bivores rather than artificially induced damage is also preferable. The induction that we describe produced large reductions in the populations of herbivorous mites. For the most part, other investigators considered only plant changes or, at most, the impact of induced changes on bioassays measuring insect performance, rather than the impact on herbivore populations. The induced changes that we describe did not dissipate for at least 12 days.

The finding that mites can induce resistance to subsequent herbivory is potentially of great importance in pest and disease control. It may be possible to inoculate a plant against various herbivores and pathogens. A more thorough understanding of the mechanism and specificity of the induced resistance should allow facilitation of a practical inoculation.

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21. No statistically significant differences were found between plants of the two treatments for six plant characteristics (R. Karban, in preparation): total stem length, stem growth during the experimental period, mean internode distance, total leaf area (including cotyledons), mean area of true leaves, and number of leaves.
22. For *T. urticae* as the first species: $F(1, 22) = 9.80$, $P < 0.01$. For *T. turkestani* as the first species: $F(1, 22) = 9.32$, $P < 0.01$.
23. For *T. urticae* as the first species: $F(1, 22) = 11.32$, $P < 0.01$. For *T. turkestani* as the first species: $F(1, 22) = 9.01$, $P < 0.01$.
24. For *T. urticae* as the first species: immatures, $F(1, 22) = 4.32$, $P < 0.05$; males, $F(1, 22) = 0.50$, not significant; and females, $F(1, 22) = 2.45$, not significant. For *T. turkestani* as the first species: immatures, $F(1, 22) = 8.40$, $P < 0.01$; males, $F(1, 22) = 1.58$, not significant; and females, $F(1, 22) = 5.17$, $P < 0.05$.
25. At the mite densities reported for the initial inoculation, control plants built up populations of mites that were approximately twice the size of mite populations on unexposed controls. The size of the final mite population buildup is inversely correlated with the size of the initial mite exposure (R. Karban, in preparation).
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Simian Sarcoma Virus–Transformed Cells Secrete a Mitogen Identical to Platelet-Derived Growth Factor

Abstract. *Normal rat kidney (NRK) cells transformed by simian sarcoma virus (SSV) release into the culture medium a biologically active mitogen with properties identical to those of human platelet–derived growth factor (PDGF). Like PDGF, the growth factor derived from SSV-NRK cells was shown to be stable to heat and sensitive to reducing agents. It was capable of inhibiting binding of labeled PDGF to the receptor on human fibroblasts. It also stimulated the phosphorylation of the same membrane protein (185 kilodaltons) in isolated plasma membranes from human fibroblasts. Immunoprecipitation of metabolically labeled proteins released by SSV-NRK cells showed that a 34-kilodalton protein was specifically precipitated by antiserum to PDGF. Upon reduction, this protein had a molecular size of 17 kilodaltons. PDGF has been shown to consist of two 14- to 18-kilodalton proteins linked by disulfide bonds.*

Investigations of human platelet–derived growth factor (PDGF), a potent mitogen for mesenchymal-derived cells (*1*), have provided insight into the mechanism by which the v-*sis* oncogene of the simian sarcoma virus brings about cell transformation. PDGF represents the major growth factor activity in human serum and normally circulates stored in the α-granules of platelets (*2*). PDGF has been isolated and characterized from serum (*3*), platelets (*4, 5*), and platelet-rich plasma (*6*). Elucidation of the amino-terminal amino acid sequence of PDGF has shown that it consists of two homologous polypeptide chains (PDGF-1 and PDGF-2) linked by disulfide bonds (*7*). Computer analysis of the partial sequence of PDGF revealed a homology with the transforming gene product $p28^{sis}$ of the simian sarcoma virus (SSV) (*8*), an acute transforming retrovirus of primate origin (*9, 10*), implying that the two proteins arose from the same or closely related genes. This relation has been confirmed by others (*11*). More recent studies (*12*) have shown that $p28^{sis}$ and PDGF show antigenic similarities and a common structural configuration.

