The results demonstrate the critical role of iodine in promoting thyroiditis in genetically susceptible chickens. Other studies have shown that iodination of Tg leads to an increased T_3 and T_4 content (*15*), an increased sedimentation coefficient (*16*), and an altered configuration (*17*) of the molecule. Such changes may be accompanied by increased immunogenicity of the molecule. This is an attractive hypothesis because treatment with iodine increases antibodies not only to Tg but also to T_3 and T_4. Alternatively, iodine may act by altering antigen presentation by the thyroid epithelial cells (*18*), modifying the function of thyroid-targeted immune cells or by other mechanisms. The thyroid abnormality of young CS chicks (*11*) may predispose them to iodine-induced thyroiditis.

These results are of relevance to the study of human autoimmune thyroiditis and suggest that elevated levels of dietary iodine may exacerbate or induce the disease in genetically susceptible individuals, such as females in families with a high incidence of thyroid disorders. In this context, it is important to note that the daily intake of iodine in the United States is two to five times the recommended allowance (*19*).

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Evidence That the v-*sis* Gene Product Transforms by Interaction with the Receptor for Platelet-Derived Growth Factor

Abstract. *A scheme for partial purification of biologically active* v-sis*–coded protein from cells transformed with simian sarcoma virus (SSV) has made possible a functional comparison of the transforming protein with platelet-derived growth factor (PDGF). The SSV-transforming gene product is capable of specifically binding PDGF receptors, stimulating tyrosine phosphorylation of PDGF receptors, and inducing DNA synthesis in quiescent fibroblasts. Each of these activities was specifically inhibited by antibodies to different regions of the* v-sis *gene product. Moreover, viral infection of a variety of cell types revealed a strict correlation between those cells possessing PDGF receptors and those susceptible to tranformation by SSV. These findings provide evidence that SSV-transforming activity is mediated by the interaction of a virus-coded mitogen with PDGF receptors.*

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The discovery that the onc gene (v-*sis*) of simian sarcoma virus (SSV) encodes a protein closely related in its predicted amino acid sequence to a major component of human platelet-derived growth factor (PDGF) provided the first evidence that onc gene products were involved in growth factor–mediated proliferative pathways (*1*). Subsequent studies have shown that the product of the v-*sis* gene is a PDGF-2–like precursor polypeptide that assumes a homodimer configuration soon after its synthesis (*2*, *3*). In contrast, human PDGF preparations contain two distinct but related polypeptide chains, one of which is encoded by the human *sis* proto-oncogene (*4*). Mitogenically active PDGF also exhibits a dimer structure, but whether it is composed of homo- or heterodimers of the two polypeptide chains is not known. Thus, while there are strong structural similarities between the processed *sis* product and PDGF, the functional relation of the SSV-transforming gene product to the growth factor is not yet established.

In the present study, we used SSV-transformed cells as the source of v-*sis*–coded protein and devised a means of functionally characterizing cell-associated molecules that can be unequivocally identified as products of the v-*sis* gene. We also sought to determine whether the transforming properties of the v-*sis* gene product are directly exerted through interaction with the cellular PDGF recep-

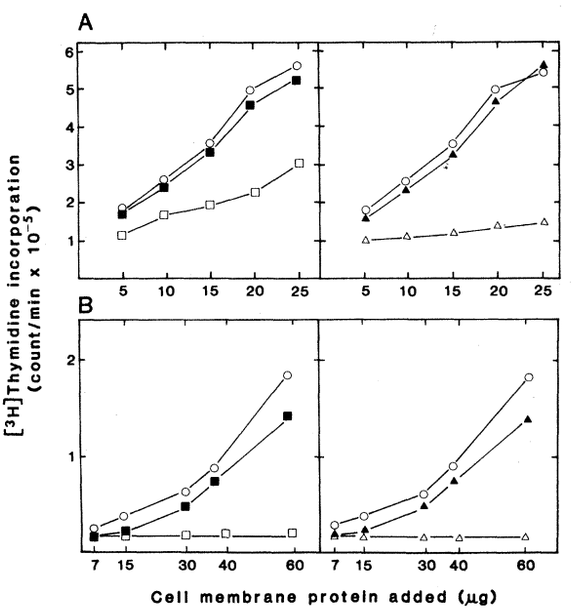

Fig. 1. Mitogenic activity of products of the v-*sis* gene. Crude membranes were prepared from hypotonic lysates of HF/SSV cells that were untreated (A) or exposed to $10^{-6}M$ monensin for 16 hours (B). Membranes were boiled, clarified, and tested for mitogenic activity after treatment with the IgG fraction of preimmune serum (○) or with antiserum to *sis*-N (□, ■) or to *sis*-C (△, ▲) bound to protein A–Sepharose beads (*17*). In some cases (■, ▲), antisera were incubated before immunoprecipitation with the homologous peptide after binding to protein A–Sepharose beads. [^{3}H]Thymidine incorporation was determined by scintillation counting.

tor. Our findings provide a demonstration of the mechanism by which a transforming gene perturbs the control of normal cellular growth.

