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Augmentation of c-*fos* mRNA Expression by Activators of Protein Kinase C in Fresh, Terminally Differentiated Resting Macrophages

DANUTA RADZIOCH, BARBARA BOTTAZZI, AND LUIGI VARESIO*

Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute Frederick Cancer Research Facility, Frederick, Maryland 21701

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Expression of c-*fos* mRNA was investigated in fresh, normal peritoneal macrophages (Mϕ), which are terminally differentiated, nonproliferating cells. The levels of c-*fos* mRNA were dramatically increased by stimulation with phorbol myristate acetate (PMA), calcium ionophore, or 1-oleoyl-2-acetoyl glycerol (OAG). Induction of c-*fos* mRNA by all the above agents followed similar kinetics, with a peak of mRNA 30 min after stimulation. These results demonstrate that c-*fos* mRNA can be augmented in fresh, terminally differentiated cells. Since the stimuli increasing c-*fos* mRNA are direct or indirect activators of protein kinase C, our data suggest that in Mϕ c-*fos* mRNA is controlled by protein kinase C activation. PMA, calcium ionophore, and OAG were biologically active in Mϕ. PMA and calcium ionophore induced respiratory burst and tumoricidal activity, respectively, whereas OAG and PMA were chemotactic for Mϕ. Interferons beta and gamma, potent Mϕ activators eliciting tumoricidal activity, did not alter the levels of c-*fos* mRNA. These results indicate that c-*fos* mRNA augmentation is a stimulus-specific rather than a function-specific response connected to activation of protein kinase C.

c-*fos* appears to be a gene with pleiotropic functions, since it may be involved in cell growth as well as differentiation. Increased levels of c-*fos* mRNA followed by proliferation were observed in bone marrow cells treated with colony-stimulating factor and in fibroblasts exposed to mitogenic stimuli (4, 7, 14, 15, 21, 28, 29). However, similar augmentation of c-*fos* expression also preceded growth arrest and terminal differentiation of myelomonocytic cell lines treated with phorbol myristate acetate (PMA) (13, 25, 26, 29) and pheochromocytoma cell lines treated with nerve growth factor (10, 21, 27). Moreover, a role for c-*fos* in the differentiation of fetal membrane has been suggested (31). It has also been reported that after transfection, c-*fos* can cause differentiation of teratocarcinoma cells and transformation of fibroblasts (24, 32). The pleiotropic effects of c-*fos* could be a lineage-specific response, proliferative in fibroblasts and differentiative in other cell types, and different biochemical pathways could lead to augmentation of c-*fos* mRNA. To address these issues, we analyzed the levels of c-*fos* mRNA in peritoneal exudate macrophages (Mϕ), fresh, terminally differentiated cells (for a review, see reference 40) which did not proliferate in response to the stimuli used. We found that the levels of c-*fos* mRNA in Mϕ were dramatically increased by stimulation with PMA, 1-oleoyl-2-acetoyl glycerol (OAG), or calcium ionophore, demonstrating for the first time that c-*fos* mRNA is susceptible to augmentation in primary cultures of terminally differentiated cells. Moreover, the agents tested are direct or indirect activators of protein kinase C (PK-C) (5, 8, 16, 18, 22, 23, 33, 34), and our data suggest that in Mϕ c-*fos* is controlled by PK-C activation.

MATERIALS AND METHODS

Macrophages. C57BL/6N male mice, obtained from the Division of Research Services, National Institutes of Health, were injected intraperitoneally with 1 ml of 1% thioglycolate medium (BBL Microbiology Systems, Cockeysville, Md.), and peritoneal exudate cells were harvested after 4 days by washing the peritoneal cavity with Eagle minimal essential medium (Gibco Laboratories, Grand Island, N.Y.). The peritoneal exudate cells were washed three times with Hanks balanced salt solution (HBSS; Gibco), and the Mϕ were purified by adherence to plastic. For the O_2^- production, [^{3}H]thymidine uptake, and cytotoxicity assays, 2×10^5 Mϕ were plated on 96-well tissue culture plates, allowed to adhere for 2 h, and washed with HBSS. For RNA extraction 2×10^7 Mϕ were plated on 15-cm ϕLux plates (Miles Scientific, Naperville, Ill.), and after 2 h of adherence the Mϕ were washed with HBSS.

