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Specific receptors for platelet-derived growth factor on cells derived from connective tissue and glia

(cultured cells/iodine-labeled tracer/binding assay/high affinity)

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ABSTRACT **A cellular receptor for platelet-derived growth factor (PDGF) was demonstrated by incubation of ^{125}I-labeled PDGF with human foreskin fibroblast cultures followed by liberation of cell-bound radioactivity with Triton X-100. The cellular binding of labeled PDGF in the presence of increasing amounts of unlabeled PDGF showed saturation; Scatchard analysis of binding data indicated a single class of receptors having $k_d = 1 \times 10^{-9}$ M. The number of PDGF binding sites was $\approx 3 \times 10^5$/cell. Labeled PDGF binding reached an apparent equilibrium after 3 hr at 4°C. At 37°C, it passed a maximum after 30 min and then decreased with time due to degradation of the tracer. A large excess of unlabeled PDGF reduced labeled PDGF binding by more than 90% whereas similar doses of epidermal growth factor, fibroblast growth factor, or insulin had no effect. It was concluded that PDGF did not share receptors with these factors. PDGF receptors were found on skin fibroblasts, normal and malignant glial cells, smooth muscle cells, and 3T3 cells but not on epithelial-derived cells, neuroblastoma cells, endothelial cells, or peripheral lymphocytes. As only the receptor-positive cells—i.e., the connective tissue- and glia-derived cells—are responsive to stimulation with PDGF, these findings imply a functional significance of the PDGF receptor.**

The human platelet-derived growth factor (PDGF) is a M_r 30,000 cationic protein, probably composed of two polypeptide chains linked by disulfide bonds (1, 2). PDGF is stored in the α-granules of platelets (3, 4) and released during the platelet-release reaction (3–5). The factor stimulates the proliferation of various cultured cells, such as human glial cells (1, 6, 7), and a number of connective tissue-derived cells, e.g., arterial smooth muscle cells (8, 9) human fibroblasts (10, 11), and mouse 3T3 cells (12–15). For these cell types, PDGF actually constitutes the major part of the growth-promoting activity of serum. The access to pure ^{125}I-labeled PDGF (1) has made it possible to study the interaction between PDGF and its target cells. In this paper, we describe a specific binding of ^{125}I-labeled PDGF to cultured cells. A preliminary report has been presented elsewhere (16).

MATERIALS AND METHODS

Growth Factors. PDGF was purified from fresh platelets as described (2). The final product was >90% pure as estimated from analytical NaDodSO$_4$/polyacrylamide gel electrophoresis. Epidermal growth factor (EGF) and fibroblast growth factor (FGF) were purchased from Collaborative Research (Waltham, MA). Insulin was obtained from Vitrum (Stockholm, Sweden).

Radiolabeling of PDGF. PDGF was labeled with ^{125}I according to Hunter and Greenwood (17) as described (1). Five micrograms of PDGF was labeled with 0.5 mCi (1 Ci = 3.7 $\times 10^{10}$ becquerels) of ^{125}I. ^{125}I-Labeled PDGF was separated from unbound radioactivity on a Sephadex G-25 column eluting with 1 M acetic acid containing serum albumin at 1 mg/ml (1). The specific activity was $\approx$16,000 cpm/ng, corresponding to $\approx$0.2 atoms of iodine per molecule of PDGF. The present labeling procedure results in preferential incorporation of ^{125}I into the 17,000-dalton chain of PDGF. ^{125}I-Labeled PDGF was stored at −20°C. It retained unchanged binding properties over a period of 2 mo.

