, A., R.A. Franklin, H. Patel, A.M. Gardner, G.L. Johnson, and E.W. Gelfand. 1994. Cross-linking of surface IgM stimulates the ras/raf-1/MEK/MAPK cascade in human B lymphocytes. J. Biol. Chem. 269:7538-7543.
3. Lazarus, A.H., K. Kawauchi, M.J. Rapoport, and T.L. Delovitch. 1993. Antigen-induced B lymphocyte activation involves the p21tm and ras/GAP signaling pathway. J. Exp. Med. 178:1765-1769.
4. Harwood, A.E., and J.C. Cambier. 1993. B cell antigen receptor cross-linking triggers rapid protein kinase C independent activation of p21tm. J. Immunol. 151:4513-4522.
5. Graziaedi, L., K. Rjabowol, and D. Bar-Sagi. 1990. Co-capping of ras proteins with surface immunoglobulins in B lymphocytes. Nature (Lond.). 347:396-400.
6. Boguski, M.S., and F. McCormick. 1993. Proteins regulating Ras and its relatives. Nature (Lond.). 366:643-654.
7. Vojtek, A.B., S.M. Hollenberg, and J.A. Cooper. 1993. Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell. 74:204-214.
8. Leevers, S.J., H.F. Paterson, and C.J. Marshall. 1994. Requirement for Ras in Raf activation is overcome by targeting Raf to the plasma membrane. Nature (Lond.). 369:411-414.
9. Stokoe, D., S.G. Macdonald, K. Cadwallader, M. Symons, and J.F. Hancock. 1994. Activation of ras as a result of recruitment to the plasma membrane. Science (Wash. DC). 264:1463-1467.
10. Kyriakis, J.M., H. App, X.F. Zhang, P. Banerjee, D.L. Bruntigan, U.R. Rapp, and J. Avruch. 1992. Raf-1 activates MAP kinase-kinase. Nature (Lond.). 358:417-421.
11. Payne, D.M., A.J. Rossomando, P. Martino, A.K. Erickson, J. Her, J. Shabanowitz, D.F. Hunt, M.J. Weber, and T.W. Sturgill. 1991. Identification of the regulatory phosphorylation sites in pp42/mitogen-activated protein kinase (MAP kinase). EMBO J. 10:885-892.
12. Pulverer, B.J., J.M. Kyriakis, J. Avruch, E. Nikolakaki, and J.R. Woodgett. 1991. Phosphorylation of c-jun mediated by MAP kinases. Nature (Lond.). 353:670-674.
13. Treisman, R. 1994. Ternary complex factors: growth factor regulated transciptional activators. Curr. Opin. Gen. Dev. 4:96-101.
14. Lange-Carter, C.A., C.M. Pleiman, A.M. Gardner, K.J. Blumer, and G.L. Johnson. 1993. A diversity in the MAP kinase regulatory network defined by MEK kinase and Raf. Science (Wash. DC). 260:315-319.
15. Bustelo, X.R., and M. Barbacid. 1992. Tyrosine phosphorylation of the vav proto-oncogene product in activated B cells. Science (Wash. DC). 256:1196-1199.
16. Gubins, E., C. Langlet, G. Baier, N. Bonnefoy-Berard, E. Herbert, A. Altman, and K.M. Coggeshall. 1994. Tyrosine phosphorylation and activation of Vav GTP/GDP exchange activity in antigen receptor-triggered B cells. J. Immunol. 152:2123-2129.
17. Gold, M.R., M.T. Crowley, G.A. Martin, F. McCormick, and A.L. DeFranco. 1993. Targets of B lymphocyte antigen receptor signal transduction include the p21ras GTPase-activating protein (GAP) and two GAP-associated proteins. J. Immunol. 150:377-386.
18. Boyer, M.J., D.H. Gutmann, F.S. Collins, and D. Bar-Sagi.
1994. Crosslinking of the surface immunoglobulin receptor in B lymphocytes induces a redistribution of neurofibromin but not p120-GAP. *Oncogene.* 9:349–357.

18a. Saxon, T.M., I. van Oostveen, D. Bowtell, R. Aebersold, and M.R. Gold. 1994. B cell antigen receptor cross-linking induces phosphorylation of the p21^TM^ oncoprotein activators SHC and mSOS1 as well as assembly of complexes containing SHC, GRB-2, mSOS1, and a 145-kDa tyrosine-phosphorylated protein. *J. Immunol.* 153:623–636.

