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COMMENTARY

PLATELET-DERIVED GROWTH FACTOR AND MALIGNANT TRANSFORMATION

HARRY N. ANTONIADES

The Center for Blood Research and the Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, U.S.A.

Investigations of human platelet-derived growth factor (PDGF), a potent mitogen for mesenchymal derived cells in culture [1–3], have provided a rational basis for the understanding of at least one mechanism involved in malignant transformation. PDGF is a heat-stable (100°), cationic (isolectric point 9.8) polypeptide [4] that circulates in blood stored in the α-granules of platelets [5]. It is released from platelets into the serum during blood clotting, constituting the major polypeptide growth factor of serum. It is suggested that *in vivo* PDGF is delivered during platelet degranulation at the site of injury where it participates in the process of wound healing by stimulating the proliferation and migration of connective tissue cells.

We have shown recently that PDGF and the transforming protein of the simian sarcoma virus (SSV), an acute transforming retrovirus of primate origin, derive from the same or closely related cellular genes [6]. This conclusion is based on the demonstration that PDGF and the SSV transforming protein [7, 8] share extensive amino acid sequence homology [6, 9, 10], have common antigenic determinants and structural conformation [11], and exert identical biological functions [12, 13]. These findings suggest that the ability of the simian sarcoma virus to induce transformation derives from the incorporation of the PDGF gene within the retroviral genome. The resulting transforming *onc* gene (v-*sis*) region within the retrovirus genome codes for a PDGF-like mitogen and is capable of inducing neoplastic transformation by the continuous production of this potent mitogen causing sustained cell proliferation.

Consistent with the findings described above is the detection of v-*sis*-related messenger RNAs in human tumors of mesenchymal origin, such as glioblastomas, fibrosarcomas, and osteosarcomas [14]. Production of PDGF-like mitogen by these human malignant cells in culture has been reported [15–17]. More recent studies have demonstrated that these cells synthesize, process, and release PDGF-like polypeptides which are recognized by specific PDGF-antisera (Graves *et al.* and Pantazis *et al.*, unpublished data).

These findings demonstrate that activation of *sis* transcription can cause the sustained abnormal proliferation of human cells which are target cells of PDGF action. Thus, *sis* activation may be involved in the process leading normal cells of mesenchymal origin toward malignancy.

Following is a brief description of the events that led to the recognition of PDGF; the current understanding of PDGF structure and function; its role in the regulation of normal cell growth; and its link to malignant transformation.

Recognition of platelet-derived growth factor

The recognition of PDGF emerged from two independent projects. One resulted in the isolation and characterization of the major growth factor polypeptide from human serum [18]. The other produced the information that the serum growth factor activity resided in platelets and could be recovered in platelet extracts [19–21]. As summarized below, the combination of these two efforts led to the present status of PDGF.

The presence of a potent cell growth factor in serum was suspected from the finding that serum is indispensable for the growth of normal cells in culture [22, 23]. In collaboration with D. Scher, we reported, in 1974 and 1975 [18, 24], the isolation and characterization of the major human serum growth factor, which turned out to be a distinct and unusual polypeptide. It was shown to be heat-stable, even after it was boiled at 100° for 10–20 min [18, 24], and it was strongly cationic, with an isoelectric point of about 9.7 [18]. Reduction with 2-mercaptoethanol abolished its mitogenic activity. Under reducing conditions, its molecular weight was about 13,000 as judged by analytical sodium dodecyl sulfate (SDS)-electrophoresis [18]. This polypeptide was present in serum in trace amounts with an estimated concentration of about 50 ng/ml serum [25, 26]. At the time of its isolation and characterization from human serum, we were unaware that this growth factor derived in serum from platelets during blood clotting.

Balk, in 1971 [19], described that the serum growth factor activity resides in blood platelets and is released into serum during blood clotting. This conclusion derived from the observation that platelet-poor plasma, unlike clotted blood serum, could not support the growth of 3T3 fibroblasts in culture. These observations were confirmed and extended in 1974 by Ross *et al.* [20] and by Kohler and Lipton [21]. In addition, these investigators demonstrated the recovery of the growth factor activity from platelet extracts, establishing conclusively that platelets are the source of the serum growth factor activity. Similar data were reported in 1975 by Westermark and Wasteson [1]. Stimulated by these important reports, we investigated the possibility of whether or

not the growth factor polypeptide we had isolated from human serum derived from platelets. Using a specific radioimmunoassay for the serum growth factor and cell culture procedures, we were able to confirm that this factor derived from platelets [25, 26]. It was present in clotted blood serum and in platelet extracts but absent from platelet-poor serum.

