Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4789–4793, July 1986
Cell Biology

Nerve growth factor rapidly induces c-fos mRNA in PC12 rat pheochromocytoma cells

(c-fos/nerve growth factor receptor/signal transduction/superinduction)

JEFFREY MILBRANDT

Division of Laboratory Medicine, Departments of Pathology and Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Paul E. Lacy, March 10, 1986

ABSTRACT The nerve growth factor (NGF)-mediated increase in c-*fos* gene expression in the rat pheochromocytoma PC12 cell line has been investigated. NGF treatment of PC12 cells results in an increased level of c-fos mRNA within 15 min. An approximately 100-fold increase in the level of c-fos mRNA occurs 30–45 min after exposure to NGF and the c-fos mRNA concentration returns to its basal level 2 hr after NGF treatment. Thus, the half-life of this RNA transcript is extremely short. In the presence of cycloheximide, the c-*fos* gene is superinduced and the increased level of c-fos mRNA persists for at least 24 hr. The induction of c-*fos* gene expression was further studied by utilizing a monoclonal antibody (mAb-192) that is directed against the NGF receptor but does not compete with NGF for binding to the receptor. Treatment of the cells with mAb-192 inhibits the NGF-stimulated elevation of c-fos mRNA, suggesting that the antibody may interfere with the receptor's ability to generate the signal required to stimulate the transcription of this gene. NGF is not the only agent capable of inducing c-*fos* gene expression in these cells; epidermal growth factor, the tumor promoter phorbol 12-myristate 13-acetate, and the calcium ionophore A23187, agents that induce the c-*fos* gene in other cell lines, are also effective in PC12 cells. The mRNA for the nuclear protein fos is rapidly induced by NGF and other agents to which PC12 cells respond. This supports the hypothesis that the *fos* gene product may play a role in signal transduction.

Nerve growth factor (NGF) is a polypeptide hormone that is necessary for the differentiation and survival of sympathetic and embryonic sensory neurons (1, 2). Sympathetic neurons cultured *in vitro* manifest an absolute requirement for NGF (3). The exposure of immature rats to anti-NGF antibodies results in the degeneration of neural crest-derived sensory neurons and almost all of the sympathetic neurons (4, 5). NGF also influences the differentiation of neurons: it stimulates the production of enzymes involved in the synthesis of neurotransmitters (6), it induces the formation of neurite outgrowths from isolated ganglia (7), and it may play a role in the regeneration of sympathetic and sensory axons after injury (8). The mechanism of action of NGF is poorly understood. However, it is clear that NGF binds to specific cell-surface receptors present on NGF-responsive cells and is subsequently internalized (9). NGF is then carried to the neuronal cell body by retrograde transport (10), but it does not appear that NGF acts as its own second messenger (11).

The PC12 cell line, derived from a rat pheochromocytoma, has proven to be a valuable tool in studies aimed at determining the actions of NGF and its role in neuronal differentiation (for review, see ref. 12). The overall effect of NGF on PC12 cells is to convert them from a population of replicating adrenal chromaffin-like cells into a population of nonreplicating sympathetic neuron-like cells. This NGF-mediated differentiation process requires RNA synthesis (13) and results in the extension of neurites, the development of electrical excitability, and the *de novo* synthesis of ornithine decarboxylase (14) and the NILE glycoprotein (15). To investigate the changes in gene expression induced by the actions of NGF and to further our understanding of the role of NGF in the developing nervous system, we have analyzed the expression of two proto-oncogenes, c-*fos* and c-*myc*, whose mRNAs are induced by the actions of other polypeptide growth hormones (16).

The expression of the c-*myc* and c-*fos* genes has been extensively characterized in several experimental systems that involve mitogenesis or differentiation (17–19). Levels of c-myc and c-fos mRNAs rise rapidly when fibroblasts are treated with platelet-derived growth-factor. The c-fos mRNA is also rapidly induced by other factors with growth promoting activities, such as epidermal growth factor (EGF), phorbol 12-myristate 13-acetate (PMA), and colony-stimulating factor. The c-*fos* gene product is also thought to play a causative role in cellular differentiation, as it is found in high concentration in fetal membranes (20) and hemopoietic stem cells (21). These data suggest that the products of these proto-oncogenes may play critical roles in the generation of hormone-induced responses. Therefore, it was of interest to determine whether or not the NGF-stimulated differentiation of PC12 cells is accompanied by the induction of these genes. In this report, I demonstrate that the treatment of PC12 cells with NGF results in the rapid transient induction of c-fos mRNA but that it has little effect on the level of c-*myc* gene expression. The induction of the c-*fos* gene is inhibited by the actions of monoclonal antibody 192 (mAb-192), an antibody directed against the NGF receptor, which does not compete with NGF for binding to the receptor (22, 23).