To investigate whether the v-*sis* gene product (or products) has functional activities known to be associated with PDGF, we first analyzed lysates of HF cells transformed with SSV (HF/SSV cells) for PDGF-like mitogenic activity. When crude hypotonic lysates of HF/SSV and uninfected control cells were tested for their ability to induce DNA synthesis in quiescent NIH 3T3 cells, both showed high levels of activity. Since PDGF activity is heat stable (*5*), we also tested the same cell lysates after heat inactivation at 100°C for 5 minutes. This treatment totally abolished mitogenic activity associated with the uninfected cell lysates. In contrast, a large fraction of the activity present in HF/SSV cell lysates survived this treatment.

We have recently shown that processing of the v-*sis* translational products occurs in association with cellular membranes (*6*). Thus, v-*sis* translational products can be quantitatively recovered in cellular membrane preparations. To determine whether the heat-stable mitogen in HF/SSV cells cofractionates with v-*sis* gene products, we subjected metabolically labeled HF/SSV cultures to subcellular fractionation and followed their recovery by immunoprecipitation. As expected, v-*sis*–coded proteins were quantitatively recovered in the membrane component, whereas little or no such proteins were detected in nuclear or cytoplasmic fractions. When the mitogenic activity of these same fractions was analyzed, 89 percent of the activity was retained in the membrane preparation. These findings establish that the heat-stable mitogen of HF/SSV cells copurifies with the v-*sis* gene products during subcellular fractionation.

To assess whether the heat-stable mitogenic activity of HF/SSV cells shows target-cell specificity, we compared its effect on NIH 3T3 and BALB/MK keratinocyte cell lines. The latter is dependent on epidermal growth factor (EGF) for growth and is not responsive to stimulus by PDGF (*7*). EGF induced a marked increase in DNA synthesis in both quiescent BALB/MK and NIH 3T3 cells (Table 1). In contrast, the heat-stable mitogenic activity in HF/SSV membrane preparations, like PDGF, was specific for stimulation of DNA synthesis by quiescent NIH 3T3 cells (Table 1).

To establish more directly that the v-*sis* product (or products) is the heat-stable mitogen in HF/SSV cells and to assess which species of *sis* protein has this activity, we used antisera to *sis* peptides that are capable of recognizing different processed forms of the v-*sis* translational product. Antisera to *sis*-N and *sis*-C both recognize the unprocessed $p28^{sis}$ monomer as well as the processed $p56^{sis}$ and $p42^{sis}$ dimers. In addition, antiserum to *sis*-C recognizes the $p35^{sis}$ dimer (*2*, *6*). Thus, our approach involved testing mitogenic activity in HF/SSV membrane preparations either before or after quantitative removal of specific v-*sis* translational products with the immunoglobulin G (IgG) fractions of antisera to *sis* peptides. Such treatment with *sis*-N antibody removed 50 percent of the mitogenic activity in the membrane preparations from HF/SSV cells, whereas treatment with *sis*-C antibody resulted in the loss of 80 percent of this activity (Fig. 1A). In each case, incubation with the homologous peptide before immunoprecipitation blocked the ability of the antibody to remove mitogenic activity from the *sis* protein–enriched membrane fraction. These findings establish that the heat-stable mitogenic activity in HF/SSV cells is directly attributable to v-*sis* gene products. Since *sis*-C antibody removed substantially more mitogenic activity than *sis*-N antibody, it is likely that $p35^{sis}$, a relatively minor species, has a comparatively high specific mitogenic activity. However, our results imply that less processed forms have biologic activity as well.

Table 1. Specificity of SSV-transformed cell mitogenic activity for fibroblasts. Membranes of HF/SSV and uninfected HF cells were prepared from cell cultures as described (*14*). In the assay for [^{3}H]thymidine incorporation, BALB/MK or NIH 3T3 cells were plated in 96-well culture dishes and grown to confluence without changing of the medium. Sixteen hours after test samples were added, the medium was supplemented with 2 μCi of [^{3}H]thymidine (New England Nuclear; specific activity, 20 Ci/mmol) per well and incubated for an additional 5 hours. Material insoluble in trichloroacetic acid was measured by scintillation counting; data are reported as counts per minute per well. Receptor-grade epidermal growth factor (EGF) and human platelet-derived growth factor (PDGF) were used (Collaborative Research).

