Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5282–5286, July 1988
Neurobiology

Induction of nerve growth factor receptors on cultured human melanocytes

(differentiation/pigmentation/melanoma)

MONICA PEACOCKE*, MINA YAAR*, CLAIRE P. MANSUR*, MOSES V. CHAO†, AND BARBARA A. GILCHREST*‡

*U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111; and †Department of Cell Biology and Anatomy and Division of Hematology/Oncology, Cornell University Medical College, New York, NY 10021

Communicated by Philip Siekevitz, February 29, 1988 (received for review September 11, 1987)

ABSTRACT **Normal differentiation and malignant transformation of human melanocytes involve a complex series of interactions during which both genetic and environmental factors play roles. At present, the regulation of these processes is poorly understood. We have induced the expression of nerve growth factor (NGF) receptors on cultured human melanocytes with phorbol 12-tetradecanoate 13-acetate and have correlated this event with the appearance of a more differentiated, dendritic morphology. Criteria for NGF receptor expression included protein accumulation and cell-surface immunofluorescent staining with a monoclonal antibody directed against the human receptor and induction of the messenger RNA species as determined by blot-hybridization studies. The presence of the receptor could also be induced by UV irradiation or growth factor deprivation. The NGF receptor is inducible in cultured human melanocytes, and we suggest that NGF may modulate the behavior of this neural crest-derived cell in the skin.**

Human melanocytes are neural crest-derived cells that synthesize and distribute pigment within the epidermis. Melanin provides basal skin color and, because of its ability to absorb electromagnetic irradiation, functions as a protective, pigmentary shield against carcinogenic UV light. When the melanocyte is exposed to sunlight, melanin synthesis is stimulated via a tyrosinase-dependent pathway. The pigment is then packaged in cytoplasmic particles known as melanosomes and distributed to neighboring keratinocytes. Current data suggest that excessive UV light exposure, especially in fair-skinned individuals, contributes to the development of malignant melanoma, but early events in the transformation of the normal melanocyte are presently undefined.

Little is known about the regulation of melanocyte growth, differentiation, or transformation in part because *in vitro* cultivation of this fastidious cell has been difficult to achieve (1–4). While a variety of growth regulatory molecules has been described for neural crest-derived cells, the best characterized is nerve growth factor (NGF). NGF is a polypeptide hormone that exerts trophic and differentiative effects upon the vertebrate nervous system (5). Early studies suggest a role for NGF as a neural crest mitogen in that NGF increased the volume of sensory and sympathetic ganglia *in vivo* (6). Subsequent studies showed, however, that this increase was the result of reduction in naturally occurring cell death during the early stages of differentiation (7). Hence, NGF functions more as a survival factor for these cells. Most recently, NGF has been shown to be important for mast-cell survival (8, 9). In some systems, NGF also functions as a differentiation factor in that it can induce a variety of enzymes—specifically, tyrosine hydroxylase (10), dopamine hydroxylase (11), choline acetyltransferase (12), phenylethanolamine *N*-methyltransferase (13), ornithine decarboxylase (14), and acetylcholinesterase (15). In the rat pheochromocytoma cell line PC 12, NGF leads to growth arrest and dramatic neurite outgrowth (16).

The diverse biologic effects of this growth factor are initiated by interaction of the ligand with a specific cell-surface receptor. NGF receptors have been demonstrated on both sensory and sympathetic neurons in a variety of animal species (17–19). As well, NGF receptors have been found on a variety of nonneuronal derivatives of the neural crest, including pheochromocytoma cells, Schwann cells, and neurofibroma cells and on several melanoma cell lines (20–22). To date, little work has been done to characterize the NGF receptor on nontransformed human cells. Based on the predicted amino acid sequence, the molecular mass of the NGF receptor is 50 kDa. This form undergoes both N-linked and O-linked glycosylation to produce a mature receptor of 70–75 kDa (23). The gene for the NGF receptor has been recently cloned (24, 25). It is located on the long arm of chromosome 17 and is transcribed as a single messenger RNA species of 3.8 kilobases (kb) (26).