In view of these findings, the serum growth factor activity was named platelet-derived growth factor (PDGF).

Properties of PDGF

The finding that the serum growth factor resides in platelets allowed its large scale isolation and purification from clinically outdated platelets [4, 27]. Purified PDGF obtained from platelets was shown to be a heat-stable, cationic (pI 9.8 to 10.2) polypeptide, sensitive to reducing agents, properties identical to those described previously for the polypeptide growth factor isolated from human serum. Subsequently, PDGF has been purified from platelet-rich plasma [28, 29], and the properties of these preparations were also similar to those obtained from human serum and platelets. The molecular weight of unreduced PDGF was estimated at 32,000–35,000. Upon reduction, the molecular weight appeared to be between 12,000 and 18,000, suggesting that biologically active, unreduced PDGF consists of two polypeptide chains [29–31]. The amino-terminal amino acid sequence of human PDGF provided additional evidence that it consists of two homologous polypeptide chains linked together by disulfide bonds [10].

Recent studies have demonstrated the presence of multiple molecular weight forms of biologically active, unreduced PDGF obtained from platelets [30] or from platelet-rich plasma [28, 29]. The two predominant forms, named PDGF-I and PDGF-II, have molecular weights of about 35,000 and 32,000 respectively. It appears that PDGF-II derives from PDGF-I by partial proteolysis which occurs during the outdating of platelets or during their handling and fractionation. Amino acid sequence data indicate that the amino-terminal regions of PDGF-I and II are similar [10].

Production of pure PDGF is limited by the small amounts present in platelets, and by heavy losses during the complicated process of fractionation. Initial yields of purified PDGF obtained from 500 units of platelets, representing 250 liters of human blood, amounted to between 20 and 100 μg [4, 27]. Improved procedures allowed better recoveries but, even so, in order to accumulate adequate amounts for physiological and structural studies it was necessary to fractionate tens of thousands of clinically outdated human platelet units.

Amino-terminal amino acid sequence of PDGF

The successful elucidation of the amino-terminal amino acid sequence of PDGF was accomplished in collaboration with M. W. Hunkapiller of Caltech [10]. The sequence data demonstrated that PDGF consists of two homologous polypeptide chains (PDGF-1 and PDGF-2) linked together by disulfide bonds. Additional sequence was obtained by cyanogen bromide fragmentation [6]. The accuracy of the sequence data was verified by the subsequent important discovery of the near identity of PDGF-2 sequence with that of the transforming protein of the simian sarcoma virus [6].

The sequence analysis of pure PDGF was limited by the low amounts available. Standard laboratory procedures for sequencing required amounts far in excess of supply. The development of a microsequenator at Caltech [32] made it possible to identify the amino-terminal amino acid sequence of PDGF using 100–300 pmoles of pure, biologically active, unreduced PDGF and of its biologically inactive reduced-alkylated products. Interpretation of the sequence data proved to be extremely complicated by overlapping sequences due to partial degradation at the amino-terminal regions of the two reduced PDGF chains. The expertise of M. Hunkapiller was a decisive factor in the successful and accurate elucidation of the amino-terminal amino acid sequence of PDGF. His early communication of our sequence data also aided the sequence work of Waterfield *et al.* [9].

Physiologic functions of PDGF

Target cells for PDGF include fibroblasts [2, 3], arterial smooth muscle cells [2, 20], and brain glial cells [1, 27]. The mitogenic effects of PDGF occur at low concentration (0.1 mM), similar to the concentrations required for the action of other hormonal polypeptides. In addition to its mitogenic activity, PDGF has been shown to be a potent chemoattractant for cultured fibroblasts [33], smooth muscle cells [34, 35], and for human neutrophils and monocytes [36]. The effects on fibroblasts and smooth muscle cells appear to be specific for PDGF since other growth factors did not stimulate their migration [33, 34].