Conditions of ^{125}I-Labeled PDGF Binding to Human Foreskin Fibroblasts. Cellular binding of ^{125}I-labeled PDGF was studied by using the human foreskin fibroblast cell line AG 1523, kindly provided by the Human Mutant Cell Repository, Institute for Medical Research (Camden, NJ). For binding experiments, cells were seeded in Linbro 12-well plates (4.5 cm^2 per well). The dishes were incubated in Eagle's minimal essential medium/5% fetal calf serum for 6 days with a medium change on day 3. The cell number was then 0.20–0.35 $\times 10^6$ per well. After a wash with binding medium (phosphate-buffered saline containing human serum albumin at 1 mg/ml, $CaCl_2 \cdot 2H_2O$ at 0.01 mg/ml, and $MgSO_4 \cdot 7H_2O$ at 0.01 mg/ml), each well received 0.5 ml of binding medium. Labeled PDGF (5 ng, 80,000 cpm) with or without various amounts of unlabeled PDGF (50% pure) was then added. Binding was allowed for various periods of time in open air. Binding at 37°C was assayed in 5% CO_2/95% air using F-10 medium/human serum albumin at 1 mg/ml. Experiments were terminated by six washes with ice-cold binding medium containing 1% calf serum rather than albumin. The cells were then lysed with 0.5 ml of 20 mM Hepes, pH 7.4/1% Triton X-100/10% (vol/vol) glycerol containing serum albumin at 0.1 mg/ml. The Triton lysate was sampled after 20 min of incubation at room temperature. Residual extracellularly bound radioactivity was solubilized from the dishes with 0.5 ml of 0.3 M sodium hydroxide. The radioactivity of the lysates was determined in a γ spectrometer; counting efficiency was $\approx$70%.

Screening of ^{125}I-Labeled PDGF Binding Cell Lines. For screening of ^{125}I-labeled PDGF binding, a number of cultured cell lines was used (see Table 2). These were maintained in Eagle's minimal essential medium/5% fetal calf serum supplemented with streptomycin at 50 μg/ml and penicillin at 100 units/ml. Cells were routinely grown in 5-cm Nunc plastic dishes, incubating at 37°C in humidified 5% CO_2/95% air. Human peripheral lymphocytes were separated on Ficoll, using freshly drawn citrate blood from healthy donors.

For the binding assay, cells were trypsinized with 0.25% trypsin (Difco) in phosphate-buffered saline. They were then suspended in culture medium and seeded into 12-well Linbro plates (4.5 cm^2 per well). The cultures were incubated for 6 days

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Abbreviations: PDGF, platelet-derived growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor.

with a medium change on day 3. Cell densities of 0.16–1.0 × 10^6 per well were attained. Binding experiments with ^{125}I-labeled PDGF were performed as described above, incubating for 3 hr at 4°C. Experiments were terminated as described above and cell-associated radioactivity was determined after solubilization with Triton X-100. Human lymphocyte preparations were incubated with labeled PDGF in suspension; the cells were then separated from unbound radioactivity by centrifugation through a layer of 25% calf serum in phosphate-buffered saline. The radioactivity of the pellet was taken as an index of cell-bound labeled PDGF. Nonspecific binding was defined as the amount of labeled PDGF bound in the presence of a 50-fold molar excess of unlabeled PDGF.

Cell Counting. Cell numbers of trypsinized cultures were determined by using a Coulter Counter.

RESULTS

Demonstration of a Fibroblast Receptor for ^{125}I-Labeled PDGF. Incubation of confluent cultures of human foreskin fibroblasts (AG 1523) with 5 ng of ^{125}I-labeled PDGF (80,000 cpm) for 3 hr at 4°C, with or without a 50-fold excess of unlabeled PDGF, led to binding of radioactivity to the cultures (Table 1). As purified PDGF is a very "sticky" protein and readily adsorbed to solid surfaces, binding to the culture vessel was anticipated. Therefore, the nonionic detergent Triton X-100 was used to solubilize the cell layer, such that cell-associated radioactivity was selectively recovered in the supernatant. Subsequent treatment of the cultures with sodium hydroxide resulted in the liberation of additional radioactivity, representing labeled PDGF adsorbed to the solid support. Control experiments showed that plastic-bound labeled PDGF was readily released by alkali but not by the detergent. The Triton-soluble radioactivity, amounting to ≈15% of total added labeled PDGF, was markedly reduced (83%) after binding of labeled PDGF in the presence of a 50-fold molar excess of unlabeled PDGF. This indicates that the cellular binding of labeled PDGF is saturable and implies the presence of a receptor for PDGF on these cells. In contrast, adsorption of the tracer to the solid substratum was only slightly affected by excess unlabeled factor.

