Interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor induce the rapid phosphorylation of the c-raf protein in the growth factor-dependent FDC-P1 and DA-3 murine myeloid cell lines. Furthermore, immunoprecipitates of c-raf isolated from growth factor-stimulated cells demonstrate a marked increase in intrinsic protein kinase activity as measured in vitro. IL-3 and granulocyte-macrophage colony-stimulating factor induce phosphorylation of c-raf at both serine and tyrosine residues. Antiphosphorylase immunoprecipitates from IL-3-stimulated cells demonstrate the rapid and coordinate phosphorylation of both c-raf and a protein co-migrating with the 140-kDa putative IL-3 receptor component. Collectively, the findings of rapid and coordinate ligand-induced phosphorylation of a potential IL-3 growth factor receptor component and cytoplasmic c-raf with concomitant c-raf activation provide a cogent sequential molecular model for linking external growth stimuli to intracellular signal transduction events.

The growth and development of normal bone marrow progenitors are orchestrated by soluble glycoproteins known as hematopoietic growth factors (1). Substantial evidence shows that these same growth factors may be important mediators of leukemic cell growth (2). Hematopoietic growth factors exert their effects on normal and leukemic progenitor cells by initially binding to specific, high affinity cell-surface receptors (3, 4). However, the components and sequence of post-receptor hematopoietic growth signal transduction are largely unknown. Current data strongly suggest that protein phosphorylation is an important early regulatory mechanism of growth signaling. Thus, a variety of transmembrane growth factor receptors contain intrinsic tyrosine kinase activity and exhibit ligand-dependent tyrosine phosphorylation, including those for PDGF, epidermal growth factor, colony-stimulating factor 1, insulin, and possibly IL-3 and GM-CSF (5-12). In addition, several transforming oncogene products which can confer growth factor independence are protein-tyrosine kinases. These include abl, fms, and src (13-15). Furthermore, the transforming potential is abrogated by mutations in the protein-tyrosine kinase domain of these molecules (16-18), underscoring the importance of this enzymatic function in cell growth. Moreover, cytosolic protein kinases are plausible links between receptor-ligand interactions and growth factor-specific cellular events (19-21). Recent evidence suggests that protein kinase C, a cytoplasmic serine/threonine kinase, may mediate some of the IL-3-induced cellular events (22). Additionally, the existence of transforming genes such as raf and mos which encode cytoplasmic serine/threonine protein kinases strengthens the evidence implicating this class of molecules in growth regulation (23-25).

c-raf is a 74-kDa cytosolic serine/threonine protein kinase present in normal hematopoietic and leukemic cells (26). The viral homolog, v-raf, is oncogenic and cooperates with myc to induce erythroleukemia and lymphoma in mice (27). The combination of v-raf and v-myc induces IL-3-independent growth in factor-dependent murine myeloid cells (28). Furthermore, phosphorylation and activation of the c-raf protein have been observed after PDGF addition to fibroblasts (29). Collectively, these data suggest that c-raf specifically may play a role in post-receptor signal transduction. Results described here demonstrate that the unique hematopoietic growth factors IL-3 and GM-CSF can mediate rapid post-receptor biochemical events which appear to involve and/or converge at the level of c-raf, suggesting that c-raf may play a pivotal role in hematopoietic growth signal transduction.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture Media—The IL-3-dependent murine myeloid cell lines FDC-P1 and DA-3 were grown and maintained as described (30). The FDC-P1-derived cell line FDDP-2 expresses a temperature-sensitive mutant v-abl protein (31, 32). FDDP-2 cell culture medium at the nonpermissive temperature (39 °C) necessarily contained IL-3 and was identical to that for FTC-P1 and DA-3 cells. FDDP-2 cells at the permissive temperature (32 °C) were IL-3-independent for growth and were cultured in RPMI 1640 medium (GIBCO) supplemented only with 10% fetal bovine serum (FBS) (GIBCO).