MATERIALS AND METHODS

Cell Culture Techniques. PC12 cells were obtained from C. Chandler and were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 5% horse serum in a humidified atmosphere containing 12% CO_2/88% air at 37°C. Drugs, growth factors, and antibodies were added to cells in exponential growth (i.e., 60–70% confluency) from concentrated stock solutions made up in culture medium. For studies involving cycloheximide, the drug was added at a final concentration of 10 μg/ml at the same time the NGF was administered. When experiments involving antibodies were performed, they were added 30 min prior to the addition of NGF and/or cycloheximide.

Analysis of RNA Levels. Total cellular RNA was isolated essentially as described by Chirgwin *et al.* (24). Samples containing 15 μg of total RNA were electrophoresed through

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NGF, nerve growth factor; mAb-192, monoclonal antibody 192; PMA, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor; kb, kilobase(s).

an agarose gel formed in 2.2 M formaldehyde/20 mM 3-(N-morpholino)propanesulfonic acid (Mops) buffer, pH 7.2/5 mM Na acetate, and transferred directly to nitrocellulose in 3 M NaCl/0.3 M Na citrate as described by Thomas (25). The filters were baked *in vacuo* at 80°C for 2 hr and pre-hybridized in 40% formamide/0.6 M NaCl/60 mM Na citrate/5× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/herring sperm DNA (100 μg/ml)/0.1% $NaDodSO_4$/0.2% Na pyrophosphate/poly(A) (1 μg/ml) at 42°C for at least 3 hr. DNA probes were then added (5×10^5 dpm/ml) and allowed to hybridize at 42°C for 12–24 hr. Filters were washed in two changes of 0.3 M NaCl/30 mM Na citrate/0.1% $NaDodSO_4$ at 25°C for 10 min and two changes of 75 mM NaCl/7.5 mM Na citrate/0.1% $NaDodSO_4$ at 50°C for 1 hr each. The filters were exposed to Kodak XAR-5 film with DuPont Cronex intensifying screens at −70°C. DNA probes were labeled with $[^{32}P]dATP$ to a specific activity of $2–5 \times 10^6$ dpm/ng by oligo labeling (26).

RNA slot blots were performed essentially as suggested by the manufacturer (Schleicher & Schuell). Briefly, the indicated amount of RNA was suspended in 100 μl of water and 300 μl of 6.15 M formaldehyde/1.5 M NaCl/0.15 M Na citrate, heated at 65°C for 15 min, cooled at 25°C for 10 min, and the samples were then vacuum-filtered onto the nitrocellulose filter equilibrated with 1.5 M NaCl/0.15 M Na citrate. The filters were then handled as described above, and quantitative analysis of the autoradiograms was performed by densitometry.

Materials. The v-fos DNA probe (27) was obtained from P. Reitsma and the human C-myc (28) and N-myc (29) probes were the gift of G. Brodeur. β-NGF and the monoclonal antibodies, mAb-192 and mAb-151, were the gifts of E. Johnson. Epidermal growth factor (EGF) was the gift of Luis Glaser. All other chemicals were purchased from Sigma.