Other functions of PDGF include its ability to stimulate synthesis of protein [37], phospholipid and cholesterol ester [38–41] and prostaglandin molecules [42–45]; stimulation of tyrosine kinase activity [46–49,*]; and stimulation of amino acid transport [37]. In addition, it modulates receptor binding of several biologically important components including low density lipoprotein [38–40] and serotonin [44]. Two functions of PDGF, one related to its possible role in atherosclerosis and the other to its role in the regulation of platelet aggregation, deserve special comment. The effects of PDGF on proliferation, migration and cholesterol ester synthesis in cultured arterial smooth muscle cells inspired a provocative hypothesis by R. Ross and associates [50, 51] for its role in atherogenesis. This hypothesis assumes that delivery of PDGF by platelets at the site of vascular injury initiates events involving vascular cell migration and proliferation leading to restoration of intact vascular morphology. Among those events are the migration and proliferation into the intima of arterial smooth muscle cells. It is suggested that subsequent formation of connective matrix by the proliferated smooth muscle and deposition of lipids both within the cells and in the connective tissue surrounding

* A. J. Owen, P. Pantazis and H. N. Antoniades, manuscript submitted for publication.

them may, under certain conditions, lead to the fibrous plaque which characterizes atherosclerosis.

The possible role of PDGF in the regulation of platelet aggregation derives from studies demonstrating that this growth factor is a potent stimulator of prostacyclin (PGI_2) synthesis by cultured arterial smooth muscle and endothelial cells [43]. Prostacyclin is the most potent naturally occurring inhibitor of platelet aggregation. Subsequent studies have shown that another platelet-derived factor, serotonin, is also a potent stimulant of PGI_2 production by arterial smooth muscle cells but not by endothelial cells [44]. Addition of PDGF to cultures of smooth muscle cells in the presence of serotonin produced a striking increase in PGI_2 production, far above the additive individual effects of serotonin or PDGF. This synergistic interaction was blocked by the addition of serotonin-receptor blocking agents suggesting that serotonin stimulates smooth muscle prostacyclin synthesis through a specific receptor-mediated mechanism modulated by PDGF [44]. The selective delivery of PDGF and serotonin by platelets at the site of vascular injury and the subsequent stimulation of PGI_2 release may provide a mechanism for protecting against excess accumulation of platelets and occlusive thrombosis in certain pathological conditions [44].

The studies described above demonstrated that PDGF stimulates many diverse functions in cultures of normal, untransformed cells. In contrast, the growth of viral-transformed cells was shown to be independent of PDGF. Extensive studies carried out by Scher *et al.* [52] demonstrated that PDGF did not affect the growth of these transformed cells and that these cells could grow equally well in serum or in platelet-poor plasma. Similar observations were made for the growth requirement of the human osteosarcoma cells (U-2 OS) in culture [15, 16]. It is now understood that these transformed cells release a variety of polypeptide growth factors in their culture media, including PDGF-like polypeptides, and apparently this autocrine secretion of cell-produced growth factors substitutes for PDGF.

Regulation of cell growth by PDGF

The major *in vivo* function of PDGF is to induce mitosis in quiescent cells such as diploid fibroblasts, arterial smooth muscle cells, and brain glial cells. This would corroborate the proposed *in vivo* function of PDGF as a wound healing factor. Studies on the action of PDGF in cultured mouse 3T3 cells demonstrated that both PDGF and platelet-poor plasma were required for DNA replication and cell division [2, 3, 53–55]. PDGF alone or platelet-poor plasma alone did not significantly stimulate the growth of the 3T3 cells in culture. The synergistic effects of PDGF and of other hormones present in plasma were required for optimal cell growth. Some of these hormones in plasma were shown to belong to the family of polypeptides with insulin-like activity [55]. These studies provided a new understanding of cell growth control. The significant finding was that the transition between the G_0/G_1 phase of the cell cycle and S phase could be subdivided into two stages. One, called competency, is controlled by PDGF and allows cells to enter the G_0/G_1 phase of the cell cycle [53–55]. The other, called progression, is controlled by factors in platelet-poor plasma which enable the progression of the PDGF-induced competent cells into the S phase [53–55].

PDGF-induced competency could be achieved by a brief exposure of the culture cells to PDGF. This was demonstrated by the fact that cells exposed to PDGF remained competent for up to 13 hr after PDGF was removed [53]. Addition of plasma to cell cultures pre-exposed to PDGF allowed the progression into S phase. Similarly, cells exposed to PDGF under conditions of amino acid restriction became competent and passed through progression into S phase when both plasma and amino acids were added to cell cultures [56]. This latter experiment also indicated that the classic "restriction" point caused by nutrient deprivation occurs during the progression phase of the G_0/G_1 to S transition [57].