In vivo, the epidermal melanocyte is situated at the dermal–epidermal junction and extends long, dendritic projections between keratinocytes into the epidermis. *In vitro*, the cell morphology depends on the culture environment, being epithelioid to bipolar in serum-free hormone-supplemented medium (3) but more dendritic in the presence of phorbol 12-tetradecanoate 13-acetate (TPA), mimicking *in vivo* appearance (2, 27). Using a combination of immunofluorescent, immunoblotting, and RNA blotting techniques, we have demonstrated the presence of NGF receptors on appropriately stimulated human melanocytes.

MATERIALS AND METHODS

Melanocyte and Melanoma Cultivation. Pure melanocyte cultures were prepared as described (4). Briefly, deep dermis and subcutaneous fat were removed from fresh neonatal foreskins or adult skin biopsies, and the remaining tissue was cut into fragments, rinsed in phosphate-buffered saline, and incubated in 0.25% trypsin (Sigma) overnight at 4°C. The epidermal portions were then separated from the dermis, incubated in 0.2% EDTA, mixed to yield a single-cell suspension, and inoculated at 1.25×10^5 cells per cm^2 (10^6 cells per 35-mm dish) on uncoated tissue culture dishes (Falcon) and maintained at 37°C in 8% CO_2/92% air.

Cells were maintained in Medium 199 (GIBCO) supplemented with 10 ng of epidermal growth factor (Collaborative Research, Waltham, MA) per ml, 1 nM triiodothyronine (Sigma), 10 μg each of transferrin (Sigma) and insulin (Sigma) per ml, 1 nM choleragen (Schwartz/Mann), and a bovine hypothalamic extract (100 μg/ml) known to contain growth-

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; TPA, phorbol 12-tetradecanoate 13-acetate.

‡To whom reprint requests should be addressed.

promoting activity for human melanocytes (3, 4, 28). In specific experiments, 50 ng of TPA (Sigma) or 25 units of NGF (Collaborative Research) per ml was added to the culture medium for periods of 3–48 hr or bovine hypothalamic extract was removed for 48 hr. In other experiments, melanocyte cultures were irradiated by using a Kratos (Westwood, NJ) LH 153 solar simulator with maximally tolerated UV doses in a previously developed protocol (29) known to induce dendricity and melanogenesis and to arrest the growth of irradiated cells.

The cell line Hs 294T (HTB-140) was obtained from the American Type Culture Collection. It was routinely grown in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal bovine serum and serially passaged at confluence.

Indirect Immunofluorescence. Cells from primary or secondary cultures were seeded on glass coverslips in standard medium and grown for 3 days. Cells on coverslips were then fixed with 4% (vol/vol) formaldehyde for 30 min and processed for immunofluorescence as described (30). The first antibody used was either the mouse monoclonal antibody ME 20.4, which specifically recognizes the human NGF receptor (21), or an irrelevant mouse monoclonal antibody. The second antibody used was a fluorescein-tagged goat anti-mouse IgG (Cooper Biomedicals, Malvern, PA) (31).

Immunoblotting. Confluent melanocyte cultures, unstimulated or stimulated with TPA for 48 hr, were extracted in 0.5 ml of 1% Triton X-100 in Tris-buffered saline containing 1 mM phenylmethylsulfonyl fluoride (32). The extracted proteins were reduced, denatured, separated by $NaDodSO_4$/7.5% PAGE, and then electrophoretically transferred to nitrocellulose paper by using a transblot apparatus (Bio-Rad) overnight at 4°C and 60 V in Tris glycine buffer with 20% (vol/vol) methanol (33). Antigens on the nitrocellulose paper were incubated with the anti-NGF receptor mouse monoclonal antibody ME 20.4 or an irrelevant control monoclonal antibody at a dilution of 1:50. Specific binding was detected by immunoperoxidase staining of the nitrocellulose paper.

DNA Probes. The plasmid pH1-3 was used for these studies (24). It contains an 800-base-pair human NGF receptor cDNA cloned into the *Eco*RI site of the vector pSP65. This cDNA sequence encodes the extracellular domain of the NGF receptor (24).