RESULTS

Kinetics of NGF-Induced c-fos mRNA Accumulation. Much evidence has accumulated implicating several of the protooncogenes in the control of cellular proliferation and differentiation. To determine the effects of the nonmitogenic hormone, NGF, on the expression of these genes, exponentially growing PC12 cells were exposed to NGF at 50 ng/ml, a level that induces neurite outgrowth within 24 hr (30), for various periods of time. An RNA blot was hybridized with the v-*fos* probe, which is ≈90% homologous to the mouse and human c-*fos* genes (31), to detect the amount of c-fos mRNA induced in response to NGF treatment. Inspection of the gel, which is displayed in Fig. 1*A*, reveals the presence of the expected 2.2-kilobase (kb) c-fos mRNA in lanes 3–6. At 2 min (lane 2), no induction is seen, while at 15 min (lane 3), a significant increase in c-fos mRNA is detectable. The peak level of accumulation is reached at 30–45 min (lanes 4 and 5). This rapid increase is accompanied by an equally rapid fall, so that after 120 min (lane 7) the level of c-fos mRNA is close to the basal level. To quantitate the level of induction of the c-fos mRNA, a slot blot analysis was performed. Samples of total RNA from each of the time points were filtered onto nitrocellulose, the blot was hybridized to the v-fos probe, and the signal strengths from the resulting autoradiogram were quantitated by densitometry. The results are plotted in Fig. 1*B* and reveal that the maximal induction of ≈100-fold over the basal level occurs between 30 and 45 min. Slot blots containing RNA samples from 0, 45, and 120 min were hybridized with the c-myc and N-myc probes. The results are shown in Fig. 1*C*. Note that c-*myc* is only increased 2-fold after 2 hr, while N-*myc*, an oncogene that is frequently amplified and whose expression has been correlated with metastasis in neuroblastomas (29), is not increased.

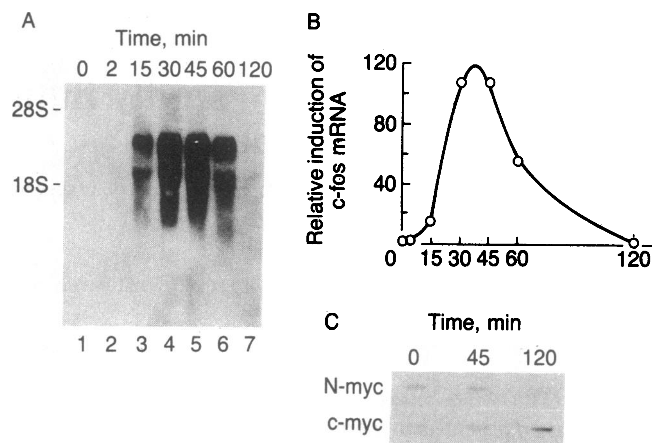

FIG. 1. (*A*) RNA blot analysis of RNA isolated from PC12 cells treated with NGF (50 ng/ml) for various periods of time. Total RNA (15 μg) was fractionated on 1.4% formaldehyde/agarose gels and transferred to a nitrocellulose membrane. The c-fos transcripts were detected by hybridization with a ^{32}P-labeled v-fos probe and subsequent autoradiography. (*B*) Kinetics of c-fos accumulation. A slot blot containing serial dilutions of total RNA from cells treated with NGF for the indicated time was hybridized to the v-fos probe. The resulting autoradiogram was quantitated by densitometry (only signals in the linear range of film sensitivity were quantitated). (*C*) RNA slot-blot analysis of N-myc and c-myc transcripts. Ten micrograms of total RNA isolated from cells treated with NGF (50 ng/ml) for the indicated length of time was applied to the nitrocellulose and hybridized with the indicated ^{32}P-labeled DNA probe.

Effects of Cycloheximide on c-fos mRNA Levels. The enhanced accumulation of hormonally induced mRNAs that occurs in cells simultaneously treated with protein synthesis inhibitors and growth factors has been termed superinduction (32). To determine whether or not c-fos is superinduced in response to NGF, a time course of c-fos induction was established in cells treated with NGF and cycloheximide (10 μg/ml). In Fig. 2*A*, RNAs from cells treated with NGF for various periods of time in the presence or absence of cycloheximide were probed with v-fos. Cycloheximide has very little effect on the rate of accumulation of c-fos mRNA at very early time points (lanes 3 and 4), but the maximal level