These studies demonstrated that a transient exposure of the cultured cells to PDGF could render the cells competent to enter the cell cycle. This implies that the intracellular signals generated by the transient exposure to PDGF are relatively stable and persist after PDGF is removed. It appears that PDGF induces stable secondary "modulators" in its target cells which render them capable of responding to progression factors. Since RNA and protein syntheses inhibitors blocked the competency response, it is possible to speculate that such "modulators" could be specific, PDGF-induced RNAs and/or proteins.

The search for the identification of intracellular signals in response to PDGF-induced competency produced evidence for the presence of PDGF-modulated proteins and RNAs in cultured mouse 3T3 cells. Pledger *et al.* [58] described the appearance of intracellular proteins and their presence correlated with the dose–response relationship for PDGF-stimulated DNA synthesis in these cells. These PDGF-induced proteins may serve as secondary modulators of the competency response. However, their functional role during competency, if any, is unknown. Cochran *et al.* [59] suggested that PDGF-induced RNAs are responsible for mediating the competency response. These studies demonstrated that PDGF was capable of inducing a significant increase in mRNA complementary to clones hybridizing with cDNA from PDGF-treated cells, but not with cDNA from quiescent cells. The primary translation products of two hybrid-selected mRNAs were two polypeptides with molecular weights of 10,000 and 18,000. These molecular weights did not correspond to those of the PDGF-induced proteins described above [58].

Recently, Kelly *et al.* [60] reported some intriguing results indicating that several growth factors, including PDGF, can induce c-myc mRNA. The concentration of c-myc mRNA was induced about 40-fold by PDGF in cultures of 3T3 fibroblasts [60]. This effect was observed within 3 hr of the addition of PDGF to the quiescent 3T3 cultures. The possibility was suggested that c-myc is involved in the progression of cells through the cell cycle. The transmitter of the PDGF signal for the induction of c-myc mRNA was suggested to represent a labile protein. Identification of the signals preceding c-myc

mRNA induction by PDGF and of the post-induction events leading to DNA replication will greatly contribute to the understanding of the role of c-myc in the regulation of cellular growth.

Early events associated with PDGF action

The following two events are associated with the initial action of PDGF: (a) binding to specific cell membrane receptors in target cells in culture, and (b) ability to stimulate tyrosine-specific kinases capable of phosphorylating cell membrane and cellular proteins at tyrosine residues.

Receptor binding has been recognized as the earliest event in the action of biologically active ligands. The presence of PDGF receptors has been demonstrated in target cells, such as cultured fibroblasts, glial cells, and arterial smooth muscle cells [61–65]. The binding of PDGF was shown to be temperature dependent. At 37° the bound PDGF is internalized and degraded with a half-life of 1–3 hr. The PDGF–receptor complexes appear to cluster at coated pits on the cell surface [66] and are internalized via the endosome pathway.

Unlike normal cells, transformed cells, such as human osteosarcoma cells, producing PDGF-like polypeptides did not exhibit a significant number of PDGF receptors [15, 16]. SSV-transformed NRK fibroblast contained a significantly lower number of PDGF receptors compared to normal untransformed NRK cells [13]. A possible explanation for the significantly lower number of PDGF receptors in these transformed cells is based on the assumption of saturation binding and/or of a state of "down regulation" of the receptor caused from the sustained secretion of PDGF-like polypeptides by the transformed cells. On the other hand, it is possible that the low number of receptors in the transformed cells indicates an "atrophy" in receptor production, if the PDGF-like polypeptides elaborated by the transformed cells express their function intracellularly, by-passing the need for extracellular membrane receptor binding. However, evidence for intracellular action of PDGF is missing.

Phosphorylation of cell membrane proteins involving tyrosine specific kinases has been implicated in the control of cell proliferation by both polypeptide growth factors and the transformation-specific proteins which are the products of several retrovirus oncogenes [67]. PDGF has been shown to stimulate kinases capable of phosphorylating cell membrane and cellular proteins at tyrosine residues [49–51,*]. The target protein in membranes of human fibroblasts appears to have a molecular weight of about 185,000. There is only indirect evidence that the PDGF-dependent kinase activity resides in the PDGF-receptor molecule. This has been demonstrated previously for the EGF [68] and insulin receptors [69] which were shown to be autophosphorylated.