RNA Isolation and Blot-Hybridization Analysis. Total cellular RNA was isolated by lysis of cells with 4 M guanidine thiocyanate, followed by centrifugation through a 5.7 M cesium chloride gradient (34, 35). To remove melanin, samples were routinely extracted twice with hot phenol, the phenol was removed with chloroform extraction, and the samples ultimately were precipitated with ethanol. The RNA was resuspended in sterile distilled water, and the concentration of RNA was determined by absorbance at 260 nm. The purity of the sample was determined by A_{260}/A_{280} and was always 2.0 or greater. The RNA was reprecipitated with 3 M sodium acetate and ethanol and stored at −70°C.

Total cellular RNA (40 μg) was size-fractionated through a 1% agarose gel containing formaldehyde (2.2 M). The RNA was then transferred to a nylon membrane (Hybond-N, Amersham) and immobilized by shortwave UV illumination. The blot was then prehybridized for 6 hr in a solution containing 50% formamide, 10% dextran sulfate, 0.6 M NaCl, 50 mM NaH_2PO_4-H_2O, 5 mM EDTA, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin, and denatured salmon sperm DNA at 10 μg/ml for 6 hr at 42°C. The NGF receptor cDNA insert was radiolabeled by nick-translation to a specific activity of 2×10^8 cpm/μg of DNA and denatured. The blot was then hybridized overnight at 42°C. The blot was then washed twice for 30 min at room temperature in 0.30 M NaCl/0.03 M sodium citrate/0.1% $NaDodSO_4$ and then twice for 30 min at 65°C in 0.015 M NaCl/0.0015 M sodium citrate/0.1% $NaDodSO_4$. The blot was then dried, and autoradiography was performed at −70°C with Kodak XAR film and intensifying screens.

Growth Assays. Melanocytes were seeded at 4×10^4 cells per 35-mm dish in standard medium alone or in medium containing NGF at 25 units or 125 units per ml. At day 7 cells were counted in a Coulter Counter.

RESULTS

Morphology. The rapid induction of morphological changes in TPA-stimulated melanocytes has been noted (27). Actively proliferating melanocytes exhibit tripolar and polygonal morphology (Fig. 1*a*), but as quickly as 1 hr after stimulation with TPA at 50 ng/ml, the cells acquire a neuron-like morphology with prominent nuclei, scarce perinuclear cytoplasm, and long projecting dendritic processes (Fig. 1*b*). These changes occur as quickly as 1 hr after stimulation and last for periods of up to 7 days, even when TPA is removed from the medium 1 hr after stimulation.

Our tissue culture system is optimized for cell growth, and the cells usually exhibit polygonal morphology. However,