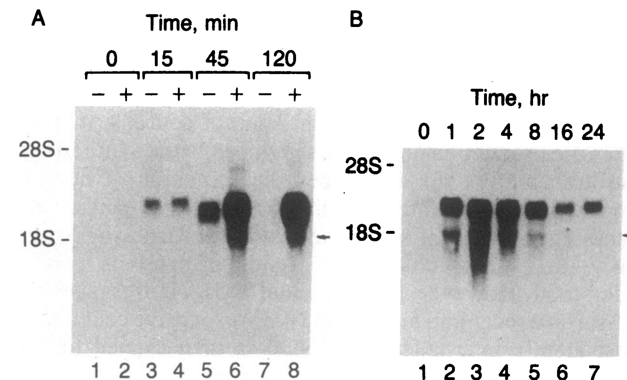

FIG. 2. (*A*) Superinduction of c-fos mRNA in NGF/cycloheximide-treated PC12 cells. Total RNA was isolated from cells treated with NGF (50 ng/ml) either in the presence (+) or absence (−) of cycloheximide (10 μg/ml) at the indicated time points. The RNA samples were fractionated by electrophoresis, transferred to nitrocellulose, and hybridized to the ^{32}P-labeled v-fos probe. (*B*) Time course of c-fos mRNA accumulation in NGF/cycloheximide-treated PC12 cells. RNA blot analysis of c-fos mRNA that has been isolated from PC12 cells grown in the presence of NGF (50 ng/ml) and cycloheximide (10 μg/ml) for the indicated period of time. Arrow denotes the 2.0-kb band, which also hybridizes to the v-fos probe (see *Results*).

attained is increased (lanes 5 and 6), and the time course of disappearance is dramatically altered (lanes 7 and 8). A 2.0-kb transcript is also detected in lanes containing RNA from the NGF-treated cells (denoted by arrow). In addition, a slight change in the mobility of the c-fos mRNA is apparent with increasing time of exposure to NGF (compare lanes 3 and 5). To further examine the alteration in the rate of c-fos mRNA decline, RNAs from cells grown in the presence of cycloheximide for up to 24 hr were analyzed for the presence of c-fos mRNA. As shown in Fig. 2*B*, the peak level of accumulation now appears to be delayed to 2 hr (lane 3); indeed, this is the time point at which c-fos mRNA levels return to baseline in the absence of cycloheximide (Fig. 1*A*, lane 7). Surprisingly, the c-fos mRNA persists at increased levels for at least 24 hr when the cells are incubated in the presence of cycloheximide (Fig. 2*B*, lane 7). These data support the hypothesis (17) that the rapid decrease in c-fos mRNA is the result of a protein(s) that under normal circumstances is responsible for the rapid degradation of the c-fos message. Therefore, preventing the synthesis of this protein, which under the nonstimulated condition must be absent from the cell, results in an extended c-fos mRNA half-life. When these blots were hybridized to the c-myc probe, only a slight increase in the level of c-myc mRNA could be detected (data not shown). Thus, unlike most other systems there is no correlation between the expression of the c-*fos* and c-*myc* proto-oncogenes in PC12 cells exposed to NGF.

EGF Stimulates c-fos Expression. In addition to NGF, PC12 cells respond to EGF by an increase in cell adhesion and induction of ornithine decarboxylase activity, but they do not extend neurites (33). To determine whether or not EGF could stimulate c-fos mRNA accumulation in PC12 cells as it does in other cell lines, RNA was prepared from cells that were treated with cycloheximide (10 μg/ml) and either EGF (100 ng/ml), NGF (50 ng/ml), or both for 3 hr. Fig. 3*A* displays an RNA blot analysis of the c-fos transcripts from these cells. EGF-mediated c-fos induction (lane 2) is increased ≈6-fold over the control cells that have been treated with cycloheximide alone (lane 1). Cells treated with NGF (lane 4) or with a combination of EGF and NGF (lane 3) each show a large increase in the amount of c-fos mRNA.

PMA and A23187 Induce c-fos in PC12 Cells. When PC12 cells are exposed to the tumor promoter PMA, several of the observed responses are similar to those obtained by treatment with EGF and NGF (34). Therefore, it was of interest to determine the magnitude of c-fos mRNA accumulation in PC12 cells treated with this agent as well as the calcium ionophore A23187. Cells were treated with PMA (50 ng/ml), A23187 (20 μM), or NGF (50 ng/ml) for 45 min and RNA was prepared as described. Untreated PC12 cells contain very few c-fos transcripts (Fig. 3*B*, lane 1); however, the level of c-fos mRNA is greatly increased by treating the cells with NGF, PMA, or A23187 (Fig. 3*B*, lanes 2–4). Comparison of the signal strengths between these lanes revealed that the amount of c-fos transcript in the PMA-treated cells was equal to that of the NGF-treated cells, while A23187 produced a slightly lower response. Thus, it appears that these agents, which have been implicated in signal transduction and increase c-fos mRNA levels in other cell lines, induce c-fos mRNA in PC12 cells to a similar degree as NGF.