In considering the early events of PDGF action in normal cells, one must keep in mind that both PDGF-receptor binding and PDGF-induced phosphorylation occur within a few minutes of exposure of PDGF to cell cultures. However, DNA synthesis, stimulated by the action of PDGF, commences 12–15 hr later. Thus, there is a significant time interval between the initial events and the expression of PDGF action. Elucidation of the intracellular signals induced by PDGF and the sequence of events leading to DNA replication will constitute a major contribution in our understanding of the mechanisms involved in PDGF-induced mitogenesis.

PDGF and the transforming protein of the simian sarcoma virus derive from the same gene(s)

Elucidation of the amino-terminal amino acid sequence of human PDGF [10] precipitated the discovery of its identity with the transforming gene product of the simian sarcoma virus [6, 9]. This retrovirus was initially isolated from a fibrosarcoma of a woolly monkey, and it is the only sarcoma virus of primate origin [70]. Characterization of its genome has localized its transforming gene to its cell-derived *onc* sequence, v-*sis* [71, 72]. The v-*sis* has been sequenced by Devare *et al.* [7], and its 28,000 molecular weight transforming protein product ($p28^{sis}$) has been identified by immunoprecipitation with antisera prepared against synthetic polypeptides corresponding to its amino terminal and carboxyl terminal regions [8]. Computer analysis revealed a near identity between the sequence of the PDGF-2 chain and the SSV *onc* gene product, $p28^{sis}$ [6].

The SSV *onc* gene codes for a protein consisting of 226 amino acid residues. The region corresponding to PDGF starts at the serine residue in position 67, which follows a double basic (Lys-Arg) sequence at positions 65–66 [6]. This appears to be the processing point yielding a polypeptide of 160 residues with a molecular size of 18,056 daltons, essentially the same size estimated for the PDGF-2 chain on the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis [10]. However, the SSV *onc* gene codes only for one chain (PDGF-2) of the PDGF polypeptide. It was important, therefore, to establish whether the *onc* gene product was functioning as a single polypeptide chain or in a conformational manner similar to the dimeric formation of biologically active PDGF. This important question has been resolved recently by the demonstration that, in SSV-transformed cells, $p28^{sis}$ undergoes a series of discrete processing steps including dimer formation and proteolytic cleavage to yield molecules structurally and immunologically resembling the disulfide linked dimeric forms of PDGF [11]. More recent studies have shown that this processed product is secreted by SSV-transformed fibroblasts into their culture media in a biologically active form which is recognized by PDGF antisera [13]. Its properties and biologic activities are identical to those of PDGF. It is heat-stable (100° for 10 min), and it is inactivated by reducing agents. Under nonreducing conditions its molecular weight was estimated at about 34,000 and after reduction at about 17,000. These properties are similar to those of biologically active PDGF and consistent with a disulfide linked dimeric form.

These studies established conclusively that the biologically active SSV *onc* gene product is a homodimer, consisting of two PDGF-2 chains linked together by disulfide bonds. Its immunologic reac-

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tivity and biologic properties are identical to those of PDGF.

PDGF and malignant transformation

The findings described above provided the missing link for the understanding of the mechanism of cell transformation induced by the *onc* gene of the simian sarcoma virus. This *onc* gene, v-*sis*, was shown to encode a PDGF-like polypeptide which is a potent mitogen for fibroblasts, arterial smooth muscle cells, and glial cells. Activation of *sis* transcription may cause the sustained abnormal proliferation of cells responsive to the mitogenic effects of the PDGF-like molecule.

Sis-related messenger RNAs have been demonstrated in human tumors of mesenhymal origin, such as glioblastoma, osteosarcoma and fibrosarcoma [14]. In some human T-cell leukemia virus (HTLV)-infected cell lines, *sis*-related mRNAs were also detected [14]. The presence of mitogenic PDGF-like polypeptides has been demonstrated in the conditioned media and in the cell lysates of cultured human malignant cells of mesenhymal origin. Heldin *et al.* [15] initially described the presence of PDGF-like polypeptides in cultures of human osteosarcoma cells. These observations were confirmed and extended by Graves *et al.* [16]. More recently, PDGF-like mitogen have been identified in the media of cultured human glioblastoma cells [17] and in the lysates and in cultured media of human glioblastoma and fibrosarcoma cells (Pantazis *et al.*, unpublished data). Immunoprecipitation with PDGF antisera of metabolically labeled lysates and cultured media derived from human osteosarcoma, glioblastoma and fibrosarcoma cell lines have demonstrated the biosynthesis, processing and release of PDGF-like polypeptides by the malignant cells (Owen *et al.* and Pantazis *et al.*, unpublished data). Similar observations were made in certain human HTLV-infected cells (Salahuddin *et al.* and Pantazis *et al.*, unpublished data). Recently, a cDNA library has been prepared from mRNA isolated from human osteosarcoma cells (U-205). One of the members of the cosmid library which hybridizes with a v-*sis* probe has been sequenced and found to code for a peptide which is over 90% homologous with the predicted carboxyl-terminal region of the v-*sis* product (Tempst *et al.*, unpublished data).