Concentration Dependence of ^{125}I-Labeled PDGF Binding to Fibroblasts. Incubation of confluent foreskin fibroblast cultures with ^{125}I-labeled PDGF and various concentrations of unlabeled PDGF demonstrated a saturable binding reaction (Fig. 1). Half-maximal binding was at 2–3 nM, and saturation was at 20–30 nM. A Scatchard plot (18) of the binding data indicated a single class of receptors (Fig. 2). The apparent K_d was ≈1 × 10^{-9} M, and the average number of receptors was ≈3 × 10^5 per cell.

Table 1. Binding of ^{125}I-labeled PDGF to human foreskin fibroblast cultures

	Addition	^{125}I-labeled PDGF bound, cpm per well: 1% Triton lysate	0.3 M NaOH-solubilized residue
Culture dishes with cells	5 ng of ^{125}I-labeled PDGF	12,938	4,378
Culture dishes with cells	5 ng of ^{125}I-labeled PDGF + 250 ng of PDGF	2,188	3,105
Control dishes without cells	5 ng of ^{125}I-labeled PDGF	296	18,798
Control dishes without cells	5 ng of ^{125}I-labeled PDGF + 250 ng of PDGF	323	14,944

Binding was performed on 12-well Linbro plates with or without a confluent culture of human foreskin fibroblasts (0.35 × 10^6 cells per well) as described in *Materials and Methods*. Briefly, the dishes were incubated at 4°C for 3 hr together with ^{125}I-labeled PDGF with or without unlabeled PDGF.

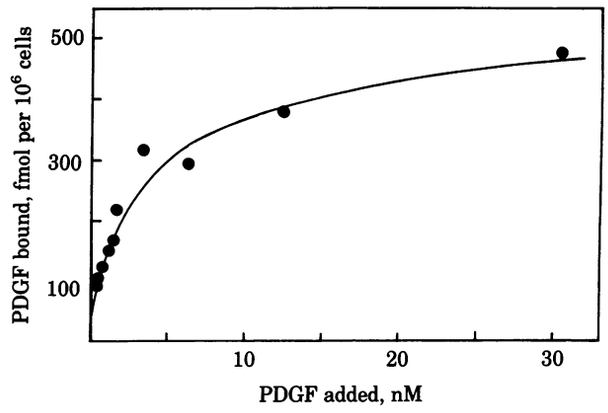

FIG. 1. Concentration dependence of ^{125}I-labeled PDGF binding to fibroblasts. Confluent cultures of human foreskin fibroblast cells were incubated for 3 hr at 4°C with labeled PDGF and various amounts of unlabeled PDGF as described in *Materials and Methods*. After washing, cell-associated radioactivity was collected by Triton-solubilization (see legend to Table 1). Nonspecific binding (≈6%), determined in the presence of a 400-fold molar excess of unlabeled PDGF, was subtracted.

Time Course and Temperature Dependence of ^{125}I-Labeled PDGF Binding. The time course of ^{125}I-labeled PDGF binding to foreskin fibroblasts at different temperatures is shown in Fig. 3. At 37°C, binding was maximal after 30 min and decreased on prolonged incubation. At lower temperatures, the binding rate was less and maximal binding was obtained later (2–4 hr). A small but reproducible decrease in cell-bound radioactivity occurred at 22°C whereas, at 4°C, binding seemed to approach a plateau.

Supernatants harvested from cultures that had been incu-

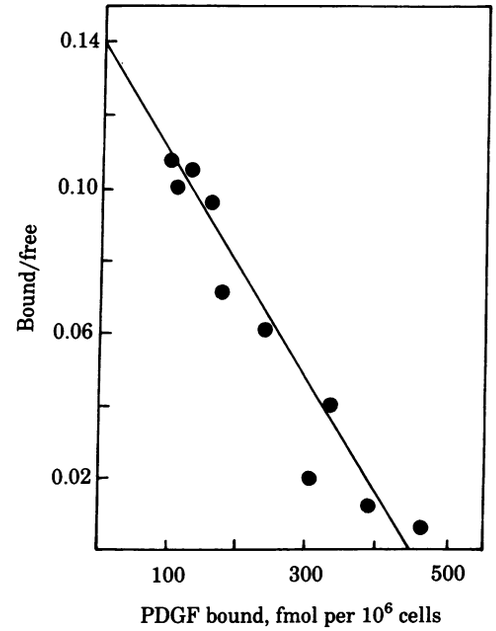

FIG. 2. Scatchard plot of data presented in Fig. 1, showing binding of ^{125}I-labeled PDGF to human foreskin fibroblasts.