19. Izquierdo, M., S.J. Leevers, C.J. Marshall, and D. Cantrell. 1993. p21^TM^ couples the T cell antigen receptor to extracellular signal-regulated kinase 2 in T lymphocytes. *J. Exp. Med.* 178:1199–1208.

20. Owaki, H., R. Varma, B. Gillis, J.T. Bruder, U.R. Rapp, L.S. Davis, and T.D. Geppert. 1993. Raf-1 is required for T cell IL2 production. *EMBO (Eur. Mol. Biol. Organ.)* J. 12:4367–4373.

21. Monroe, J.G., A.J. Yellen-Shaw, and V.L. Seyfert. 1993. Molecular basis for unresponsiveness and tolerance induction in immature stage B lymphocytes. *Adv. Mol. Cell. Immunol.* 1B:1–32.

22. Perez-Castillo, A., C. Pipaon, I. Garcia, and S. Alemany. 1993. NGFI-A gene expression is necessary for T lymphocyte proliferation. *J. Biol. Chem.* 268:19445–19450.

23. Tsai-Morris, C.H., X.M. Cao, and V.P. Sukhatme. 1988. 5' flanking sequence and genomic structure of Egr-1, a murine mitogen inducible zinc finger encoding gene. *Nucleic Acids Res.* 16:8835–8846.

24. Luckow, B., and G. Schutz. 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. *Nucleic Acids Res.* 15:5490.

25. Tabin, C., S. Bradley, C. Bargmann, R. Weinberg, A. Papageorge, E. Scolnick, R. Dhar, D. Lowy, and E. Chang. 1982. Mechanism of activation of a human oncogene. *Nature (Lond.)* 300:143–149.

26. Feig, L.A., and G.M. Cooper. 1988. Inhibition of NIH 3T3 cell proliferation by a mutant ras protein with preferential affinity for GDP. *Mol. Cell. Biol.* 8:3235–3243.

27. Schaap, D., J. van der Wal, L.R. Howe, C.J. Marshall, and W.J. van Blitterswijk. 1993. A dominant-negative mutant of ras blocks mitogen-activated protein kinase activation by growth factors and oncopgenic p21^{TM}. J. *Biol. Chem.* 268:20232–20236.

28. Seyfert, V.L., S. McMahon, W. Glenn, X. Cao, V.P. Sukhatme, and J.G. Monroe. 1990. Egr-1 expression in surface Ig-mediated B cell activation. Kinetics and association with protein kinase C activation. *J. Immunol.* 145:3647–3653.

28a. McMahon, S.B. and J.G. Monroe. 1995. Transient transfection of murine B lymphocyte blasts as a method for examining gene regulation in primary B cells. *J. Immunol. Meth.* In press.

29. Hill, C.S., R. Marais, S. John, J. Wynne, S. Dalton, and R. Treisman. 1993. Functional analysis of a growth factor-responsive transcription factor complex. *Cell.* 73:395–406.

30. Gulbins, E., M. Coggeshall, G. Baier, D. Telford, C. Langlet, G. Baier-Bitterlich, N. Bonnefoy-Berard, P. Burn, A. Wittinghofer, and A. Altman. 1994. Direct stimulation of Vav guanine nucleotide exchange activity for Ras by phorbol esters and diglycerides. *Mol. Cell. Biol.* 14:4749–4758.

31. Kharbanda, S., A. Saleem, M. Hirano, Y. Emoto, V. Sukhatme, J. Blenis, and D. Kufe. 1994. Activation of early growth response 1 gene transcription and pp90^{SRK} during induction of monocytic differentiation. *Cell Growth Differ.* 5:259–265.

32. DeFranco, C., D.H. Damon, M. Endoh, and J.A. Wagner. 1993. Nerve growth factor induces transcription of NGF1 through complex regulatory elements that are also sensitive to serum and phorbol 12-myristate 13-acetate. *Mol. Endocrinol.* 7:365–379.

33. Rim, M., S.A. Qureshi, D. Gius, J. Nho, V.P. Sukhatme, and D.A. Foster. 1992. Evidence that activation of the Egr-1 promoter by v-Raf involves serum response elements. *Oncogene.* 7:2065–2068.