The findings described above are consistent with the suggestion that *sis*-activation might be involved in the process leading normal cells of certain types towards malignancy.

General comments

Studies on PDGF described here represent the collective effort of several teams of investigators over the past 10 years. These studies produced an abundance of information concerning the nature and structure of PDGF; its role in cell growth; and its diverse functions affecting cell migration, metabolic processes and receptor modulation. This work also led to an important discovery linking this potent mitogen to the transforming protein of the simian sarcoma virus, providing a basis for the understanding of the processes involved in transformation induced by the SSV *onc* gene. There are many questions yet to be answered. For example, PDGF has been localized in the α-granules of platelets. It is hypothesized that it is synthesized in megakaryocytes, but evidence for this is lacking. The action of PDGF is associated with its transient exposure to cultured fibroblast making these cells competent to enter the cell cycle. The intracellular events between PDGF-induced competency and DNA replication are still unknown. Progress is being made in relating selective intracellular protein synthesis and RNA to this process. PDGF-induced c-myc mRNAs in cells made competent by PDGF has attracted attention because for the first time there is a connection between PDGF and oncogene activation. PDGF appears to initiate its action through binding to its specific membrane receptor. This mechanism is valid in normal, untransformed cells. In transformed cells that produce and process PDGF-like mitogen, the action of PDGF may involve a direct intracellular signal without a need for prior secretion and membrane receptor binding. This information is important in developing a strategy for the control of the transforming *onc* gene. The biologically active processed product of the SSV transforming gene has been identified as the disulfide linked homodimer of the PDGF-2 chain. It is possible that other *onc*-gene(s) code for the homodimer of the PDGF-1 chain which has been shown to share about 50% homology with the PDGF-2 chain. The mechanisms involved in *sis*-activation and the role of the other oncogenes and/or "carcinogens" in these processes remain to be explored.

These are examples of some of the basic issues that require further investigation. The first 10 years of PDGF research were productive. In the next 10 years, with young investigators joining the old teams, progress on PDGF will be spectacular.

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REFERENCES

1. B. Westermark and A. Wasteson, *Adv. Metab. Disord.* **8**, 85 (1975).
2. R. Ross and A. Vogel, *Cell* **14**, 203 (1978).
3. C. D. Scher, R. C. Shepard, H. N. Antoniades and C. D. Stiles, *Biochim. biophys. Acta* **560**, 217 (1979).
4. H. N. Antoniades, C. D. Scher and C. D. Stiles, *Proc. natn. Acad. Sci. U.S.A.* **76**, 1809 (1979).
5. D. R. Kaplan, F. C. Chao, C. D. Stiles, H. N. Antoniades and C. D. Scher, *Blood* **53**, 1043 (1979).
6. R. F. Doolittle, M. W. Hunkapiller, L. E. Hood, S. G. Devare, K. C. Robbins, S. A. Aaronson and H. N. Antoniades, *Science* **221**, 275 (1983).
7. S. G. Devare, E. P. Reddy, J. D. Law, K. C. Robbins and S. A. Aaronson, *Proc. natn. Acad. Sci. U.S.A.* **80**, 731 (1983).
8. K. C. Robbins, S. G. Devare, E. P. Reddy and S. A. Aaronson, *Science* **218**, 1131 (1982).
9. M. D. Waterfield, G. T. Scrace, N. Whittle, P. Stroobant, A. Johnson, A. Wasteson, B. Westermark, C. H. Heldin, J. S. Huang and T. F. Deuel, *Nature, Lond.* **304**, 35 (1983).