Hematopoietic Growth Factors—Synthetic murine IL-3 was produced and assayed as described (30). Murine GM-CSF was synthesized by an automated method analogous to that used for human GM-CSF (34). [3H]Thymidine incorporation assays were performed in DA-3 and FDC-P1 cells as described (22), except that cells were not growth factor-deprived prior to IL-3 or GM-CSF addition. ATP, [γ-32P]orthophosphate acid, [γ-32P]ATP, 14C-labeled staphylococcal protein A, and [3H]thymidine were obtained commercially (Amersham Corp.). Chicken erythrocyte histone H5 was a generous gift of Dr. E. Moudrianakis (The Johns Hopkins University). All other chemicals used were reagent-grade and were obtained from commercial sources unless otherwise specified.
**IL-3 and GM-CSF Mediate c-raf Activation**

**Antibodies**—Immunoprecipitation of the c-raf protein was performed using a polyclonal rabbit antisera (aSP63) as described (35). IgG mouse monoclonal antiphosphotyrosine antibody covalently linked to Sepharose beads was used as described according to the manufacturer's instructions (Oncogene Sciences) (6). Anti c-raf Immunoblotting—Cell lysates, c-raf, and antiphosphotyrosine immunoprecipitates were resolved by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes as described (36). Membranes were preincubated in isotonic 10 mM Tris-buffered saline (pH 7.2) (TBS) containing 15% milk and 0.05% Tween 20 (Sigma) at 37°C for 2 h to saturate nonspecific protein-binding sites. Nitrocellulose filters were then rinsed in TBS and probed with aSP63 diluted 1:500 in TBS containing 0.005% EDTA, 0.01% Nonidet P-40 (Sigma), and 0.7% gelatin at 37°C for 4 h. Immunodetection was performed using a colorimetric assay employing biotinylated alkaline phosphatase according to the manufacturer’s instructions (Amersham Corp.) or using 125I-labeled staphylococcal protein A as previously described (22).

**Metabolic Labeling and Immunoprecipitation**—Exponentially growing cells were washed three times and resuspended at 1 x 10⁸ cells/ml in RPMI 1640 medium containing either 1 or 10% FBS. Incubations were performed from 2 to 14 h at the designated temperatures (see “Results”) to deprive cells of IL-3. After growth factor deprivation, cells were washed and resuspended at 1 x 10⁸ cells/ml in phosphate-free RPMI 1640 medium and equilibrated with ortho[32P]phosphoric acid at 100 μCi/ml for 60 min. Radiolabeled cells were pulsed with growth factor for the times indicated (see “Results”) and then immediately plunged into 10 volumes of ice-cold TBS. Cell pellets were washed twice in TBS at 4°C and then lysed in TBS containing 1% Triton X-100 (Sigma), 20 μg/ml leupeptin, 1.9 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 100 μM sodium metavanadate, and 20 mM sodium fluoride for 60 min at 4°C. Extracts were centrifuged for 15 min in a Microfuge (Beckman Instruments), and the clarified lysates were incubated for 30 min with 100 μl of staphylococcal protein A-Sepharose beads (Calbiochem) per ml. The protein A-Sepharose beads were removed by centrifugation in a Microfuge, and the precleared lysate was subjected to quantitative protein determination using a bicinchoninic acid method according to the manufacturer’s instructions (Pierce Chemical Co.). Lysates were normalized for protein content prior to immunoprecipitation using aSP63 (anti-c-raf) or antiphosphotyrosine antibodies. For aSP63 immunoprecipitation, 1 ml of lysate was incubated with 10 μl of aSP63 antiserum and 100 μl of protein A-Sepharose beads for 14 h at 4°C. c-raf antigen excess control immunoprecipitation experiments employed 10 μl of aSP63 antiserum preincubated with 5 μg of SP63 peptide for 30 min at 37°C. This antigen/antibody mixture was then used for immunoprecipitation as described above. Immunoprecipitates were washed five times in ice-cold TBS containing 1% Triton X-100, 100 μM sodium metavanadate, and 20 mM sodium fluoride. The washed immune complex-containing beads were suspended in 150 μl of SDS sample buffer, boiled for 5 min, and microcentrifuged. The resulting supernatants were resolved by SDS-PAGE and analyzed by autoradiography using Kodak X-Omat film. Immunoadsorption and elution of phosphotyrosine-containing proteins were performed according to the manufacturer’s instructions. Eluates were boiled in SDS sample buffer, resolved by SDS-PAGE, and analyzed by immunoblotting and autoradiography as described above.

**In Vitro Kinase Reactions**—Cell lysates were prepared and immunoprecipitated with aSP63 with or without excess SP63 antigen as described above. Washed immunoprecipitates containing c-raf were resuspended in 100 μl of kinase buffer consisting of 100 μg of chicken erythrocyte histone H5, 5 mM MgCl₂, and 5 mM dithiothreitol in TBS. Kinase reactions were initiated by the addition of 50 μM ATP and 1 μCi of [γ-32p]ATP. Reaction mixtures were agitated at room temperature for 5 min and then terminated by boiling in SDS sample buffer. Samples were microcentrifuged, and the supernatants were resolved by SDS-PAGE and analyzed by autoradiography as described above. Optical densitometry was performed on the phosphorylated histone peptides observed on the resulting autoradiographs.