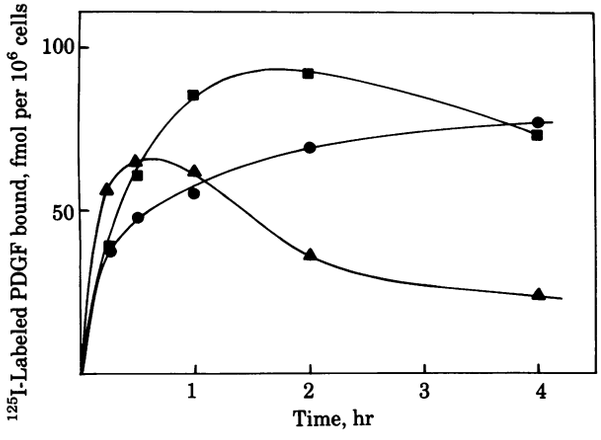

FIG. 3. Time course of binding of ^{125}I-labeled PDGF to human foreskin fibroblasts. Confluent cultures of cell line AG 1523 were incubated with labeled PDGF at 4°C (●), 22°C (■), or 37°C (▲) as described in *Materials and Methods*. Binding was terminated at various times, and cell-associated radioactivity was determined after solubilization with Triton X-100 (Table 1).

bated for 4 hr at 37°C were added to fresh fibroblast cultures, and these were assayed for bound radioactivity after 3 hr of incubation at 4°C. Although the harvested medium contained >90% of the original radioactivity, binding was very low (namely, <10% of that with fresh ^{125}I-labeled PDGF). Gel chromatography on Bio-Gel P-10 (not shown) indicated that most of the radioactivity of the spent supernatant was contained in a low molecular weight fraction.

Lack of Crossreactivity of ^{125}I-Labeled PDGF-Receptors for Other Growth Factors. Incubation of foreskin fibroblasts with a fixed amount of ^{125}I-labeled PDGF and increasing amounts of unlabeled PDGF resulted, as expected, in progressive inhibition of tracer binding; at the highest dose (400-fold excess), the displacement was >90% (Fig. 4). In contrast, the presence of similar amounts of unlabeled EGF, FGF, or insulin had no effect on the binding of labeled PDGF to the cells. It was concluded that the labeled PDGF receptor did not crossreact with the indicated factors. The addition of various basic proteins (bacitracin, cytochrome *c*, lysozyme, chymotrypsinogen A, or ribonuclease; 4 μg/ml) similarly failed to affect labeled PDGF binding (not shown).

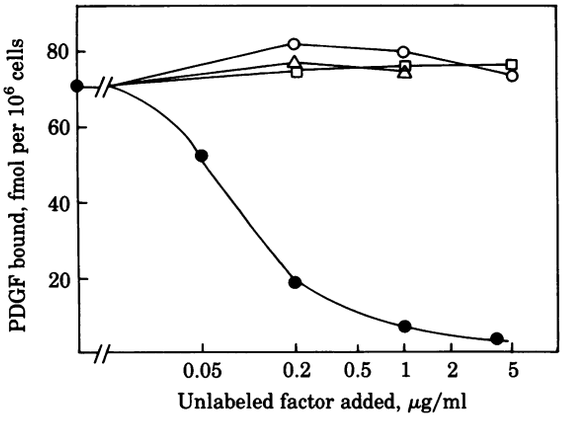

FIG. 4. Specificity of ^{125}I-labeled PDGF binding to human foreskin fibroblasts. Confluent cultures of cell line AG 1523 (0.30×10^6 cells) were incubated at 4°C for 3 hr with 5 ng of labeled PDGF (68,000 cpm) and various amounts of unlabeled PDGF (●); EGF (○); FGF (△), or insulin (□). Binding was terminated and cell-bound radioactivity was determined as described in *Materials and Methods*.