These studies provided a basis for understanding the process by which the v-*sis* gene induces cell transformation, apparently involving the constitutive expression of a PDGF-like protein. However, it is not yet known whether the action of the v-*sis* product is mediated through an extra- or intracellular signal. The extracellular signal would require the release of the gene product into the extracellular space with subsequent binding to specific cell membrane receptors in a manner similar to that established for PDGF. We now report that SSV-transformed cells release a biologically active mitogen that has properties similar to those of human PDGF.

The conditioned medium obtained from SSV-NRK (normal rat kidney) cells stimulates the incorporation of [^{3}H]thymidine into quiescent BALB/3T3 cells (clone A31) (*4*) (Table 1). When approximately 50 μl of conditioned medium was concentrated to one-fifth of its original volume, the activity of the concentrate was similar to that of 5 ng of pure PDGF. Conditioned medium obtained from untransformed NRK cells did not exhibit significant activity. The heat stability and sensitivity to reducing agents of the SSV-NRK mitogen and PDGF were compared (Table 1). Both the SSV-NRK mitogen and PDGF retained 90 percent of their activity after being boiled for 10

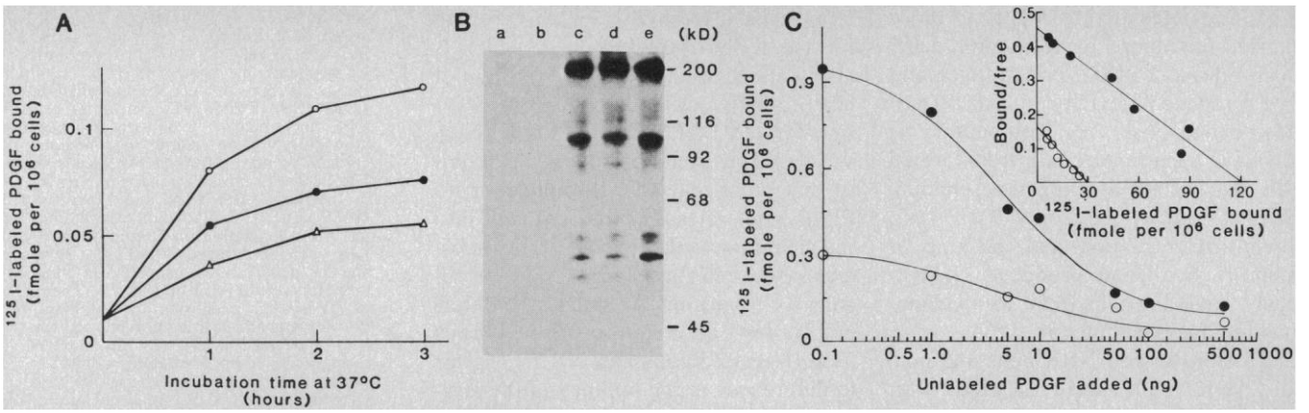

Fig. 1. Interaction of the SSV-NRK mitogen with the cellular PDGF receptor. (A) Inhibition of the binding of ^{125}I-labeled PDGF to GM-10 human diploid fibroblasts by medium conditioned with SSV and by unlabeled PDGF. (○) Iodine-125–labeled PDGF alone; (●) binding in the presence of 50 μl of medium conditioned with concentrated (one-fifth volume) SSV-NRK per well; (△) binding in the presence of 5 ng of unlabeled PDGF per well. (B) Stimulation of PDGF-dependent membrane protein kinase activity by medium conditioned with SSV-NRK. Phosphorylation reactions were carried out in the presence of 20 or 60 μl of medium conditioned with concentrated (one-tenth volume) NRK (lanes a and b), 20 or 60 μl of medium conditioned with concentrated SSV-NRK (lanes c and d), or 5 ng of purified human PDGF (lane e). (C) Analysis of binding of ^{125}I-labeled PDGF to NRK cells (●) and SSV-transformed NRK cells (○) (*18*).

minutes but were completely inactivated by reduction with 2-mercaptoethanol. Both results are identical to those reported for PDGF (*3–5*).