**Phosphoamino Acid Analysis**—The 32P-labeled c-raf protein bands identified on polyacrylamide gels were excised, and phosphoamino acid analysis was performed as described (36).

**RESULTS**

**IL-3 Induces Rapid Phosphorylation and Activation of c-raf Protein**—Growth factor-deprived FDC-P1 cells exhibit a rapid increase in c-raf protein phosphorylation after IL-3 addition (Fig. 1, upper). An increase in the apparent molecular mass of the c-raf protein from 74 to 78 kDa accompanies phosphorylation, a feature consistent with c-raf phosphorylation also observed by others (29). The c-raf peptide SP63, to which the aSP63 antibody was raised, completely blocks the immunoprecipitation of this protein, confirming antibody specificity for c-raf. The immunoprecipitation of two phosphoproteins of Mₛ ≈ 105 and ≈ 88 is also blocked by the peptide. These proteins are not detected by aSP63 immunoblotting (data not shown), suggesting that their immunoprecipitation with c-raf is not due to a shared epitope, but rather may result from avid association with the c-raf protein. The significance of these potentially associated phosphoproteins in signal transduction, if any, remains to be determined.

**IL-3-induced c-raf Protein Phosphorylation Is Time- and Dose-dependent**—To determine whether c-raf activation rep
resists a physiologically significant event, time- and dose-response studies were performed. Results demonstrate that IL-3 induces rapid and time-dependent c-raf phosphorylation in growth factor-deprived FDC-P1 cells (Fig. 2, upper). c-raf immunoblotting indicates that the increase in c-raf phosphorylation is not the result of increased c-raf protein content after IL-3 addition (Fig. 2, center). c-raf phosphorylation occurs at biologically significant concentrations of IL-3, and the extent of phosphorylation correlates with mitogenicity as measured by [3H]thymidine incorporation (Figs. 2 (lower) and 3); that is, FDC-P1 cells demonstrate increased c-raf phosphorylation after treatment with 0.1 unit/ml IL-3, and phosphorylation is approximately half-maximal after treatment with 1.0 unit/ml IL-3.

**IL-3 and GM-CSF Induce Phosphorylation and Activation of c-raf in DA-3 Cells**—To determine whether the stimulatory effect on c-raf was unique and limited to IL-3, GM-CSF was also studied. Using DA-3 cells, where growth can be supported by either IL-3 or GM-CSF (Fig. 3), we find that both growth factors can induce rapid, time-dependent phosphorylation of c-raf (Fig. 4, upper). Furthermore, either of these growth factors can stimulate c-raf protein kinase activity as demonstrated by increased in vitro histone phosphorylation (Fig. 4, lower). Histone phosphorylation by α6P63 immunoprecipitates prepared in the presence of excess SP63 antigen is nearly undetectable, confirming that this in vitro kinase activity is specifically associated with c-raf.

**IL-3- and GM-CSF-induced c-raf Protein Phosphorylation Occurs at Both Serine and Tyrosine Residues**—Both IL-3 and GM-CSF can mediate the rapid tyrosine phosphorylation of proteins in hematopoietic cells (6, 12). Additionally, IL-3 has been shown to induce the serine phosphorylation of several intracellular substrates in IL-3-dependent cells (22). Characterization of the amino acid sites on c-raf which are targeted for phosphorylation by IL-3 and GM-CSF could provide fundamental information regarding the mechanism(s) of c-raf activation. Therefore, phosphoamino acid analysis was performed on 32P-labeled c-raf after treatment with either IL-3 or GM-CSF. Results demonstrate that the growth factors induce a marked increase in both phosphoserine and phosphotyrosine content compared with c-raf protein from untreated cells (Fig. 5). These findings suggest a similar mechanism for IL-3- and GM-CSF-induced c-raf phosphorylation.