Tissue Specificity of ^{125}I-Labeled PDGF Receptors. The tissue specificity of ^{125}I-labeled PDGF binding was analyzed by using a number of cultured cells of different histogenetic origin (Table 2). Specific binding was estimated by subtracting the amount of labeled PDGF bound in the presence of a 50-fold excess of unlabeled PDGF. A high degree of binding was found on skin fibroblasts, normal and malignant glial cells, smooth muscle cells, and mouse 3T3 cells. No significant specific binding was found on epithelial-derived cells, neuroblastoma cells, endothelial cells, or peripheral blood lymphocytes. One of the human osteosarcoma cell lines (U-393 OS) displayed binding sites whereas the other one (U-2 OS) did not.

DISCUSSION

In recent years, several polypeptides have been described that stimulate the growth of cultured cells (27). For some of these, specific receptors have been described. Thus, the group of growth hormone-dependent factors (insulin-like growth factors and the somatomedins) and the related multiplication-stimulating activity of rat liver cells bind to a receptor of cultured fibroblasts (28–30). Binding of these factors can be displaced by high concentrations of insulin (28–30). The most detailed information on the interaction between a polypeptide growth factor and its target cell is that from experiments on EGF (31). High-affinity EGF receptors have been found on several cultured cell types—e.g., fibroblasts (32), 3T3 cells (33), and human glial cells (34).

Our results demonstrate binding of PDGF to human foreskin fibroblasts. Binding of ^{125}I-labeled PDGF was a saturable reaction, and Scatchard analysis at equilibrium showed only one class of high-affinity binding sites. The specificity of the reaction was tested in several ways. More than 90% of the tracer binding was abolished by simultaneous addition of a high concentration of PDGF, whereas the addition of nonrelated basic proteins had no effect. The PDGF receptor appeared distinct from the EGF receptor and the group of insulin/somatomedin receptors, as shown by the lack of competition by high concentrations of EGF and insulin. A specific cell binding of FGF has never been demonstrated; the present data show that the PDGF receptor must be separate from the hypothetical FGF receptor.

The foreskin fibroblasts were found to have a relative abundance of PDGF receptors ($\approx 3 \times 10^5$ per cell). This would indicate that each fibroblast has at least three times more binding sites for PDGF than for EGF (32). However, the kinetic data should be taken only as estimates as they are subject to several sources of errors (such as the determination of the specific activity of the ^{125}I-labeled PDGF preparations). Ample evidence has shown that EGF is internalized and degraded after binding at 37°C (23, 32, 35). This reaction is accompanied by a significant reduction in the number of EGF receptors (32, 33) ("down regulation") (36). Prolonged incubation of fibroblasts at 37°C in the presence of ^{125}I-labeled PDGF led to a decrease of cell-associated radioactivity. Analysis of the extracellular radioactivity indicated a large fraction of low molecular weight components, presumably degradation products of labeled PDGF. In conclusion, the decrease in cell-bound labeled PDGF observed under the present conditions can be explained by proteolytic inactivation of the tracer; whether down regulation of PDGF receptors also occurred remains to be determined.

The cell specificity of the mitogenic activity of PDGF has not been fully explored. Previously, only fibroblasts, 3T3 cells, vascular smooth muscle cells, and glial cells in culture have been shown to respond to the factor. This is in agreement with our demonstration of a specific binding exclusively to human skin fibroblasts, 3T3 cells, arterial smooth muscle cells, and normal and malignant human glia-derived cell lines. Human peripheral