A series of experiments was then performed to determine whether the SSV-NRK mitogen could interact with the cellular PDGF receptor. The results indicated that 50 μl of the concentrated SSV-NRK medium were slightly less effective than 5 ng of PDGF in blocking the binding of ^{125}I-labeled PDGF to human fibroblasts (Fig. 1A) (*13*). Concentrated SSV-NRK medium was tested for its ability to stimulate the PDGF receptor-associated kinase activity of human fibroblast cell membranes (Fig. 1B). Medium from transformed cells (Fig. 1B, lanes c and d) but not from normal cells (Fig. 1B, lanes a and b) stimulated the phosphorylation of a membrane protein of 185 kilodaltons (kD) (*14*). Purified PDGF stimulated phosphorylation of the same protein (Fig. 1B, lane e). The molecular size of the receptor associated with kinase activity of human fibroblast membranes has been determined as 185 kD (*15*). The first step in the action of PDGF on normal cells is the interaction of the mitogen with specific cell surface receptors (*16, 17*). Thus, the release of PDGF-like mitogens by SSV-transformed cells would have little functional significance unless those cells also had receptors. NRK cells and SSV-transformed NRK cells were tested for their ability to bind labeled PDGF (Fig. 1C). Both the normal and transformed NRK cells showed a component of ^{125}I-labeled PDGF binding that could be specifically displaced by the addition of increasing amounts of unlabeled PDGF (*18*). These results indicated that, when cultured under the same conditions, SSV-transformed NRK cells bound specifically about one-fourth as much PDGF as untransformed cells. In order to determine more quantitatively the changes in PDGF binding, the binding experiments were repeated over a wider range of concentrations of ^{125}I-labeled PDGF (*18*). A Scatchard analysis of these data (Fig. 1C, inset) indicated that SSV-transformed NRK cells had about 18,000 receptors per cell with a dissociation constant (K_d) of 1.7×10^{-10}, while NRK cells had about 72,000 receptors per cell with a K_d of 2.2 ×

Table 1. Properties of the mitogenic activity released by SSV-transformed and normal NRK cells. In experiment 1, serum-free medium conditioned by SSV-transformed NRK cells (SSV-NRK) or normal NRK cells was collected, concentrated to one-fifth of its original volume, and then tested for its ability to stimulate acid-insoluble [^{3}H]thymidine incorporation into quiescent BALB/3T3 cells (*25*). In experiment 2, both concentrated SSV-NRK and purified human PDGF were heated at 100°C for 10 minutes or reduced with 10 percent 2-mercaptoethanol by volume and assayed for mitogenic activity (*4*).

Growth stimulant	[^{3}H]thymidine incorporation (10^3 count/min)
Experiment 1	
SSV-NRK (μl)	
0	21
10	64
25	83
50	112
NRK (μl)	
10	19
25	22
50	27
PDGF (ng/ml)	
1.0	80
5.0	140
Experiment 2	
SSV-NRK (μl)	
0	18
50	103
50*	96
50†	18
PDGF (ng/ml)	
5	128
5*	123
5†	20

*Heat-treated. †Reduced with 10 percent 2-mercaptoethanol.

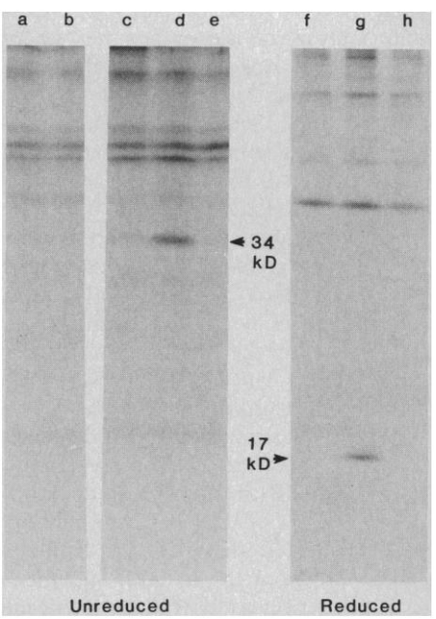

Fig. 2. Antiserum to PDGF recognizes the SSV-oncogene product released into the medium by SSV-transformed NRK cells. (Lanes a to e) SDS-PAGE performed under nonreducing conditions (*20*). Immunoprecipitation of proteins from medium conditioned by untransformed NRK cells with control rabbit serum (lane a) and antiserum to PDGF (lane b). Immunoprecipitation of proteins from medium conditioned by SSV-transformed NRK cells with control serum (lane c), antiserum to PDGF (lane d), and antiserum to PDGF in the presence of 500 ng of unlabeled PDGF (lane e). (Lanes f, g, and h) SDS-PAGE performed under reducing conditions. Proteins in medium conditioned by SSV-transformed NRK cells were immunoprecipitated with control serum (lane f), antiserum to PDGF (lane g), or antiserum to PDGF in the presence of 500 ng of excess unlabeled PDGF (lane h).