Effects of the Anti-NGF Receptor mAb-192 on NGF-Stimulated c-fos Expression. mAb-192 was raised against solubilized PC12 cell plasma membranes and has been shown to be directed against the NGF receptor (22, 23). This antibody does not compete with NGF for binding to the NGF receptor, but instead it increases the apparent affinity of NGF binding 2.5- to 4-fold to the fast NGF receptors. Previous studies had shown that this antibody could inhibit NGF-induced neurite regeneration in primed PC12 cells at low NGF concentrations (23). In an attempt to determine whether NGF-stimulated c-fos expression may play a role in the NGF-mediated differentiation of these cells, PC12 cells were exposed to a saturating concentration (50 nM) of this antibody (or mAb-151, a monoclonal antibody that is directed against the PC12 EGF receptor) 30 min prior to the addition of NGF. The cells were treated with the indicated amount of NGF (in the presence of the mAbs) for 45 min and RNA was isolated as described above. To detect the c-fos mRNA in cells treated with no NGF or low concentrations of NGF, a rat c-fos cDNA clone was isolated from a PC12 cell cDNA library. Nucleotide sequencing was used to confirm the identity of this clone as a c-fos cDNA, and it was then used to detect the c-fos mRNA in an RNA blot analysis as shown in Fig. 4. Lanes 10–12 and 13–15 contain RNA from cells treated with NGF (0.05 ng/ml) and no NGF, respectively. Very little c-fos mRNA is present under these conditions, so it was not possible to assess the effect of the antibodies on cells treated in this manner. PC12 cells treated with NGF (0.5 ng/ml) (lane 7) contain an increased amount of c-fos mRNA, but preincubation of the cells with mAb-192 inhibits this induction process (lane 8). The inhibition of mAb-192 on NGF-stimulated c-fos mRNA induction is most evident in lanes 4–6. The signals in lanes 4 (NGF at 5 ng/ml) and 6 (NGF at

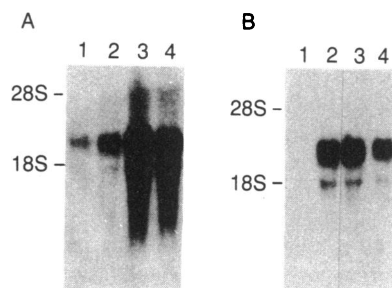

FIG. 3. (*A*) The effect of EGF on c-*fos* gene expression in PC12 cells. The v-fos probe was hybridized to an RNA blot containing RNA isolated from PC12 cells treated for 3 hr with cycloheximide (10 μg/ml) and no addition (lane 1), EGF at 100 ng/ml (lane 2), EGF and NGF (lane 3), or NGF at 50 ng/ml (lane 4). (*B*) The effect of PMA and A23187 on c-fos expression. An RNA blot containing RNA isolated from PC12 cells treated for 45 min with no addition (lane 1), NGF at 50 ng/ml (lane 2), PMA at 50 ng/ml (lane 3), or 20 μM A23187 (lane 4) was hybridized to the v-fos probe.

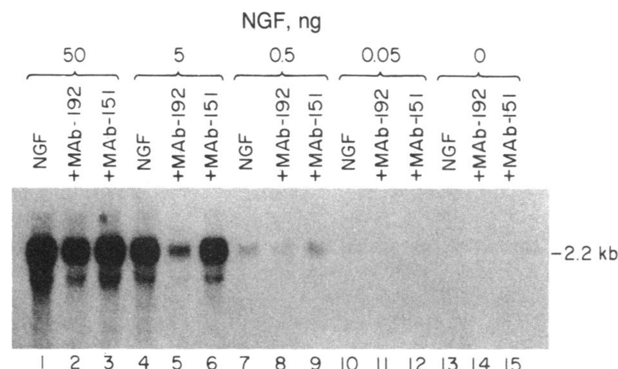

FIG. 4. The inhibition of NGF-induced c-*fos* gene expression by mAb-192. The indicated antibodies were added to the PC12 cells for 30 min prior to the addition of the NGF. Total RNA was isolated from cells 45 min after the addition of the following amounts of NGF: lanes 1–3, 50 ng/ml; lanes 4–6, 5 ng/ml; lanes 7–9, 0.5 ng/ml; lanes 10–12, 0.05 ng/ml; lanes 13–15, control (no NGF). The RNAs were fractionated by electrophoresis, transferred to nitrocellulose, and hybridized to the ^{32}P-labeled rat c-fos cDNA probe. mAb-192 is directed against the NGF receptor and mAb-151 is directed against the EGF receptor.