**IL-3 Induces Rapid and Coordinate Phosphorylation of c-raf and a 140-kDa Protein**—Studies were performed to directly compare the kinetics of IL-3-mediated c-raf tyrosine phosphorylation with the known rapid tyrosine phosphorylation of a 140-kDa IL-3 receptor component (5). Antiphosphotyrosine immunoprecipitation demonstrates that IL-3 rapidly induces phosphorylation of several proteins in DA-3 cells (Fig. 6, upper). Three phosphoproteins predominate, which include a 74- and 55-kDa band as well as a protein which co-migrates with a 140-kDa putative IL-3 receptor component (5). Phosphorylation of these three proteins is coordinate and rapid, apparent 1 min after IL-3 addition and peaking at 5 min. Whereas some of the protein phosphorylation seen in Fig. 6A (upper) likely occurs on serine or threonine residues, the specificity of the antibody for phosphotyrosyl residues indicates that tyrosine phosphorylation accounts, at least in part, for the results observed. Anti-c-raf immunoblot detection of anti-phosphotyrosine immunoprecipitates confirms the identity of the 74-kDa phosphoprotein as c-raf (Fig. 6, center). Whereas IL-3-induced tyrosine phosphorylation of c-raf in DA-3 cells peaks at 5 min and rapidly diminishes, total c-raf phosphorylation, reflecting phosphorylation of both serine and tyrosine residues, continues to increase up to 60 min (Fig. 6, lower). Thus, it appears that increased tyrosine phosphorylation of c-raf is rapid and transient, whereas increased serine phosphorylation may be sustained.

**Murine Myeloid Cells Expressing v-abl Are IL-3 Growth-independent and Demonstrate Constitutive c-raf Protein Phosphorylation**—Since several oncogenes which encode protein-tyrosine kinases including abl, fms, and src can abrogate cellular dependence on IL-3 for growth (19-15), we reasoned that such a regulatory perturbation might occur at a proximal intermediate step in the growth signal transduction pathway, i.e. c-raf phosphorylation. Such an effect might thereby circumvent the otherwise obligatory early growth factor-mediated signal(s) such as ligand-induced tyrosine kinase activation. To directly test this hypothesis, we examined the effects of a
FIG. 3. Synthetic murine (sm) IL-3 and GM-CSF are mitogenic to growth factor-dependent murine myeloid cell lines. FDC-P1 or DA-3 cells were washed and resuspended in RPMI 1640 medium supplemented with 1% FBS in 96-well microtiter plates. Growth factors were added to the cultures in serial 2-fold dilutions. After 8 h, [3H]thymidine (1 μCi/well) was added, and [3H]thymidine incorporation was determined 16 h later as described under “Experimental Procedures.”

FIG. 4. IL-3 and GM-CSF induce phosphorylation and activation of c-raf. DA-3 cells were growth factor-deprived for 3 h. Upper, cells were metabolically labeled with ortho[32P]phosphoric acid and then incubated with 10 units/ml IL-3 or GM-CSF for the times indicated. The 0 lane represents the untreated control. Immunoprecipitates prepared using αSP63 with or without excess SP63 antigen as described under “Experimental Procedures” were resolved by 10% SDS-PAGE, and c-raf phosphorylation was assessed by autoradiography. Lower, cells were treated with 10 units/ml IL-3 or GM-CSF for 15 min. Immunoprecipitates prepared using αSP63 with or without excess SP63 antigen were added to in vitro kinase reaction mixtures containing chicken erythrocyte histone H5 and [γ-32P]ATP as described under “Experimental Procedures.” Reaction mixtures were resolved by 15% SDS-PAGE and analyzed by autoradiography. Histone phosphorylation was estimated using optical densitometry.

FIG. 5. IL-3- and GM-CSF-induced c-raf phosphorylation occurs at both serine and tyrosine residues. DA-3 cells were growth factor-deprived for 3 h, metabolically labeled with ortho[32P]phosphoric acid, and stimulated with 10 units/ml IL-3 or GM-CSF for 15 min. αSP63 immunoprecipitates were prepared and resolved by 10% SDS-PAGE. 32P-Labeled c-raf protein was identified on the gels by autoradiography, excised, and subjected to phosphoamino acid analysis as described under “Experimental Procedures.”