Reagents. The following reagents were used: PMA (Sigma Chemical Co., St. Louis, Mo.), L-α-1-oleoyl-2-acetoyl-*sn*-3-glycerol (OAG) (Avanti Polar Lipids, Inc., Birmingham, Ala.), calcium ionophore A 23187 (Sigma), murine beta interferon (IFN-β) (kindly provided by K. Pauker), murine recombinant gamma interferon (IFN-γ) (kindly provided by Patrick Gray, Genentech Laboratory, San Francisco, Calif.), [^{3}H]thymidine (Amersham Corp., Arlington Heights, Ill.), ferricytochrome *c* (type III), superoxide dismutase type I (Sigma), and lipopolysaccharide (LPS) (Difco Laboratories, Detroit, Mich.).

Biological assays. (i) Superoxide production assay. O_2^- production was measured by cytochrome *c* reduction assay after 0.5, 1, 2, 3, and 4 h of stimulation as described previousy (35). The amount of O_2^- produced per well was calculated from the extinction coefficient (*E*) for the absorption of reduced cytochrome *c* minus oxidized cytochrome *c* as read at 550 nm by the formula: $E_{550} = 2.1 \times 10^4\ M^{-1}\ cm^{-1}$.

(ii) Cytotoxicity assay. Macrophages were treated for 18 h with various activating agents, washed, and tested for cytotoxic activity against indium-111 (^{111}In)-labeled L5178Y lymphoma target cells, as described previously (37). The results were expressed as percentage ^{111}In release, calculated from the average cpm of triplicate samples as [(cpm

* Corresponding author.

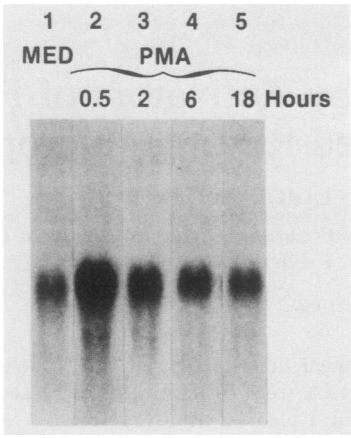

FIG. 1. Induction of c-*fos* mRNA expression by PMA. Northern blot of RNA extracted from Mϕ cultured in medium (MED, lane 1) or medium containing 30 nM PMA for 0.5 h (lane 2), 2 h (lane 3), 6 h (lane 4), or 18 h (lane 5) and hybridized with a v-*fos* probe.

with treated Mϕ − cpm with untreated Mϕ)/(total cpm incorporated in target cells)] × 100. The spontaneous release of ^{111}In from target cells cultured alone was between 10 and 15% of the total radioactivity incorporated. The effector-target ratio used was 40:1.

(iii) Chemotaxis assay. Chemotactic activity was assayed by the micropore filter technique (12). Briefly, 25 μl of chemoattractant or control medium was seeded in the lower wells of a microchamber (Neuroprobe, Cabin John, Md.). A polycarbonate filter (5-μm pore size; Neuroprobe) was layered onto the wells, and 50 μl of cell suspension (3×10^6 Mϕ/ml in RPMI 1640 [ABI Advanced Biotechnology, Inc., Silver Spring, Md.] supplemented with 10% fetal calf serum [FCS; Gibco Laboratories, Chagrin Falls, Ohio]) were seeded in the upper wells. After 4 h of incubation at 37°C, the filters were removed, stained with Giemsa, and counted. Three replicate filters per experimental group were read, and results are expressed as mean ± standard error for cells in five oil immersion fields.