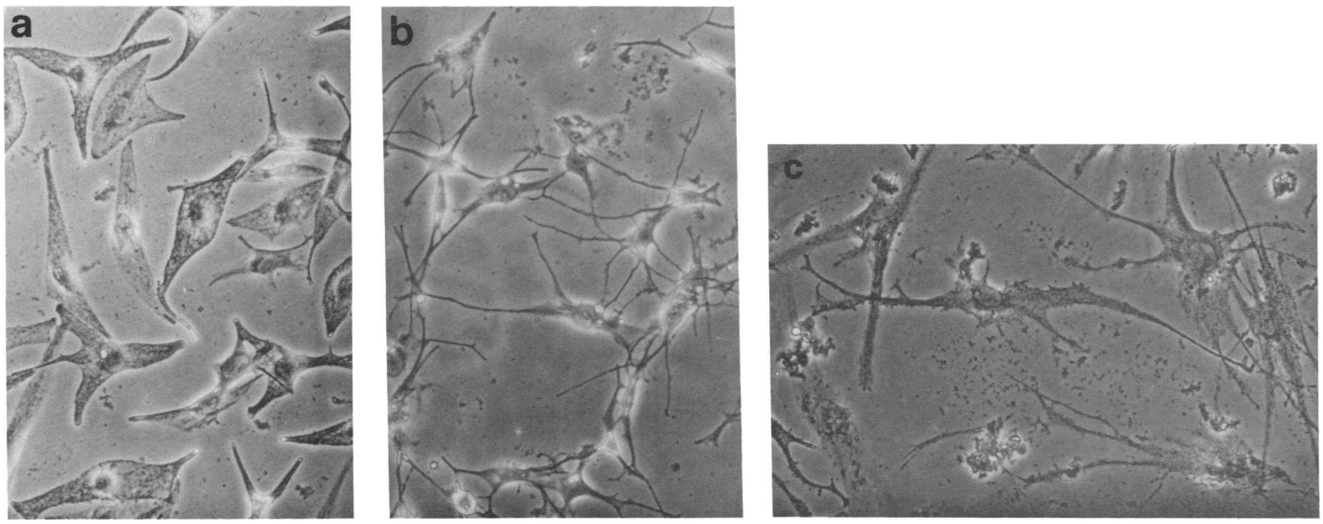

FIG. 1. Melanocyte morphology. Phase-contrast micrographs of human melanocytes grown in standard medium (*a*), stimulated for 48 hr with TPA at 50 ng/ml (*b*), or deprived of the bovine hypothalamic extract, the major mitogen source in this medium, for 48 hr (*c*). Note the long, projecting dendrites in the TPA-stimulated cells and the nearly comparable dendricity in the mitogen-deprived cells. (*a* and *b*, ×180; *c*, ×140.)

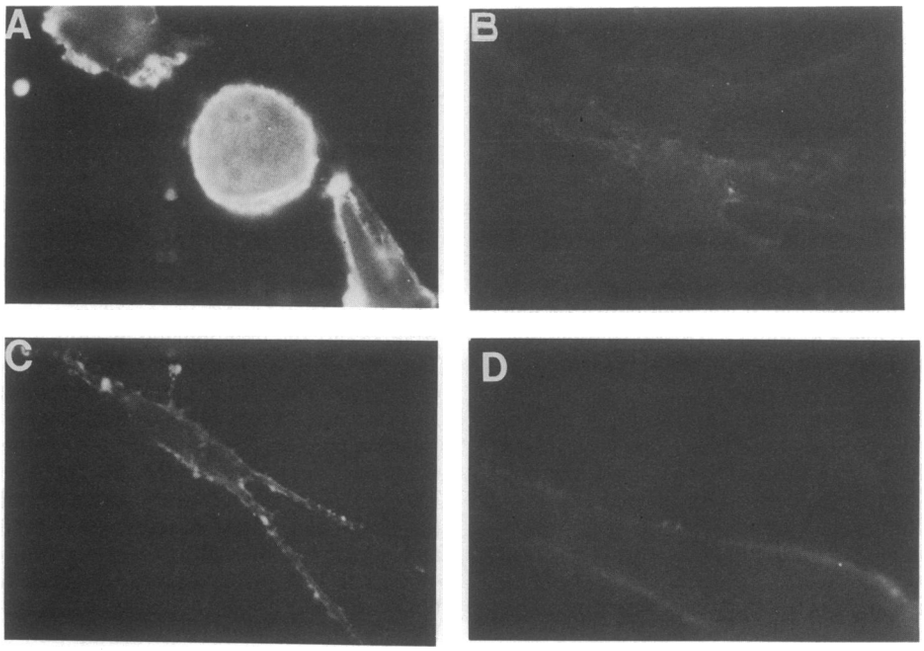

FIG. 2. Immunofluorescence of melanocytes. Immunofluorescent staining of cells stained with anti-NGF receptor antibody. (*A*) Hs 294T melanoma cell line. (*B*) Proliferating melanocytes in complete medium. (*C*) TPA-stimulated melanocytes. (*D*) UV-irradiated melanocytes. Note the bright rim fluorescence of the melanoma cells and of the TPA-treated or UV-irradiated cells in contrast to the lack of fluorescence in the unstimulated melanocytes. (*A–C*, ×1200; *D*, ×1600.)

when the cells are growth factor-deprived by removal of serum or bovine hypothalamic extract from the culture, melanocytes become increasingly dendritic over several days (Fig. 1*c*). UV-irradiated cells similarly become more dendritic (29), with a morphology intermediate between that of cells in the basal medium and in TPA-supplemented medium. Addition of NGF to the cultures has no morphologic effect during 7 to 10 days.