5 ng/ml + mAb-151) are essentially equivalent, while the signal in lane 5 (NGF at 5 ng/ml + mAb-192) is greatly reduced. A similar result is seen in lanes 1–3. However, c-fos mRNA is synthesized in the presence of mAb-192 at this higher concentration of NGF (50 ng/ml). A slot blot analysis of these RNA samples was also performed (data not shown) and densitometry revealed that the percentage inhibition at 0.5, 5, and 50 ng of NGF per ml is ≈5-, ≈8-, and ≈2-fold, respectively. These results demonstrate that, although the binding of mAb-192 to the NGF receptor does not prevent the simultaneous binding of NGF, it does inhibit the NGF-mediated induction of c-fos mRNA accumulation. To ensure that mAb-192 is inhibiting NGF-induced c-*fos* gene expression, rather than delaying it, similar experiments were carried out in the presence of cycloheximide. RNAs were harvested after 3 hr and RNA blot analysis revealed a similar inhibition of c-fos induction (data not shown). In contrast, mAb-151, a monoclonal antibody that also binds to the surface of the PC12 cell, had no effect.

DISCUSSION

The neuronal differentiation induced by NGF has been difficult to study because it has not been possible to maintain NGF-responsive neurons *in vitro* in the absence of the growth factor. The NGF-responsive PC12 cell line provides a system that can be experimentally manipulated to study NGF-mediated changes in gene expression. Although RNA transcription is necessary for neurite development, the analysis of proteins from untreated or from NGF-stimulated PC12 cells by two-dimensional electrophoresis revealed only minor qualitative changes (35). However, more recent studies, in which a small subset of the total cellular proteins were analyzed, have demonstrated the NGF-mediated induction of several proteins (36, 37). To further investigate these alterations in gene expression, we have utilized specific DNA probes to measure the levels of various mRNAs in PC12 cells both before and after exposure to NGF.

The discovery that the expression of certain proto-oncogenes was stimulated by treating cells with specific peptide growth factors prompted us to examine their expression in NGF-treated PC12 cells. The results presented here demonstrate that NGF rapidly induces the synthesis of c-fos mRNA to levels ≈100-fold greater than are present in the uninduced state. In contrast, the level of c-myc mRNA displays only a 2-fold increase and the level of N-myc mRNA does not change in response to NGF. In addition to the expected 2.2-kb c-fos transcript, a 2.0-kb transcript was also detected in RNA samples derived from cells treated with NGF. These hybridizations were performed under conditions of reduced stringency. Therefore, this signal may be the result of hybridization to the 2.0-kb transcripts of the r-*fos* gene, which is induced in fibroblasts exposed to platelet-derived growth factor (38). In addition, a slight decrease in the apparent length of the induced c-fos mRNA with time was consistently observed in these studies (compare lanes 3 and 5 in Fig. 2A). This may be due to further processing of the c-fos mRNA, such as a shortening of the poly(A) tail, or it may be the result of the initial steps involved in eliminating c-fos mRNA from the cell. The kinetics and magnitude of the induction and the superinduction of c-fos mRNA levels in NGF-treated PC12 cells are both similar to those reported for platelet-derived growth factor-stimulated fibroblasts. In the presence of cycloheximide and NGF, the level of c-fos mRNA is increased 5- to 10-fold over NGF alone at early time points (Fig. 2A, lanes 5 and 6). Thus, cycloheximide may prevent the synthesis of a labile repressor protein as has been suggested (17). Alternatively, cycloheximide could also be preventing the synthesis of an enzyme that is responsible for regulating the level of this mRNA and thereby increasing the half-life of the c-fos mRNA. This is supported by three observations: (*i*) the level of c-fos mRNA after 15 min of induction with NGF is unaffected by cycloheximide treatment; (*ii*) 2 hr after NGF treatment without cycloheximide, the level of c-fos mRNA has returned to baseline, while in the presence of the drug c-fos mRNA levels are maximal at this time point; and (*iii*) the level of c-fos mRNA remains significantly increased for at least 24 hr in cells treated with cycloheximide.