(iv) Thymidine incorporation. DNA synthesis was measured as previously described (42). Mϕ were incubated for 18 h with the activating agents, pulsed for 4 h with 1 μCi of [*methyl*-^{3}H]thymidine (^{3}H-Thy; New England Nuclear Corp.) per ml, solubilized with sodium hydroxide, and precipitated with cold trichloroacetic acid (10% solution in water). The amount of radioactivity incorporated was measured by liquid scintillation counting. The results were expressed as mean incorporation for quadruplicate samples.

RNA extraction. Total cellular RNA was isolated from Mϕ solubilized with guanidinium isothiocyanate and was purified by centrifugation through a cushion of CsCl (6) with some modifications detailed elsewhere (39, 41).

Northern blotting analysis. The mRNA levels were analyzed by hybridization of Northern or dot blots with ^{32}P-labeled probes (38). The following cDNA probes were used: v-*fos* probe (kindly provided by I. Verma [11]), actin (purchased from Oncor, Inc., Gaithersburg, Md.), "25-46" probe recognizing 18S rRNA, and "1-19" probe recognizing 28S rRNA (kindly provided by N. Arnheim [1]). Purified inserts labeled with [^{32}P]dCTP (Amersham Corp.) by nick translation were always used as probes (specific activity of the probes was 5×10^8 cpm/μg). Each RNA sample was tested by Northern and dot blot analysis at least three times in independent experiments.

RESULTS

Initial experiments were performed to determine whether PMA, which is a potent inducer of c-*fos* mRNA in proliferating or differentiating cell lines (13, 14, 28) was able to induce c-*fos* mRNA expression in fresh Mϕ (Fig. 1). Mϕ constitutively express low but significant levels of c-*fos* mRNA. After stimulation with 30 nM PMA, a peak of c-*fos* mRNA was observed after 30 min of treatment. For 10 experiments performed, a 6- to 10-fold increase in c-*fos* mRNA was consistently detected in PMA-treated Mϕ. The initial augmentation of c-*fos* mRNA expression was followed by a return to baseline levels 2 to 6 h after PMA treatment (Fig. 1). Dose-response experiments demonstrated that induction of c-*fos* mRNA reached plateau levels at a PMA concentration of 30 nM (data not shown). These data showed that c-*fos* mRNA is inducible by PMA in freshly harvested peritoneal macrophages. The blot shown in Fig. 1 was rehybridized with a probe specific for actin mRNA. Similar levels of actin mRNA were expressed in control and stimulated macrophages (data not shown), indicating that the increased c-*fos* mRNA expression in response to PMA was not the result of a general augmentation of mRNA levels.

Since PMA is a potent activator of PK-C, we explored the possibility that c-*fos* mRNA augmentation was induced by PK-C activation. For this purpose we tested the effects of OAG, a synthetic diacylglycerol able to activate specifically PK-C in intact cells (23), on the expression of c-*fos* mRNA by Mϕ. As demonstrated by the results of a representative experiment (Fig. 2A), increasing levels of c-*fos* mRNA were induced by OAG in a dose-dependent manner. Maximal induction of c-*fos* mRNA was observed at 125 μM OAG. The kinetics of c-*fos* mRNA induction by OAG was similar to that observed in PMA-treated Mϕ, with a peak after 30 min of treatment followed by a decline to normal levels thereafter (Table 1). These results show that OAG is a potent inducer of c-*fos* mRNA expression in Mϕ and imply the involvement of PK-C activation in this process.

To test whether augmentation of intracellular calcium could affect the levels of c-*fos* mRNA, we used calcium ionophore, which caused influx of calcium into the cytoplasm. Calcium ionophore A 23187 at concentrations ranging between 1 and 3 μM induced the accumulation of c-*fos* mRNA in Mϕ (Fig. 2B). Concentrations of calcium ionophore higher than 5 μM were toxic if Mϕ were treated for longer than 12 h. Maximum c-*fos* mRNA expression was observed after 30 min and was followed by a decline to basal levels after 2 h (Table 1). These results showed that calcium-dependent pathways were involved in the regulation of c-*fos* mRNA and were consistent with the possibility of a role for PK-C.