Immunofluorescence. The monoclonal antibody ME 20.4 (21) was used to detect NGF receptors in Hs 294T, a human melanoma cell line known to express high levels of NGF receptor (36). An intense cell-surface staining pattern was observed (Fig. 2*A*). In contrast, when cultured human melanocytes were seeded in standard medium in the presence of fetal bovine serum (2–20%) and/or bovine hypothalamic extract (100 μg/ml), there was no cell-surface staining for the receptor (Fig. 2*B*). After 24 hr of exposure to TPA, melanocytes exhibited the characteristic staining pattern (Fig. 2*C*) indicative of cell-surface expression of NGF receptors. Melanocytes deprived of growth factors or serum also exhibited cell-surface staining, but the proportion of cells stained in the culture was smaller—approximately half. Similarly, the more highly dendritic melanocytes in UV-irradiated cultures also expressed the NGF receptor (Fig. 2*D*). These data confirm an earlier suggestion that cultured melanocytes may stain with anti-NGF receptor antibodies (37).

Immunoblotting. Melanocytes were grown in standard medium until nearly confluent. Paired cultures were either harvested directly or treated for 48–72 hr with TPA at 50 ng/ml. With the TPA-treated cells, the monoclonal antibody ME 20.4 (21) recognized a 70- to 75-kDa protein (Fig. 3), entirely in keeping with the known molecular mass of the human NGF receptor (23). This band was absent in the untreated controls.

RNA Blotting. For these studies, melanocytes were grown in standard medium until nearly confluent. The cells were then harvested directly or at various times after stimulation with TPA at 50 ng/ml. Examination of the autoradiograph (Fig. 4) revealed the high levels of the 3.8-kb messenger RNA species in the control cell line Hs 294T. Receptor mRNA species were undetectable in unstimulated cells, but after 12 hr of TPA stimulation, melanocytes displayed a striking increase in the steady-state level of NGF receptor mRNA.

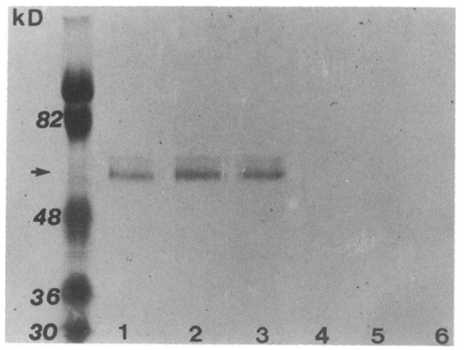

FIG. 3. Immunoblot analysis of melanocytes, showing TPA-stimulated cells (lanes 1, 2, and 3) and paired unstimulated control cells (lanes 4, 5, and 6). Note the presence of a 70- to 75-kDa protein in the stimulated cells that is absent in the unstimulated cells. kD, kDa.

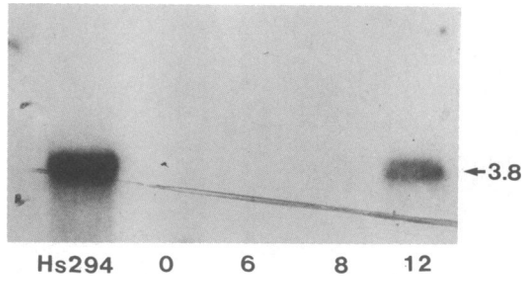

FIG. 4. RNA blot-hybridization analysis of melanocytes, showing the presence of a 3.8-kb transcript in Hs 294T (Hs294) control cells. The transcript is undetectable in unstimulated cells, but appears 12 hr after TPA stimulation. Equal amounts of total cellular RNA (40 μg) were loaded in each lane as assessed by ethidium bromide staining.

Table 1. Lack of NGF effect on melanocyte growth

NGF, units/ml	Day	Cell number (2 SD)
None	7	210,960 (13576)
25	7	215,760 (13237)
125	7	185,760 (5657)

Growth Assays. Melanocytes in paired dishes were seeded in standard medium containing NGF at 0, 25, or 125 units per ml. In three experiments, there was no increase in cell number 7 days after seeding (Table 1).