Table 2. Binding of ^{125}I-labeled PDGF to cultured cells

Cell line	Reference	Origin	Cell type	Specific binding of ^{125}I-labeled PDGF*, fmol per 10^6 cells	Binding in the presence of excess PDGF, % of total
AG 1523		Human foreskin	Fibroblast	48	16
911S†		Human skin	Fibroblast	43	22
U-1508 CG	19, 20	Human brain	Glia like cell	18	28
U-251 MG	21	Human glioblastoma	Glioma cell	12	25
		Porcine renal artery	Smooth muscle cell	49	25
U-2 OS	22	Human osteosarcoma	Osteosarcoma cell	<2	80
U-393 OS‡		Human osteosarcoma	Osteosarcoma cell	10	27
PTh 33§		Porcine thyroid gland	Follicular cell	<2	86
SW 1736¶		Human thyroid cancer	Carcinoma cell	<2	70
U-1752‖		Human squamous carcinoma of the lung	Carcinoma cell	<2	83
A-431	23, 24	Human epidermal cancer	Carcinoma cell	<2	75
SH**	25	Human neuroblastoma	Neuroblast	<2	68
HEC††		Human umbilical vein	Endothelial cell	<2	87
3T3	26	Mouse embryo	Fibroblast-like	25	24
		Human blood	Lymphocytes	<2	96

Binding was performed as described in *Materials and Methods*, using a 5 ng of ^{125}I-labeled PDGF (60,000–80,000 cpm) per well for 3 hr at 4°C.

* Specific binding was calculated by subtracting binding in the presence of 50-foldmolar excess of unlabeled PDGF.
† Kindly provided by M. Jondal, Dept of Tumor Biology, Karolinska Institute (Stockholm).
‡ Unpublished cell line of the Wallenberg Laboratory (Uppsala).
§ Unpublished cell line of the Wallenberg Laboratory (Uppsala).
¶ Kindly provided by J. Fogh, Sloan Kettering (Rye, NY).
‖ Kindly provided by J. Bergh, the Wallenberg Laboratory (Uppsala).
** Provided through S. Påhlman, the Wallenberg Laboratory (Uppsala).
†† Kindly provided by C. Busch, the Wallenberg Laboratory (Uppsala).

blood lymphocytes, neuroblastoma cells, endothelial cells, and a variety of epithelial cells did not bind. The finding that endothelial cells did not bind is in agreement with the lack of mitogenic response of these cells to PDGF (37, 38).

An interesting observation was that one osteosarcoma cell line (U-2 OS) did not bind PDGF whereas another (U-393 OS) displayed specific PDGF binding. We have previously reported that U-2 OS cells—but not U-393 OS cells—release a growth factor (osteosarcoma-derived growth factor) that is similar, if not identical, to PDGF (39). Probably, the PDGF receptors of the U-2 OS cells are blocked by the endogenously produced growth factor. Cells transformed with murine sarcoma virus release a growth factor, sarcoma growth factor, that blocks EGF binding in an analogous manner (40, 41).

The present data imply that PDGF has a more restricted target-cell specificity than EGF, which is known to bind to a number of epithelial cells in addition to fibroblasts, vascular smooth muscle cells, and glial cells. PDGF may thus be a growth factor exclusive for connective tissue-derived cells and glial cells. This target specificity of PDGF, in conjunction with the fact that it is transported and released by the platelets, fits well with the idea that PDGF may have a role in wound healing (42) or other proliferative reactions of connective tissue.