Mϕ respond to many stimuli with phenotypic and biochemical changes. The demonstration that OAG, PMA, and calcium ionophore are inducers of c-*fos* mRNA raised the question of whether augmentation of c-*fos* mRNA was a common event associated with Mϕ stimulation. To address this point we examined the effect of IFN-β and IFN-γ on c-*fos* expression, since both cytokines are potent activators of Mϕ (for a review, see reference 40), although they utilize different pathways (2). Mϕ were treated with 20 U of IFN-γ or 1,000 U of IFN-β per ml, in the presence of 10 ng of LPS per ml, for 0.5, 4, and 18 h at 37°C and tested for c-*fos* mRNA levels (Fig. 3). No significant changes in the levels of c-*fos* mRNA were observed in response to IFN, indicating that augmentation of c-*fos* mRNA was not a general feature

of stimulation but a selective response to PMA, OAG, and calcium ionophore.

Since c-*fos* mRNA augmentation could not be associated with proliferation or differentiation in our system, we investigated whether it could be related to the expression of other specific Mϕ functions. Mϕ were activated in vitro and tested for superoxide production (an early Mϕ response that is evident 2 h after stimulation), chemotaxis (which can be measured 4 h after stimulation), and cytolytic activity against tumor target cells (an assay which requires 6 to 12 h of activation followed by contact of the activated Mϕ with the target cells for 18 to 24 h) (Table 2). Different biological responses followed stimulation of Mϕ with the various agents: chemotactic response was elicited by PMA and OAG, superoxide production by PMA, and cytotoxic activity by IFN-β, IFN-γ, and calcium ionophore. None of the stimuli induced ^{3}H-Thy uptake by Mϕ. These results showed that the stimuli used to treat Mϕ were all biologically active since they were able to trigger various Mϕ functions and confirmed that they were not mitogenic. However, we did not find correlations in the ability of a stimulus to induce a specific biological function and to augment c-*fos* mRNA expression (the latter data are also summarized in Table 2).

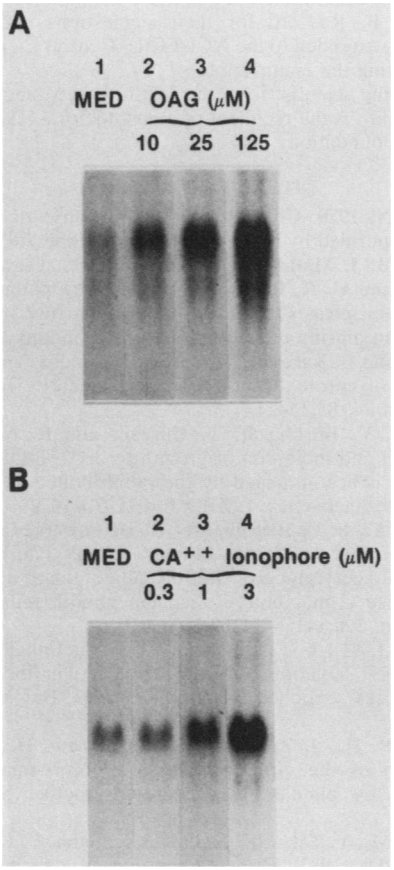

FIG. 2. (A) Induction of c-*fos* mRNA by OAG. Northern blot analysis of RNA extracted from Mϕ treated for 30 min with medium (lane 1) or with 10 μM (lane 2), 25 μM (lane 3), or 125 μM (lane 4) OAG and hybridized with the v-*fos* probe. (B) Induction of c-*fos* mRNA by calcium ionophore. Northern blot analysis of RNA extracted from Mϕ treated for 0.5 h in medium (lane 1) or in medium containing 0.3 μM (lane 2), 1 μM (lane 3), or 3 μM (lane 4) calcium ionophore and hybridized with the v-*fos* probe.

TABLE 1. Kinetics of c-*fos* mRNA augmentation

Activator	Increase in mRNA level (fold) at:				
	0 h	0.5 h	2 h	6 h	18 h
OAG[a]	1	10	2	1	1
Calcium ionophore[b]	1	8	3	1	1

[a] Mϕ were treated with medium alone or medium containing 125 μM OAG for 0.5, 2, 6, or 18 h and analyzed by dot blot and Northern blot analysis. The results are expressed in comparison to the basal level.