10^{-10}. These results are similar to those reported for human osteosarcoma cells, which release a PDGF-like mitogen and have a reduced capacity to bind PDGF added exogenously (*16*). Comparable results have been reported for transformed cells that secrete a mitogen resembling epidermal growth factor (EGF) (*19*). Downward regulation and masking of receptors by ligand produced endogenously have been invoked as explanations for this phenomenon.

SSV-transformed NRK cells and normal NRK cells were labeled with [^{35}S]cysteine that was added to the medium (*20*), and the proteins released into the medium were immunoprecipitated with antiserum to PDGF to determine which of the intracellular forms of the v-*sis* product is released from the cells. The immunoprecipitates were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography under both nonreducing and reducing conditions (*20*) (Fig. 2). No proteins were specifically precipitated from medium conditioned by NRK cells (Fig. 2, lanes a and b). In contrast, immunoprecipitation of medium conditioned by SSV-transformed NRK cells and analysis of the precipitates under nonreducing conditions revealed that antiserum (Fig. 2, lane d) but not control serum (Fig. 2, lane c) precipitated a 34-kD protein. The immunoprecipitation of this protein was blocked by excess unlabeled PDGF (Fig. 2, lane e). When the immunoprecipitates were analyzed under reducing conditions, a 17-kD protein was precipitated by antiserum (Fig. 2, lane g) but not by control serum (Fig. 2, lane f) or in the presence of excess PDGF (Fig. 2, lane h). The 34-kD species was not observed under reducing conditions, an indication that it is a disulfide-linked dimer of the 17-kD protein. Both purified PDGF (*7*) and some of the intracellular forms of the v-*sis* gene product (*12*) have been shown to be disulfide-linked dimers of 15- to 20-kD subunits.

The intracellular form of the v-*sis* gene product has been established as a 28-kD protein that dimerizes to a 56-kD species. The 56-kD protein is then processed by proteolysis at the amino- and carboxyl-terminals to yield the 46-, 34-, 30-, and 24-kD dimeric proteins. Monomers with molecular sizes of 28 and 20 kD were found under reducing conditions (*12*). Since only the 34-kD dimeric species could be detected in the medium, its release may be the result of a specific process rather than a nonspecific release of cytoplasmic contents caused by cell death and lysis. Further, human osteosarcoma cells (*21*) and human glioblastoma cells (*22*) have been observed to release a PDGF-like mitogen, which is also a disulfide-linked dimeric protein of 30 to 35 kD. Cell extracts from SSV-transformed BALB/3T3 cells have been shown (*23*) to contain a mitogenic activity that cross-reacted with antiserum to PDGF, but release of the mitogen into the medium was not detected. The differences between those results and the results we report may be due to the different strains of SSV-transformed cells used (NIH/3T3 cells and NRK cells) or to differences in the potency of the antiserum to PDGF.

Whether the v-*sis* gene product operates by an intracellular mechanism, or whether release into the medium followed by uptake into the cell via the PDGF receptor is an obligatory step, cannot be established directly from our results. Since a PDGF-like mitogen is released by the transformed cells, and since the cells retain PDGF receptors, self-stimulation of SSV-transformed NRK cells by an autocrine mechanism (*24*) may be a means by which the cells could sustain a high growth rate.