PC12 cells respond to EGF and PMA with an increase in cell adhesion and ornithine decarboxylase activity, but they do not extend neurites. Surprisingly, co-administration of PMA and EGF resulted in the extension of long slender cellular processes. In addition, the phosphorylation of a 30-kDa chromosomal protein has been demonstrated in PC12 cells treated with either PMA, EGF, or NGF (33). In this study, treatment with EGF increased the level of c-fos mRNA ≈6-fold in PC12 cells grown in the presence of cycloheximide. The characteristics of PMA-treated PC12 cells bear several similarities to those studied in NGF-treated cells. Therefore, it was of interest to observe that PMA rapidly and substantially induced the expression of c-fos in these cells. Indeed, the levels of c-fos mRNA induced by PMA were equal to the maximal level observed in NGF-treated cells. The calcium ionophore A23187 also stimulates c-fos expression in PC12 cells, as it does in A431 cells. The stimulation of c-fos expression by PMA and A23187 may be achieved by virtue of their ability to stimulate protein kinase C (39). It is interesting to speculate that some of the transcription-dependent effects of NGF may be mediated through diacylglycerol formation and the resulting activation of protein kinase C.

The inhibition of NGF-mediated c-fos mRNA induction by the anti-NGF receptor antibody (mAb-192) is interesting because this antibody has previously been shown to inhibit another biological response to NGF—namely, neurite outgrowth. In this study, the expression of the c-*fos* gene is induced ≈5-fold at 0.5 ng of NGF per ml and ≈50-fold at 5 ng of NGF per ml (see Fig. 4). Thus, c-fos mRNA induction is observed at concentrations of NGF above what would be expected if the effect were mediated by the high-affinity (slow) receptor sites (2). This induction process is inhibited by mAb-192 (see Fig. 4), an antibody that increases the affinity of the fast (low affinity) receptor for NGF. Taken together, these observations suggest that the fast receptor may play a role in the activation of the c-*fos* gene by NGF. It has also been observed that this antibody is internalized and retrogradely transported with the same kinetics as NGF *in vivo* (22). In addition, an antibody–ricin A-chain hybrid is capable of killing PC12 cells. Therefore, the antibody must also be internalized in PC12 cells (40). Thus, it is possible that the inhibitory activity of this antibody is related to its ability to interfere with the transduction of the NGF signal to the nucleus by virtue of inducing a conformational change in the receptor, by inhibiting the interaction of a receptor regulatory protein, or by becoming internalized along with the NGF receptor.

The possible role of c-fos in differentiation and development has been suggested (20). The activation of the c-*fos* gene is one of the earliest responses to NGF exposure. Thus, it is possible that the *fos* gene product may play an important role in the NGF-mediated differentiation of PC12 cells. However, it is also apparent from these studies that the induction of the c-*fos* gene is not sufficient by itself to induce neurite formation in PC12 cells, since agents that do not promote neurite formation are capable of stimulating the expression of this gene. The activation of the c-*fos* gene by NGF and these agents may provide new tools for determining the nature of

the transduction signal and the mechanism by which NGF exerts its multitude of effects.

While this manuscript was in preparation, results similar to some of those reported here have appeared elsewhere (41–43).

I thank Tim Fahrner for his valuable technical assistance and Drs. Eugene Johnson, Peter DiStefano, and Megumi Taniuchi for their thoughtful comments on this work and their gifts of NGF, mAb-192, and mAb-151. I gratefully acknowledge the helpful advice and encouragement of Dr. John Lowe. This work was supported by Clinical Investigator Development Award NS01018 from the National Institute of Neurological and Communicative Disorders and Stroke, Grant RG1779-A-1 from the Multiple Sclerosis Society, and a grant from the Center for Molecular and Cellular Neurobiology at Washington University.