DISCUSSION

Cell-surface receptors for growth factors play a central role in normal signal transduction and are an important means of modulating cell growth and differentiation. As well, certain oncogenes demonstrate strong homology to growth factor receptors, such as the avian erythroblastosis virus *erbB* oncogene and the receptor for epidermal growth factor (36), and the oncogene *fms* and the receptor for colony-stimulating factor (38).

Our observations indicate that NGF receptor mRNA and cell-surface protein are inducible in cultured human melanocytes. To date, the NGF receptor is the only growth factor receptor documented on human melanocytes. Its appearance on the melanocyte cell surface correlates with a characteristic, dendritic neuron-like morphology that can be induced most strikingly by TPA but also by culture manipulations such as serum or growth-factor deprivation, and by UV-irradiation. Because TPA is known to induce a broad range of differentiated behaviors in cultured cells (39, 40), it is intriguing to postulate that this receptor is a marker of differentiation in these cells and that NGF may mediate one or more melanocyte behaviors *in vivo*. NGF-receptor induction after UV-irradiation or withdrawal of serum and hypothalamic extract may be interpreted as a differentiation response to slowed growth or as a distress signal by cells now damaged or lacking the major survival factors previously present in their culture environment.

The regulation of growth, differentiation, or transformation of human melanocytes *in vivo* or *in vitro* is poorly understood at present. Only recently have advances in selective tissue culture techniques permitted large-scale cultivation of this cell type. Thus far, reported growth-promoting activities for human melanocytes *in vitro* include serum (2, 3, 28), choleragen and other agents that increase cAMP (2, 3, 28), phorbol esters (2), and, most recently, basic fibroblast growth factor (41). Growth-stimulating activities for human melanocytes have also been described in various conditioned media and cell or tissue extracts (4, 42–44), but these factors remain incompletely characterized. Our present observations agree with earlier results (3, 28) that NGF is not a mitogen for human melanocytes. However, the fact that melanocytes express the NGF receptor when they are injured or deprived of growth factors suggests that NGF may function as a survival factor for these cells. It is of interest that perineural Schwann cells, also neural crest-derived, appear to express NGF receptors in adult skin *in vivo* (21) and in rat sciatic nerve after axotomy (45), yet cultured Schwann cells seem not to require NGF for growth or survival, nor does Schwann cell morphology change in response to NGF (45–48).

The mechanism of induction of NGF receptors in cultured human melanocytes is unknown. Tumor-promoting phorbol esters such as TPA activate protein kinase C, the acknowledged receptor for TPA (49). Protein kinase C is a phospholipid-dependent, calcium-activated kinase that phosphorylates proteins on serine and threonine residues. TPA has been shown to induce rapid reorganization of the cytoskeleton (50, 51). It is interesting that the morphological changes in the melanocytes occurred as quickly as 1 hr after TPA stimulation, while the NGF receptor mRNA appeared only 10–12 hr later, suggesting that TPA may act directly on the cytoskeletal proteins already present in the cell or on the expression of genes involved in neurite outgrowth. The appearance of the receptor may then be required for the transmission of signals important to the differentiated, dendritic melanocyte. Melanin synthesis and distribution are major functions of melanocytes *in vivo*, and we hypothesize that NGF and its receptor may play a crucial role in this process, either at the level of induction of the enzyme tyrosinase or by transducing critical signals for melanogenesis from adjacent keratinocytes. The former mechanism would be appealing in view of the known induction of tyrosinase and enhanced pigment production after, for example, UV exposure of intact skin, while the latter mechanism is consonant with the recent demonstration of NGF in developing murine epithelium (52).

The NGF receptor is known to be abundantly expressed in many melanoma cell lines (21, 22). In our tissue culture system, the tumor promoter TPA induces receptor expression by normal melanocytes. Thus, induction of the NGF receptor may represent the first in a series of changes that ultimately can result in the development of melanoma. This speculation is strengthened by the observation that cell-surface staining with anti-NGF receptor antibody has been reported (21) to be positive on 100% of nevus cells in frozen sections of a dysplastic nevus, in contrast to 10% or less of nevus cells in sections of five benign nevi. Melanoma has been a long-standing focus of both clinical and laboratory-based research because of its grave prognosis and its propensity to affect young adults. Over the past several decades, melanoma has increased more than 11-fold in incidence and is now increasing more rapidly than any other malignancy, except lung cancer in women (53, 54). Therefore, it is of the utmost importance to investigate all of the early changes associated with the development of melanoma to elucidate the mechanism leading to the malignant transformation of pigment cells in the skin.