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1. Heldin, C.-H., Westermark, B. & Wasteson, Å (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3722–3726.
2. Heldin, C.-H., Westermark, B. & Wasteson, Å (1981) *Biochem. J.* **193**, 907–913.
3. Kaplan, D. R., Chao, F. C., Stiles, C. D., Antoniades, H. N. & Scher, C. D. (1979) *Blood* **53**, 1043–1052.
4. Witte, L.D., Kaplan, K. L., Nossel, H. L., Lages, B. A., Weiss, H. J. & Goodman, D. S. (1978) *Circulation Res.* **42**, 402–409.
5. Busch, C., Wasteson, Å & Westermark, B. (1976) *Thromb. Res.* **8**, 493–500.
6. Westermark, B. & Wasteson, Å. (1976) *Exp. Cell Res.* **98**, 170–174.
7. Heldin, C.-H., Wasteson, Å. & Westermark, B. (1977) *Exp. Cell Res.* **109**, 429–437.
8. Ross, R., Glomset, J., Kariya, B. & Harker, L. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1207–1210.
9. Ross, R. & Vogel, A. (1978) *Cell* **14**, 203–210.
10. Castor, C. W., Ritchie, J. C., Scott, M. E. & Whitney, S. L. (1977) *Arthritis Rheum.* **20**, 859–868.
11. Slayback, J. R. B., Cheung, L. W. Y. & Geyer, R. P. (1977) *Exp. Cell Res.* **110**, 462–466.
12. Antoniades, H. N., Stathakos, D. & Scher, C. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2635–2639.
13. Antoniades, H. N. & Scher, C. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1973–1977.
14. Antoniades, H. N., Scher, C. D. & Stiles, C. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1809–1813.
15. Kohler, N. & Lipton, A. (1974) *Exp. Cell Res.* **87**, 297–301.
16. Heldin, C.-H., Wasteson, Å. & Westermark, B. (1981) in *Biology of Human Growth*, eds. Ritzen, M. & Larsson, A. (Raven, New York), pp. 23–30.
17. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496.
18. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.* **51**, 660–672.
19. Pontén, J. & Macintyre, E. (1968) *Acta Path. Microbiol. Scand. Sect. A* **74**, 465–486.
20. Pontén, J., Westermark, B. & Hugosson, R. (1969) *Exp. Cell Res.* **58**, 393–400.
21. Westermark, B., Pontén, J. & Hugosson, R. (1973) *Acta Pathol. Microbiol. Scand. Sect. A* **81**, 791–805.
22. Pontén, J. & Saksela, E. (1967) *Int. J. Cancer* **2**, 434–447.
23. Haigler, H., Ash, J. F., Singer, S. J. & Cohen, S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3317–3321.
24. Todaro, G. J. & De Larco, J. E. (1978) *Cancer Res.* **38**, 4147–4154.

25. Biedler, J. L., Helson, L. & Spengler, B. A. (1973) *Cancer Res.* **33,** 2643–2652.
26. Todaro, G. J. & Green, H. (1963) *J. Cell Biol.* **17,** 299–313.
27. Gospodarowicz, D. & Moran, J. S. (1976) *Annu. Rev. Biochem.* **45,** 531–558.
28. Raizada, M. K. & Perdue, J. F. (1976) *J. Biol. Chem.* **251,** 6445–6455.
29. Rechler, M. M., Nissley, S. P., Podskalny, J. M., Moses, A. C. & Fryklund, L. (1977) *J. Clin. Endocrinol. Metab.* **44,** 820–831.
30. Rechler, M. M., Podskalny, J. M. & Nissley, S. P. (1977) *J. Biol. Chem.* **252,** 3898–3910.
31. Cohen, S. (1962) *J. Biol. Chem.* **237,** 1555–1562.
32. Carpenter, G., Lembach, K. J., Morrison, M. M. & Cohen, S. (1975) *J. Biol. Chem.* **250,** 4297–4304.
33. Aharonov, A., Pruss, R. M. & Herschman, H. R. (1977) *J. Biol. Chem.* **252,** 3970–3977.
34. Westermark, B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1619–1621.
35. Carpenter, G. & Cohen, S. (1976) *J. Cell Biol.* **71,** 159–171.
36. Raff, M. (1976) *Nature (London)* **259,** 265–266.
37. Haudenschild, C. C., Zahniser, D., Folkman, J. & Klagsbrun, M. (1976) *Exp. Cell Res.* **98,** 175–183.
38. Ross, R., Vogel, A., Davies, P., Raines, E., Kariya, B., Rivest, M. J., Gustafson, C. & Glomset, J. (1979) in *Hormones and Cell Culture,* Cold Spring Harbor Conferences on Cell Proliferation, eds. Sato, G. & Ross, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 3–16.
39. Heldin, C.-H., Westermark, B. & Wasteson, Å. (1980) *J. Cell Physiol.,* **105,** 235–246.
40. De Larco, J. E. & Todaro, G. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4001–4005.
41. Todaro, G. J. & De Larco, J. E. (1980) in *Control Mechanisms in Animal Cells: Specific Growth Factors,* eds. Jimene de Asua, L., Levi-Montalcini, R., Shields, R. & Iacobelli, S. (Raven, New York), Vol. 1, pp. 223–243.
42. Balk, S. D. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 271–275.