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13. Human fetal fibroblasts (GM-10) were plated in tissue culture dishes (24 wells) and grown to confluence. Binding of ^{125}I-labeled PDGF (20,000 dis/min per nanogram) was performed under the conditions described (*18*). Incubation and competitive inhibition were performed at 37°C.
14. For phosphorylation in vitro [B. Ek, B. Westermark, A. Wasteson, C. H. Heldin, *Nature (London)* **294**, 419 (1982); J. Nishimura, J. S. Huang, T. F. Deuel, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4303 (1982); L. J. Pike, D. F. Bowen-Pope, R. Ross, E. G. Krebs, *J. Biol. Chem.* **258**, 9383 (1983)], membranes were prepared from confluent, quiescent GM-10 human fibroblasts [D. Thom, A. J. Powell, C. W. Lloyd, D. A. Rees, *Biochem. J.* **168**, 187 (1977)]. Approximately 30 μg of membrane protein in 1 percent human serum albumin–Dulbecco's phosphate-buffered saline (HSA-DPBS) was placed in centrifuge tubes containing 20 or 60 μl of a concentrated (one-tenth volume) conditioned medium from cultures of untransformed NRK cells or SSV-NRK cells. Controls consisted of 5 ng of PDGF in 1 percent HSA-DPBS and 1 percent HSA-DPBS alone. The total volume in all tubes was 1 ml. After incubation for 20 minutes at 4°C, the tubes were centrifuged (30,000*g*) for 30 minutes, and the pellet was resuspended in 30 μl of 20 m*M* Hepes buffer–0.1 m*M* $MnCl_2$. Then 20 μCi of ^{32}P-labeled adenosine triphosphate (6 Ci/mmole) was added to each tube, and the tubes were incubated at 4°C for 20 minutes. The reaction was stopped by addition of 25 μl of SDS sample buffer and boiling for 3 minutes. Samples were analyzed by SDS-PAGE and autoradiography [P. Pantazis and W. M. Bonner, *J. Biol. Chem.* **256**, 4669 (1981)].
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18. NRK cells and SSV-NRK cells were plated in tissue culture dishes (24 wells) and grown to confluence. The cells were depleted by changing the serum content of the medium to 0.5 percent fetal bovine for 48 hours before assay. The binding buffer consisted of 1 percent HSA and 0.1 ng of ^{125}I-labeled PDGF (20,000 dis/min per nanogram) dissolved in DPBS. For Scatchard analysis of PDGF-binding to NRK and SSV-NRK cells, ^{125}I-labeled PDGF was adjusted to a specific activity of 2000 count/min per nanogram, and the binding to each cell type was determined over the range of 0.5 to 50 ng/ml. Nonspecific uptake of label was determined by measuring the binding of labeled PDGF in the presence of 500 ng/ml of unlabeled PDGF.
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20. NRK and SSV-NRK cells were metabolically labeled in serum-free, cysteine-free MEM (minimum essential medium) cultures containing 200 μCi of [^{35}S]cysteine per milliliter. The cultures were incubated for 4 hours at 37°C. The medium was collected, clarified by centrifugation, and freeze-dried. The dried material was dissolved in a minimum volume of radioimmunoprecipitation buffer and precipitated [M. Essex *et al.*, *Science* **221**, 1061 (1983)]. The precipitate was suspended in SDS sample buffer with or without reducing agent (100 m*M* dithiothreiotol) and placed in boiling water for 3 minutes. The suspension was microfuged, and the clarified supernatant was subjected to SDS electrophoresis on 14 percent acrylamide slab gels and analyzed by autoradiography [P. Pantazis and W. M. Bonner, *J. Biol. Chem.* **256**, 4669 (1981)].
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25. NRK cells and SSV-transformed NRK cells were obtained from K. C. Robbins and S. A. Aaronson, National Cancer Institute, Bethesda, Md.). The serum-free media were collected from the cultures after an 18- to 24-hour incubation period, lyophilized, reconstituted to one-fifth of the original volume, and dialyzed to remove excess ions. Assays were performed as described (*4*).
26. We thank K. Robbins and S. A. Aaronson for providing the SSV-transformed and untransformed NRK cells; and S. Williams, J. MacLaren, and C. Keller-McGandy for technical assistance. Supported by the National Heart, Blood, and Lung Institute (HL-27607), the National Cancer Institute (CA-30101), and the American Cancer Society.

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