The authors gratefully acknowledge Michael Vrabel, Deborah Woodward, and Jacalyn Gordon for expert technical assistance; Toby Kauffman for help with manuscript preparation; and Jessica Jahnen for helpful discussions. This work was supported in part by a fellowship of the Dermatology Foundation, Westwood Pharmaceutical–Lawrence Gelb Research Foundation (to M.P.); by National Institutes of Health Grants AG00354 (to M.Y.) and CA45687 (to B.A.G.); and by the Agriculture Research Service of the U.S. Department of Agriculture. M.V.C. is supported by National Institutes of Health Grant NS21072, the Cornell Scholars Program, and the Hirschl/Weill-Caulier Trust.

1. Klaus, S. N. (1980) *Methods Cell Biol.* **21,** 277–285.
2. Eisenger, M. & Marko, O. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2018–2022.
3. Wilkins, L. M., Szabo, G., Connell, L., Gilchrest, B. A. & Maciag, T. (1982) *Cold Spring Harbor Conf. Cell Proliferation* **9,** 929–936.
4. Gilchrest, B. A., Vrabel, M. A., Flynn, E. & Szabo, G. (1984) *J. Invest. Dermatol.* **83,** 370–376.
5. Thoenen, H. & Barde, Y.-A. (1980) *Physiol. Rev.* **60,** 1284–1335.
6. Levi-Montalcini, R. & Hamburger, V. (1953) *J. Exp. Zool.* **123,** 233–288.
7. Hendry, I. A. (1975) *J. Neurocytol.* **5,** 337–349.
8. Aloe, L. & Levi-Montalcini, R. (1977) *Brain Res.* **133,** 358–366.
9. Bohm, A. & Aloe, L. (1986) *Atti Accad. Naz. Lincei Mem. Cl. Sci. Fis. Mat. Nat. Sez. 3* **80,** 1–6.
10. Thoenen, H., Angeletti, P. U., Levi-Montalcini, R. & Kettler, R. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 1598–1602.
11. Chun, L. L. Y. & Patterson, P. H. (1977) *J. Cell Biol.* **75,** 694–704.