We have observed that IL-3 induces c-raf protein phosphorylation as well as a significant increase in associated temperature-sensitive v-abl into FDC-P1 cells (31). At the nonpermissive temperature (39 °C), temperature-sensitive v-abl has low tyrosine kinase activity, and FDDP-2 cells are dependent on IL-3 for growth. Under these conditions, IL-3 addition to cells induces rapid c-raf phosphorylation (Fig. 7). However, at the permissive temperature (32 °C), temperature-sensitive v-abl tyrosine kinase activity is increased 11-fold (32), and the cells become independent of IL-3 for growth (31). In this instance, c-raf is found to be constitutively phosphorylated, and the addition of IL-3 has no further effect (Fig. 7). No temperature-associated changes in specific IL-3 surface binding or c-raf content are seen in FDDP-2 cells (data not shown). Phosphoamino acid analysis of 32P-labeled c-raf protein isolated from FDDP-2 cells incubated at the permissive temperature reveals high levels of both phospho-tyrosine and phosphoserine, consistent with the increased protein-tyrosine kinase activity of temperature-sensitive v-abl at 32 °C (data not shown).
IL-3 and GM-CSF Mediate c-raf Activation

**Fig. 6.** IL-3 induces rapid coordinate phosphorylation of c-raf and a 140-kDa protein. DA-3 cells were growth factor-deprived for 3 h, metabolically labeled with ortho[P]phosphoric acid, and incubated with 10 units/ml IL-3 for the times indicated. The 0 lane represents the untreated controls. Cell equivalents (5 x 10^6) were used per lane. Upper, immunoprecipitates prepared using IgG antiphosphotyrosine antibody were resolved by 10% SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane as described under "Experimental Procedures." Phosphorylation was assessed by autoradiography. The exposure of the 5-min lane has been lessened to allow discrimination of individual bands. The expected positions of a 140-kDa putative IL-3 receptor and the c-raf protein are indicated by arrowheads. Center, the nitrocellulose membrane prepared as described (upper) was probed with αSP63 antiserum, and secondary immunodetection was performed using a colorimetric method as described under "Experimental Procedures." Lower, αSP63 immunoprecipitates were resolved by 7.5% SDS-PAGE and analyzed by autoradiography. c-raf phosphorylation was estimated using optical densitometry.

**Fig. 7.** Murine myeloid cells expressing v-abl demonstrate constitutive c-raf protein phosphorylation. FDDP-2 cells were IL-3-deprived in RPMI 1640 medium supplemented with 10% FBS for 14 h at 32 or 39°C. Cells were then metabolically labeled with ortho[P]phosphoric acid and treated with 10 units/ml IL-3 for 15 min at the temperatures indicated. αSP63 immunoprecipitates were resolved by 10% SDS-PAGE and analyzed by autoradiography as described under "Experimental Procedures." Intrinsic protein kinase activity. Furthermore, IL-3-induced c-raf protein phosphorylation and activation occur very rapidly and at IL-3 concentrations relevant to its physiological effects (growth and mitogenesis). These findings form the basis for a model of early molecular events in cell growth whereby a specific ligand/receptor interaction at the plasma membrane provokes a biochemical reaction, i.e. c-raf phosphorylation, resulting in a potential phosphorylation cascade at the cytoplasmic level. This rapid activation step can also be mediated by GM-CSF, suggesting a general role for c-raf in hematopoietic growth signal transduction. c-raf-phosphorylation and activation have been observed by others (29) following the addition of PDGF and the phorbol ester mitogen phorbol 12-myristate 13-acetate to murine fibroblasts. Our results extend these findings. Collectively, these data suggest that the c-raf protein may play a pivotal role in growth signal transduction, perhaps serving as a cytoplasmic focal point in the transmission of a variety of mitogenic stimuli.

Many growth factor receptors are known to be ligand-activated tyrosine kinases including those for PDGF, epidermal growth factor, insulin, colony-stimulating factor 1, and possibly IL-3 and GM-CSF (5-12). This enzymatic activity appears to be necessary for cell growth as mutations which interrupt tyrosine kinase activity abrogate growth factor-mediated cellular responses (11, 37-39). Significantly, IL-3- and GM-CSF-induced c-raf phosphorylation occurs at both serine and tyrosine residues. IL-3-induced c-raf protein tyrosine phosphorylation is very rapid, occurring by 1 min, and is coordinately precisely with the phosphorylation of a protein co-migrating with the 140-kDa putative IL-3 receptor (Fig. 6 and Refs. 6 and 7). This finding raises the possibility that IL-3-induced c-raf tyrosine phosphorylation may be mediated directly by an IL-3 receptor-associated tyrosine kinase. In support of such an hypothesis, others (40) have found that the c-raf protein is directly associated with the PDGF receptor in whole fibroblasts and that the PDGF receptor can bind the c-raf protein and mediate its tyrosine phosphorylation in vitro. Although we (5) and others (41) have previously reported the putative IL-3 receptor to be a phosphotyrosine-containing protein, no consensus sequence for protein-tyrosine kinase activity is present in a recently cloned, low affinity IL-3-binding protein (42). These data could suggest that an associated protein-tyrosine kinase activity, distinct from the IL-3-binding component but activated by ligand binding may be responsible for the apparent ligand-induced tyrosine phosphorylation of the receptor and other proteins including c-raf. These intriguing possibilities remain to be determined.