1. Shelton, D. & Reichardt, L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7951–7955.
2. Yankner, B. & Shooter, E. (1982) *Annu. Rev. Biochem.* **51**, 845–868.
3. Chun, L.-Y. & Patterson, P. (1977) *J. Cell Biol.* **75**, 705–711.
4. Levi-Montalcini, R. & Booker, B. (1960) *Proc. Natl. Acad. Sci. USA* **46**, 384–391.
5. Johnson, E., Gorin, P., Brandeis, L. & Pearson, J. (1980) *Science* **210**, 916–918.
6. Otten, U., Schwab, M., Gagnon, C. & Thoenen, H. (1977) *Brain Res.* **133**, 291–303.
7. Levi-Montalcini, R. & Hamburger, V. (1953) *J. Exp. Zool.* **123**, 233–288.
8. Yip, H. & Grafstein, B. (1982) *Brain Res.* **238**, 329–339.
9. Andres, R., Jeng, I. & Bradshaw, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2785–2789.
10. Hendry, I., Stoeckel, L., Thoenen, H. & Iversen, L. (1974) *Brain Res.* **68**, 103–121.
11. Seeley, P., Keith, C., Shelanski, M. & Greene, L. (1983) *J. Neurosci.* **3**, 1488–1494.
12. Greene, L. & Tischler, A. (1982) in *Advances in Cellular Neurobiology*, eds. Fedoroff, S. & Hertz, L. (Academic, New York), Vol. 3, pp. 374–414.
13. Burstein, D. & Greene, L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6059–6063.
14. Greene, L. & McGuire, J. (1978) *Nature (London)* **276**, 191–193.
15. McGuire, J., Greene, L. & Furano, A. (1978) *Cell* **15**, 357–365.
16. Muller, R., Bravo, R., Burckhardt, J. & Curran, T. (1984) *Nature (London)* **312**, 716–720.
17. Kelly, K., Cochran, B., Stiles, C. & Leder, P. (1983) *Cell* **35**, 603–610.
18. Reitsma, P., Rothberg, P., Astrin, S., Trial, J., Bar-Shavitz, Z., Hall, A., Teitelbaum, S. & Kahn, A. (1983) *Nature (London)* **306**, 492–494.
19. Bravo, R., Burckhardt, J., Curran, T. & Muller, R. (1985) *EMBO J.* **4**, 1193–1197.
20. Muller, R., Slamon, D., Tremblay, J., Cline, M. & Verma, I. (1982) *Nature (London)* **299**, 640–644.
21. Muller, R., Muller, D. & Guilbert, L. (1984) *EMBO J.* **3**, 1887–1890.
22. Taniuchi, M. & Johnson, E. (1985) *J. Cell Biol.* **101**, 1100–1106.
23. Chandler, C., Parsons, L., Hosang, M. & Shooter, E. (1984) *J. Biol. Chem.* **259**, 6882–6889.
24. Chirgwin, J., Przybyla, A., MacDonald, R. & Rutter, W. (1979) *Biochemistry* **18**, 5294–5300.
25. Thomas, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
26. Feinberg, A. & Vogelstein, B. (1984) *Anal. Biochem.* **67**, 15–28.
27. Curran, T., Peters, G., Van Beveran, C., Teich, N. & Verma, I. (1982) *J. Virol.* **44**, 674–682.
28. Rothberg, P., Erisman, M., Dieh., R., Rovigatti, U. & Astrin, S. (1984) *Mol. Cell. Biol.* **4**, 432–439.
29. Brodeur, G., Seeger, R., Schwab, M., Varmus, H. & Bishop, J. (1984) *Science* **224**, 1121–1124.
30. Greene, L. & Tischler, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2424–2428.
31. Van Beveran, C., van Straaten, F., Curran, T., Muller, R. & Verma, I. (1983) *Cell* **32**, 1241–1255.
32. Cochran, B., Reffel, A. & Stiles, C. (1983) *Cell* **33**, 939–947.
33. Huff, K., End, D. & Guroff, G. (1981) *J. Cell Biol.* **88**, 189–198.
34. End, D., Tolson, N., Yu, M.-Y. & Guroff, G. (1982) *J. Cell. Physiol.* **111**, 140–148.
35. Garrels, J. & Schubert, D. (1979) *J. Biol. Chem.* **254**, 7978–7985.
36. Greene, L., Liem, R. & Shelanski, M. (1983) *J. Cell Biol.* **96**, 76–83.
37. Richter-Landsberg, C., Greene, L. & Shelanski, M. (1985) *J. Neurosci.* **5**, 468–476.
38. Cochran, B., Zullo, J., Verma, I. & Stiles, C. (1984) *Science* **226**, 1080–1082.
39. Nishizuka, J. (1984) *Science* **225**, 1365–1370.
40. DiStefano, P., Schweitzer, J., Taniuchi, M. & Johnson, E. (1985) *J. Cell Biol.* **101**, 1107–1114.
41. Curran, T. & Morgan, J. (1985) *Science* **229**, 1265–1268.
42. Greenberg, M., Greene, L. & Ziff, E. (1985) *J. Biol. Chem.* **260**, 14101–14110.
43. Kruijer, W., Schubert, D. & Verma, I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7330–7334.