12. Greene, L. A. & Rein, G. (1977) *Nature (London)* **268,** 349–351.
13. Liuzzi, A., Foppen, F. H. & Kopin, I. J. (1977) *Brain Res.* **138,** 309–315.
14. MacDonell, P. G., Nagaich, K., Lakshmanan, J. & Guroff, G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4681–4685.
15. Reiger, F., Shelanski, M. L. & Greene, L. A. (1980) *Dev. Biol.* **76,** 238–243.
16. Greene, L. A. & Tischler, A. S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2424–2428.
17. Banerjee, S. P., Snyder, S. H., Cuatrecasas, P. & Greene, L. A. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2519–2523.
18. Herrup, K. & Shooter, E. M. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3884–3888.
19. Frazier, W. A., Boyd, L. F. & Bradshaw, R. A. (1974) *J. Biol. Chem.* **249,** 5513–5519.
20. Landreth, G. E. & Shooter, E. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4751–4755.
21. Ross, A. H., Grob, P., Bothwell, M. A., Elder, D. E., Ernst, C. S., Marano, N., Ghrist, B. F. D., Slemp, C. C., Herlyn, M., Atkinson, G. & Koprowski, H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6681–6685.
22. Fabricant, R. F., DeLarco, J. E. & Todaro, G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 565–569.
23. Grob, P. M., Ross, A. H., Koprowski, H. & Bothwell, M. (1985) *J. Biol. Chem.* **260,** 8044–8049.
24. Johnson, D., Lanahan, A., Buck, C. R., Sehgal, A., Morgan, C., Mercer, E., Bothwell, M. & Chao, M. V. (1986) *Cell* **47,** 545–554.
25. Chao, M. V., Bothwell, M. A., Ross, A. H., Koprowski, H., Lanahan, A., Buck, C. R. & Sehgal, A. (1986) *Science* **232,** 418–421.
26. Huebner, K., Isobe, M., Chao, M. V., Bothwell, M., Ross, A. H., Finian, J., Hoxie, J. A., Sehgal, A., Buck, C. R., Lanahan, A., Nowell, P. C., Koprowski, H. & Croce, C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1403–1407.
27. Gilchrest, B. A. & Friedmann, P. S. (1987) *Struct. Func. Mel.* **4,** 1–13.
28. Wilkins, L. M., Gilchrest, B. A., Szabo, G., Weinstein, R. & Maciag, T. (1985) *J. Cell. Physiol.* **122,** 350–361.
29. Friedmann, P. S. & Gilchrest, B. A. (1987) *J. Cell. Physiol.* **133,** 88–94.
30. Stanley, J. R., Hawley-Nelson, P., Poirier, M., Katz, S. I. & Yuspa, S. H. (1980) *J. Invest. Dermatol.* **75,** 183–186.
31. Foidart, J. M., Bere, E. W., Jr., Yaar, M., Renard, S. I. Guillino, M., Martin, G. I. & Katz, S. I. (1980) *Lab. Invest.* **42,** 336–340.
32. Stanley, J. R., Hawley-Nelson, P., Yaar, M., Martin, G. R. & Katz, S. I. (1982) *J. Invest. Dermatol.* **78,** 456–459.
33. Stanley, J. R., Woodley, D. T. & Katz, S. I. (1984) *J. Invest. Dermatol.* **82,** 108–112.
34. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
35. Glisin, V. R., Crkvenjakov, R. & Byus, C. (1979) *Biochemistry* **13,** 2633–2637.
36. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307,** 521–527.
37. Herlyn, M., Thurin, J., Balaban, G., Bennicelli, J. L., Herlyn, D., Elder, D. E., Bondi, E., Guerry, D., Nowell, P., Clark, W. H. & Koprowski, H. (1985) *Cancer Res.* **45,** 5670–5676.
38. Sherr, C. J., Rettenmeier, C. W., Sacca, R., Roussel, M. E., Look, A. T. & Stanley, E. R. (1985) *Cell* **41,** 665–676.
39. Weinstein, I. B., Lee, L. S., Fisher, P. B., Mufson, A. & Yamasaki, H. (1979) *J. Supramol. Struct.* **12,** 195–208.
40. Nishizuka, Y. (1986) *Science* **233,** 305–312.
41. Halaban, R., Ghosh, S. & Baird, A. (1987) *In Vitro* **23,** 47–52.
42. Eisenger, M., Marko, O., Ogata, S.-I. & Old, L. J. (1985) *Science* **229,** 984–986.
43. Gilchrest, B. A., Karassik, R. L., Wilkins, L. M., Vrabel, M. A. & Maciag, T. (1983) *J. Cell. Physiol.* **117,** 235–240.
44. Nielsen, H. I. & Don, P. (1984) *Br. J. Dermatol.* **110,** 569–580.
45. Taniuchi, M., Clark, H. B. & Johnson, E. M., Jr. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4094–4098.
46. Zimmerman, A. & Sutter, A. (1983) *EMBO J.* **2,** 879–885.
47. Rohrer, H. & Sommer, I. (1983) *J. Neurosci.* **3,** 1683–1699.
48. Rohrer, H. (1985) *Dev. Biol.* **111,** 95–107.
49. Neidel, J. E., Kuhn, L. J. & Vandenbark, G. R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 36–41.
50. Fey, E. G. & Penman, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4409–4413.
51. May, W. S., Lapetina, E. G. & Cuatrecasas, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1281–1284.
52. Davies, A. M., Brandtlow, C., Heumann, R., Korsching, S., Rohrer, H. & Thoenen, H. (1987) *Nature (London)* **326,** 353–358.
53. Jensen, O. M. & Bolander, A. M. (1980) *World Health Stat. Q.* **33,** 2–6.
54. Elwood, J. M. & Lee, J. A. H. (1975) *Semin. Oncol.* **2,** 149–154.