10. H. N. Antoniades and M. W. Hunkapiller, *Science* **220**, 963 (1983).
11. K. C. Robbins, H. N. Antoniades, S. G. Devare, M. W. Hunkapiller and S. A. Aaronson, *Nature, Lond.* **305**, 605 (1983).
12. T. F. Deuel, J. S. Huang, S. S. Huang, P. Stroobant and M. D. Waterfield, *Science* **221**, 1348 (1983).
13. A. J. Owen, P. Pantazis and H. N. Antoniades, *Science*, in press.
14. A. Eva, K. Robbins, P. R. Anderson, A. Srinivasan, S. R. Tronick, E. P. Reddy, N. W. Ellmore, A. J. Gallen, J. A. Lautenberger, T. S. Papas, E. H. Westin, F. Wong-Staal, R. Gallo and S. A. Aaronson, *Nature, Lond.* **295**, 116 (1982).
15. C. H. Heldin, B. Westermark and A. Wasteson, *J. cell. Physiol.* **105**, 235 (1980).
16. D. T. Graves, A. J. Owen and H. N. Antoniades, *Cancer Res.* **43**, 83 (1983).
17. M. Nister, C. H. Heldin, A. Wasteson and B. Westermark, *Proc. natn. Acad. Sci. U.S.A.* **81**, 926 (1984).
18. H. N. Antoniades, D. Stathakos and C. D. Scher, *Proc natn. Acad. Sci. U.S.A.* **72**, 2535 (1975).
19. S. Balk, *Proc. natn. Acad. Sci. U.S.A.* **68**, 271 (1971).
20. R. Ross, J. Glomset, B. Kariya and L. Harker, *Proc. natn. Acad. Sci. U.S.A.* **71**, 1207 (1974).
21. N. Kohler and A. Lipton, *Expl. Cell Res.* **87**, 297 (1984).
22. G. J. Todaro, G. K. Lazar and H. Green, *J. cell. comp. Physiol.* **66**, 365 (1965).
23. H. W. Temin, *J. natn. Cancer Inst.* **37**, 167 (1966).
24. C. D. Scher, D. Stathakos and H. N. Antoniades, *Nature, Lond.* **247**, 279 (1974).
25. H. N. Antoniades and C. D. Scher, *Proc. natn. Acad. Sci. U.S.A.* **74**, 1973 (1977).
26. H. N. Antoniades and C. D. Scher, *Natn. Cancer Inst. Monog.* **48**, 137 (1977).
27. C. H. Heldin, B. Westermark and A. Wasteson, *Proc. natn. Acad. Sci. U.S.A.* **76**, 3722 (1979).
28. T. F. Deuel, J. S. Huang, R. T. Proffit, J. V. Baenziger, D. Chang and B. B. Kennedy, *J. biol. Chem.* **256**, 8896 (1981).
29. E. W. Raines and R. Ross, *J. biol. Chem.* **257**, 5154 (1982).
30. H. N. Antoniades, *Proc. natn. Acad. Sci. U.S.A.* **78**, 7314 (1981).
31. C. H. Heldin, B. Westermark and A. Wasteson, *Biochem. J.* **193**, 907 (1981).
32. R. M. Hewick, M. W. Hunkapiller, L. E. Hood and W. J. Dreyer, *J. biol. Chem.* **256**, 7990 (1981).
33. G. R. Grotendorst, H. E. J. Seppa, H. K. Kleinman and G. R. Martin, *Proc. natn. Acad. Sci. U.S.A.* **78**, 3669 (1981).
34. H. Seppa, G. Grotendorst, S. Seppa, E. Schiffmann and G. Martin, *J. Cell. Biol.* **92**, 584 (1982).
35. L. R. Bernstein, H. N. Antoniades and B. R. Zetter, *J. Cell. Sci.* **56**, 71 (1982).
36. T. F. Deuel, R. M. Senior, J. S. Huang and G. L. Griffin, *J. clin. Invest.* **69**, 1046 (1982).
37. A. J. Owen, R. P. Geyer and H. N. Antoniades, *Proc. natn. Acad. Sci. U.S.A.* **79**, 3203 (1982).
38. A. Chait, R. Ross, J. J. Albers and E. L. Bierman, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4804 (1980).
39. L. D. Witte and J. A. Cornicelli, *Proc. natn. Acad. Sci. U.S.A.* **77**, 5962 (1980).
40. L. D. Witte, J. A. Cornicelli, R. W. Miller and D. S. Goodman, *J. biol. Chem.* **257**, 5392 (1982).