[b] RNAs were extracted from Mϕ treated with medium alone or with 3 μM calcium inonphore and analyzed as described above.

These results suggest that augmentation of c-*fos* mRNA is a stimulus-specific rather than function-related response.

DISCUSSION

We have found that PMA, calcium ionophore, and OAG induce a rapid and transient increase in c-*fos* mRNA expression in peritoneal Mϕ. A peak of c-*fos* mRNA expression was detectable after 30 min and was followed by a return to basal level after 2 to 6 h. The kinetics of the responses were similar to those described for other experimental systems in which c-*fos* mRNA augmentation was followed by proliferation or differentiation. Our results on peritoneal Mϕ demonstrate that these cellular activities can be dissociated from augmentation of c-*fos* mRNA, although they do not exclude a role for c-*fos* in the differentiation or proliferation of cells that are still potentially endowed with such functions.

In an attempt to see whether the expression of specific Mϕ functions correlated with the stimulation of c-*fos* mRNA expression, activated Mϕ populations were tested for chemotactic response, superoxide production, and tumoricidal activity. However, we did not observe direct correlations between the ability of a stimulus to elicit c-*fos* mRNA augmentation and a specific Mϕ function. In fact, depending on the activating agent, c-*fos* mRNA augmentation was followed by O_2^- production, cytotoxic activity, or chemotactic response. These results suggested that the augmentation of c-*fos* mRNA in Mϕ was a stimulus-specific rather than a function-specific event, linked to the enzymatic pathways triggered by PMA, OAG, and calcium ionophore. It has been shown that PMA binds and activates PK-C (8,

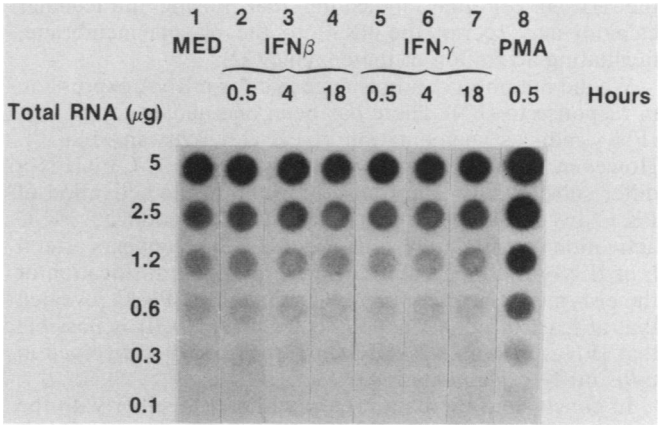

FIG. 3. c-*fos* mRNA expression after treatment of Mϕ with IFN. Mϕ were treated for 0.5, 4, or 18 h with 20 U of IFN-γ in the presence of LPS (10 ng/ml), 1,000 U of IFN-β in the presence of LPS (10 ng/ml), or with 30 nM PMA and tested for c-*fos* mRNA expression by dot blot analysis.

TABLE 2. Biological response of Mϕ to stimulation

Activator[a]	Chemotactic activity[b] (mean no. of cells ± SE)	Relative increase in O_2^- production[c]	Avg cytotoxicity[d] (%)	c-*fos* mRNA[e]	^{3}H-Thy (cpm)
None	15 ± 7	1.0	4	+	220
PMA	89 ± 3[g]	9.5[g]	5	+ + +	196
OAG	104 ± 2[g]	0.9	1	+ + +	185
Calcium ionophore	4 ± 1	1.5	42	+ + +	232
IFN-γ	NT[h]	1.4	32	+	207
IFN-β	NT	1.1	37	+	210

[a] Macrophages were treated with medium alone or medium containing 30 nM PMA, 125 μM OAG, 3 μM calcium ionophore, or 20 U of IFN-γ per ml in the presence of LPS (10 ng/ml) or 1,000 U of IFN-β per ml in the presence of LPS (10 ng/ml).