Additional evidence implicating c-raf tyrosine phosphorylation in hematopoietic cell growth regulation may be gleaned from an investigation of oncogene products which abrogate the growth requirement of FDC-P1 cells for IL-3. Oncogenes within this class such as v-fms, v-abl, and v-src encode protein-tyrosine kinases (13-15). Mutations which interrupt the tyrosine kinase activity also abrogate the physiological effects of these proteins, underscoring the importance of this enzymatic activity in growth regulation (16-18). The FDDP-2 cell line provides a unique, "reversible" system whereby a direct comparison of the subcellular events in IL-3-dependent growth can be made with those occurring during functional v-abl expression and IL-3-independent growth. At the non-permissive temperature (39°C), the temperature-sensitive v-abl protein has low tyrosine kinase activity, and the cells are dependent on IL-3 for growth (31, 32). Under these conditions, IL-3 induces c-raf phosphorylation. However, at 32°C, where temperature-sensitive v-abl protein-tyrosine kinase activity is high and cell growth is IL-3-independent, a constitutive phosphorylation of c-raf is observed (Fig. 7). In this case, IL-3 has little to no effect on c-raf phosphorylation. This finding supports a potential role for c-raf in cell growth regulation.
c-raf activation is necessary and/or sufficient to transduce membrane, may be transmitted to the interior of the cell. Whether protein kinase C to c-raf serine phosphorylation and activation physiological role for c-raf serine phosphorylation is yet known. One possibility involves c-raf autophosphorylation. However, several lines of evidence suggest that this is unlikely to be the only explanation for the substantial serine phosphorylation. First, although in vitro histone phosphorylation observed was specifically c-raf-associated (Fig. 4, lower 4th bar), at no time did we observe in vitro phosphorylation of a 74-kDa protein in the anti-c-raf immunoprecipitates derived from growth factor-stimulated cells. Second, even in the absence of histone in the kinase reaction, which might be a competitive substrate for activated c-raf, no c-raf autophosphorylation could be detected (data not shown). Third, it has been recently reported (43) that c-raf immunoprecipitates derived from fibroblasts demonstrate little autokinase activity. An alternative explanation for c-raf serine phosphorylation involves the action of another serine/threonine protein kinase which is distinct from c-raf. Two observations support protein kinase C as a potential activator. First, protein kinase C has been strongly implicated in the mechanisms of action for several growth factors including IL-3 (22, 44) and PDGF (45), suggesting at least a circumstantial association between this serine/threonine kinase and the c-raf protein. Second, two mitogenic activators of protein kinase C can induce c-raf phosphorylation and activation. Bryostatin 1, a macrocyclic lactone derived from the marine organism Bugula neritina, is a potent activator of protein kinase C (46) and is mitogenic in FDC-P1 cells (22). In addition, this compound induces rapid phosphorylation and activation of the c-raf protein in FDC-P1 cells. Recently, others (29) have observed that another protein kinase C activator, phorbol 12-myristate 13-acetate, also induces c-raf protein phosphorylation and activation. As such, these data are intriguing, but no direct evidence linking protein kinase C to c-raf serine phosphorylation and activation has been demonstrated. Although recently published studies (40) suggest that in vitro PDGF receptor-mediated c-raf tyrosine phosphorylation triggers c-raf kinase activation, no physiological role for c-raf serine phosphorylation is yet known. The aforementioned effects of protein kinase C activators indicate that c-raf phosphorylation at serine may also serve to activate its intrinsic kinase function.

In summary, these results, demonstrating IL-3- and GM-CSF-mediated phosphorylation and activation of cytosolic c-raf, suggest a potential rapid regulatory mechanism by which hematopoietic growth signals, generated at the plasma membrane, may be transmitted to the interior of the cell. Whether c-raf activation is necessary and/or sufficient to transduce these mitogenic stimuli remains to be determined.

1 M. Carroll, U. R. Rapp, and W. S. May, unpublished data.
Interleukin-3 and granulocyte-macrophage colony-stimulating factor mediate rapid phosphorylation and activation of cytosolic c-raf.
M P Carroll, I Clark-Lewis, U R Rapp and W S May

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