41. C. C. Leslie, H. N. Antoniades and R. P. Geyer, *Biochim. biophys. Acta* **711**, 290 (1982).
42. W. T. Shier, *Proc. natn. Acad. Sci. U.S.A.* **77**, 137 (1980).
43. S. R. Coughlin, M. A. Moskowitz, B. R. Zetter, H. N. Antoniades and L. Levine, *Nature, Lond.* **288**, 600 (1980).
44. S. R. Coughlin, M. A. Moskowitz, H. N. Antoniades and L. Levine, *Proc. natn. Acad. Sci. U.S.A.* **78**, 7134 (1981).
45. A. H. Tashjian, E. L. Hohmann, H. N. Antoniades and L. Levine, *Endocrinology* **111**, 118 (1982).
46. B. Ek, B. Westermark, A. Wasteson and C. H. Heldin, *Nature, Lond.* **295**, 419 (1982).
47. B. Ek and C. H. Heldin, *J. biol. Chem.* **257**, 10486 (1982).
48. J. Nishimura, J. S. Huang and T. F. Deuel, *Proc. natn. Acad. Sci. U.S.A.* **79**, 4303 (1982).
49. J. A. Cooper, D. F. Bowen-Pope, E. Raines, R. Ross and T. Hunter, *Cell* **31**, 263 (1982).
50. R. Ross and J. Glomset, *New Engl. J. Med.* **295**, 369 (1976).
51. R. Ross, *Atherosclerosis* **1**, 293 (1981).
52. C. D. Scher, W. J. Pledger, P. Martin, H. N. Antoniades and C. D. Stiles, *J. Cell Physiol.* **97**, 371 (1978).
53. W. J. Pledger, C. D. Stiles, H. N. Antoniades and C. D. Scher, *Proc. natn. Acad. Sci. U.S.A.* **74**, 4481 (1977).
54. W. J. Pledger, C. D. Stiles, H. N. Antoniades and C. D. Scher, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2839 (1978).
55. C. D. Stiles, G. T. Capone, C. D. Scher, H. N. Antoniades, J. J. Van Wyk and W. J. Pledger, *Proc. natn. Acad. Sci. U.S.A.* **76**, 1279 (1979).
56. C. D. Stiles, R. R. Isberg, W. J. Pledger, H. N. Antoniades and C. D. Stiles, *J. cell. Physiol.* **99**, 395 (1979).
57. A. Pardee, *Proc. natn. Acad. Sci. U.S.A.* **71**, 1286 (1974).
58. W. J. Pledger, C. A. Hart, K. L. Locatell and C. D. Scher, *Proc. natn. Acad. Sci. U.S.A.* **78**, 4358 (1981).
59. B. H. Cochran, A. C. Reffel and C. D. Stiles, *Cell* **33**, 939 (1983).
60. K. Kelly, B. H. Cochran, C. D. Stiles and P. Leder, *Cell* **35**, 603 (1983).
61. C. H. Heldin, B. Westermark and A. Wasteson, *Proc. natn. Acad. Sci. U.S.A.* **78**, 3664 (1981).
62. C. H. Heldin, B. Ek and L. Ronnstrand, *J. biol. Chem.* **258**, 10054 (1983).
63. D. F. Bowen-Pope and R. Ross, *J. biol. Chem.* **257**, 5161 (1982).
64. J. S. Huang, S. S. Huang, B. Kennedy and T. F. Deuel, *J. biol. Chem.* **257**, 8130 (1982).
65. L. T. Williams, P. Tremble and H. N. Antoniades, *Proc. natn. Acad. Sci. U.S.A.* **79**, 5887 (1982).
66. J. Nilsson, J. Thyberg, C. H. Heldin, B. Westermark and B. Wasteson, *Proc. natn. Acad. Sci. U.S.A.* **80**, 5592 (1983).
67. T. Hunter and B. Sefton, *Proc. natn. Acad. Sci. U.S.A.* **77**, 1311 (1980).
68. S. Cohen, G. Carpenter and L. King, *J. biol. Chem.* **255**, 4834 (1980).
69. M. Kasuga, F. A. Karlsson and C. R. Kahn, *Science* **215**, 185 (1982).
70. G. J. Thielen, D. Gould, M. Fowler and D. L. Dungworth, *J. natn. Cancer Inst.* **47**, 881 (1971).
71. K. C. Robbins, S. G. Devare and S. A. Aaronson, *Proc. natn. Acad. Sci. U.S.A.* **78**, 2918 (1981).
72. E. P. Gelmann, F. Wong-Staal, R. A. Krammer and R. C. Gallo, *Proc. natn. Acad. Sci. U.S.A.* **78**, 3373 (1981).