[b] Chemotaxis was assayed by the micropore filter technique. Incubation was done for 4 h at 37°C, then filters were removed and stained with Giemsa and counted. Results are expressed as mean cell number for five oil immersion fields in triplicate filters.

[c] Quadruplicate samples were run in each assay, and Student's *t* test was used for statistical analysis. Results are expressed as increase in O_2^- production relative to medium-treated macrophages.

[d] Mϕ were activated for 18 h and tested for cytotoxic activity against ^{111}In-labeled L5178Y lymphoma target cells. Results are expressed as the average percent cytotoxicity of quadruplicate samples.

[e] c-*fos* mRNA was measured by Northern and dot blot analysis. Mϕ were activated for 30 min before harvesting. Symbols: +, basal level of c-*fos* mRNA; + + +, 6- to 10-fold increase over the basal level.

[f] Mϕ were incubated for 18 h with the activating agents and pulsed for 4 h with 1 μCi of ^{3}H-Thy per ml before harvesting. The results are expressed as mean incorporation for quadruplicate samples.

[g] Statistically significant ($P < 0.01$) as evaluated by Student's *t* test.

[h] NT, Not tested.

34). Under physiological conditions, PK-C is activated by diacylglycerols, which are produced from phospholipase C activity, and PMA is able to substitute for diacylglycerol (33, 34, 43). In intact cells, synthetic diacylglycerols such as OAG have been shown to be specific activators of PK-C (5, 16, 23). Therefore, the demonstration that OAG is a potent inducer of c-*fos* mRNA expression and that the kinetics of induction are similar to those observed with PMA provided strong evidence for a direct connection between activation of PK-C and augmentation of c-*fos* mRNA.

We have found that calcium ionophore, which promotes calcium influx into the cell, is also a good inducer of c-*fos* mRNA in Mϕ. These results showed that calcium-dependent pathways are important in the augmentation of c-*fos* mRNA and are consistent with a role for PK-C activation. In vitro studies showed that the catalytic activity of PK-C has an absolute requirement for calcium (36). Calcium influx in Mϕ could activate PK-C by providing free calcium needed for PK-C activity or by increasing the potency and the rate of action of PK-C constitutively stimulated by endogenous diacylglycerols. This possibility is supported by studies on the HL-60 cell line, indicating that raising intracellular calcium may recruit the PK-C to the plasma membrane, facilitating activation of the enzyme (22).

We did not observe any change in c-*fos* mRNA expression in response to IFN. There has been one update report that IFN-γ induces augmentation of PK-C activity in Mϕ (17). However, the characteristics of activation of PK-C by IFN-γ differ substantially from those reported in the activation of PK-C by PMA or growth factors. IFN-γ-induced PK-C activation is a relatively slow process, which appears after 4 h of IFN-γ treatment and does not involve translocation of the enzyme to the membrane. Cloning of the PK-C revealed that at least three distinct PK-C exist (9, 19). It is possible that IFN-γ activates a PK-C different from that involved in c-*fos* mRNA augmentation.

In conclusion, the data presented here, together with the current interpretation of the mechanism of action of OAG, indicate that in Mϕ c-*fos* expression is tightly coupled to the activation of PK-C. However, c-*fos* oncogene expression does not seem to be a signal sufficient to induce the manifestation of biological activities in these cells.

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LITERATURE CITED

1. **Arnheim, N.** 1979. Characterization of mouse ribosomal gene fragments purified by molecular cloning. Gene **7**:83–96.
2. **Blasi, E., B. J. Mathieson, L. Varesio, J. L. Cleveland, P. A. Borchert, and U. R. Rapp.** 1985. Selective immortalization of murine macrophages from fresh bone marrow by a *raf/myc* recombinant murine retrovirus. Nature (London) **318**:667–670.
3. **Blasi, E., and L. Varesio.** 1984. Role of protein synthesis in the activation of cytotoxic mouse macrophages by lymphokines. Cell. Immunol. **85**:15–24.
4. **Bravo, R., Y. Burkhardt, T. Curran, and R. Müller.** 1985. Stimulation and inhibition of growth by EGF in different A431 cell clones is accompanied by the rapid induction of c-*fos* and c-*myc* proto-oncogenes. EMBO J. **4**:1193–1197.
5. **Brock, T. A., S. E. Rittenhouse, C. H. Powers, L. S. Ekstein, M. A. Gimbrone, and R. W. Alexander.** 1985. Phorbol ester and 1-oleoyl-12-acetylglycerol inhibit angiotensin activation of phospolipase C in cultured vascular smooth muscle cells. J. Biol. Chem. **260**:14158–14162.
6. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**: 5294–5299.
7. **Cochran, B. H., J. Zullo, L. M. Verma, and D. Stiles.** 1984. Expression of the c-*fos* gene and of a *fos*-related gene is stimulated by platelet-derived growth factor. Science **226**: 1080–1082.
8. **Costagna, M., Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa, and Y. Nishizuka.** 1982. Direct activation of calcium activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. Biol. Chem. **257**:7847–7851.
9. **Coussens, L., P. J. Parker, L. Rhee, T. L. Yang-Feng, E. Chen, M. D. Waterfield, U. Francke, and A. Ullrich.** 1986. Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. Science **233**:859–866.
10. **Curran, T., and J. Morgan.** 1985. Superinduction of c-*fos* by nerve growth factor in the presence of peripherally active

benzodiazepines. Science **229**:1256–1268.
11. **Curran, T., G. Peters, C. H. Van Beveren, N. M. Teich, and I. M. Verma.** 1982. FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. J. Virol. **44**:674–682.
12. **Falk, W., R. H. Goodwin, Jr., and E. J. Leonard.** 1980. A 48 well microchemotaxis assembly for rapid and accurate measurement of leukocyte migration. J. Immunol. Methods **33**:239–247.
13. **Gonda, T. J., and D. Metcalf.** 1984. Expression of *myb*, *myc* and *fos* proto-oncogenes during the differentiation of a murine myeloid leukemia. Nature (London) **310**:249–251.
14. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (London) **311**:433–437.
15. **Habenicht, A., J. A. Glomset, W. C. King, C. Nist, C. D. Mitchell, and R. Ross.** 1981. Early changes in phosphatidylinositol and arachidonic acid metabolism in quiescent Swiss 3T3 cells stimulated to divide by platelet-derived growth factor. J. Biol. Chem. **256**:12329–12335.
16. **Halenda, S. P., G. B. Zavoico, and M. B. Feinstein.** 1985. Phorbol esters and oleoyl acetoyl glycerol enhance release of arachidonic acid in platelets stimulated by Ca^{2+} ionophore A23187. J. Biol. Chem. **260**:12484–12491.
17. **Hamilton, T. A., D. L. Becton, S. D. Somers, P. W. Gray, and D. O. Adams.** 1985. Interferon-γ modulates protein kinase C activity in murine peritoneal macrophages. J. Biol. Chem. **260**:1378–1381.
18. **Hannum, Y. A., C. R. Loomis, and R. M. Bell.** 1986. Protein kinase C activation in mixed micelles. J. Biol. Chem. **261**:7184–7190.
19. **Knopf, J. L., M. H. Lee, L. A. Sultzman, R. W. Kriz, C. R. Loomis, R. M. Hewick, and R. M. Bell.** 1986. Cloning and expression of multiple protein kinase C cDNAs. Cell **46**: 491–502.
20. **Kruijer, W., J. A. Cooper, T. Hunter, and I. M. Verma.** 1984. Platelet derived growth factor induces rapid but transient expression of c-*fos* gene and protein. Nature (London) **312**: 711–715.
21. **Kruijer, W., D. Schubert, and I. M. Verma.** 1985. Induction of the protooncogene *fos* by nerve growth factor. Proc. Natl. Acad. Sci. USA **82**:7330–7334.
22. **May, W. S., N. Sahaoun, M. Wolf, and P. Cuatrecasas.** 1985. Role of intracellular calcium mobilization in the regulation of protein kinase C-mediated membrane processes. Nature (London) **317**:549–551.
23. **McCaffrey, P. G., B. Friedman, and M. R. Rosner.** 1984. Diacylglycerol modulates binding and phosphorylation of the epidermal growth factor receptor. J. Biol. Chem. **259**: 12502–12507.
24. **Miller, A. D., T. Curran, and I. M. Verma.** 1984. c-*fos* protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. Cell **36**:51-60.
25. **Mitchell, R. L., C. Henning-Chubb, E. Huberman, and I. M. Verma.** 1986. c-*fos* expression is neither sufficient nor obligatory for differentiation of monomyelocytes to macrophages. Cell **45**:497–504.
26. **Mitchell, R. L., L. Zokas, R. D. Schreiber, and I. M. Verma.** 1985. Rapid induction of the expression of protooncogene *fos* during human monocytic differentiation. Cell **40**:209–217.
27. **Morgan, J. I., and T. Curran.** 1986. Role of ion-flux in the control of c-*fos* expression. Nature (London) **322**:552–555.
28. **Muller, R., R. Bravo, J. Burckhardt, and T. Curran.** 1984. Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. Nature (London) **312**:716–720.
29. **Muller, R., T. Curran, D. Muller, and L. Guilbert.** 1985. Induction of c-*fos* during myelomonocytic differentiation and macrophage proliferation. Nature (London) **314**:546–548.
30. **Muller, R., D. Muller, and L. Guilbert.** 1984. Differential expression of c-*fos* in hematopoietic cells: correlation with differentiation of monomyelocytic cells in vitro. EMBO J. **3**:1887–1890.
31. **Muller, R., and I. M. Verma.** 1984. Expression of cellular oncogenes. Curr. Top. Microbiol. Immunol. **112**:74–103.
32. **Muller, R., and E. F. Wagner.** 1984. Differentiation of F9 teratocarcinoma stem cells after transfer of c-*fos* protooncogenes. Nature (London) **311**:438–442.
33. **Nishizuka, Y.** 1983. Phospholipid degradation and signal translation for protein phosphorylation. Trends Biochem. Sci. **8**:13–16.
34. **Nishizuka, Y.** 1984. The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature (London) **308**:693–698.
35. **Pick, E., and D. Mizel.** 1981. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. J. Immunol. Methods **46**:211–226.
36. **Takai, Y., A. Kishimoto, Y. Iwasa, Y. Kawahara, T. Mori, and Y. Nishizuka.** 1979. Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J. Biol. Chem. **254**:3692–3695.
37. **Taramelli, D., H. T. Holden, and L. Varesio.** 1980. Endotoxin requirement for macrophage activation by lymphokines in a rapid microcytotoxicity assay. J. Immunol. Methods **37**:225–232.
38. **Thomas, P.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
39. **Varesio, L.** 1985. Imbalanced accumulation of ribosomal RNA in macrophages activated in vivo or in vitro to a cytolytic stage. J. Immunol. **134**:1262–1267.
40. **Varesio, L.** 1985. Induction and expression of tumoricidal activity by macrophages, p. 381–407. *In* R. T. Dean and W. Jesup (ed.), Mononuclear phagocytes: physiology and pathology. Elsevier, London.
41. **Varesio, L., E. Blasi, G. B. Thurman, J. E. Talmadge, R. H. Wiltrout, and R. B. Herberman.** 1984. Potent activation of mouse macrophages by recombinant interferon γ. J. Cancer Res. **44**:4465–4469.
42. **Varesio, L., and A. Eva.** 1980. A microsystem to evaluate the synthesis of [^{3}H]leucine labeled proteins by macrophages. J. Immunol. Methods **33**:231–238.
43. **Yamamoto, S., H. Gotoh, E. Aizu, and R. Kato.** 1985. Failure of 1-oleoyl-2-acetylglycerol to mimic the cell-differentiating action of 12-*O*-tetradecanoylphorbol 13-acetate in HL-60 cells. J. Biol. Chem. **260**:14230–14234.