Mitogen	Protein added (μg)	[^{3}H]Thymidine incorporation	
		BALB/MK	NIH 3T3
None		2,315	28,868
EGF	0.001	195,320	362,514
PDGF	0.003	2,485	255,621
HF/SSV	5	2,154	175,920
	10	2,684	259,810
	20	2,976	497,111
HF	20	2,710	26,125

Previous findings have shown that monensin treatment of SSV-transformed cells inhibits the proteolytic processing of $p56^{sis}$, the most immature and abundant dimer of the v-*sis* gene product detectable in HF/SSV cells (*2*, *6*). To establish more conclusively that $p56^{sis}$ is itself biologically active, we analyzed *sis* proteins in the membrane-enriched fraction of monensin-treated HF/SSV cells for mitogenic activity. Under these conditions, $p56^{sis}$ was the only v-*sis* product detectable. This preparation had readily detectable mitogenic activity, all of which was removed by treatment with IgG fractions of antiserum to either *sis*-N or *sis*-C (Fig. 1B). The specificity of these antibodies for $p56^{sis}$ was demonstrated by blocking experiments with the homologous peptides (Fig. 1B). These results show that the $p56^{sis}$ molecule has mitogenic activity.

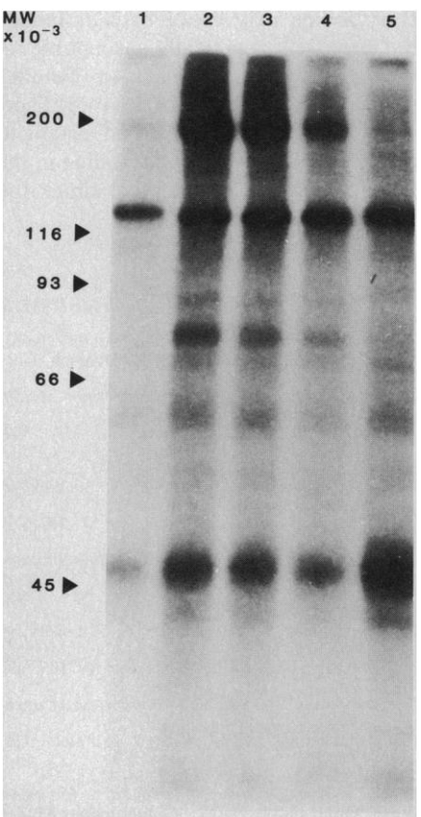

Fig. 2. PDGF receptor autophosphorylation in response to membrane extracts from SSV-transformed cells. Intact BALB/c 3T3 cells were loaded with ^{32}P-labeled orthophosphate and incubated alone (lane 1), with 150 ng (lane 2), or 20 ng (lane 3) of PDGF per milliliter, with 12.5 μg per milliliter of membrane protein from SSV-transformed marmoset cells (lane 4), or with uninfected marmoset cells (lane 5). Cell extracts were prepared and immunopurified with Sepharose-linked antibody to phosphotyrosine and analyzed by sodium dodecyl sulfate–polyacrylamide (7.5 percent) gel electrophoresis and autoradiography as described (*18*).

The growth stimulatory activity of PDGF is mediated by its interaction with specific membrane receptors of certain cell types (*8, 9*). To determine whether the v-*sis*–coded proteins specifically interact with PDGF receptors, we assayed the v-*sis* protein–enriched fraction of HF/SSV cells for its ability to compete with ^{125}I-labeled PDGF for binding to PDGF receptors. The untreated preparation completely inhibited binding of ^{125}I-labeled PDGF to cell membranes (Table 2). In control experiments to determine the specificity of *sis* protein blocking activity, removal of p56^{sis}, p42^{sis}, and p28^{sis} by treatment with the *sis*-N antibody or removal of these proteins plus p35^{sis} with the *sis*-C antibody reduced the ability of the preparation to block PDGF binding by 46 and 83 percent, respectively.

Binding of PDGF to its cell-surface receptor is associated with rapid tyrosine phosphorylation of the receptor (*10*). To determine whether v-*sis* gene products have this functional activity, we tested the v-*sis* protein–enriched membrane fraction of HF/SSV cells as well as the analogous fraction from uninfected cells for their ability to induce PDGF receptor autophosphorylation. Membrane preparations of HF/SSV cells induced tyrosine phosphorylation of a 185-kilodalton protein associated with the surface of intact BALB/3T3 cells (Fig. 2). The molecular size of this protein was not different from that of the PDGF receptor phosphorylated in response to PDGF (Fig. 2). In contrast, the membrane fraction of control HF cells induced no phosphorylation of the receptor. These results show that v-*sis*–coded products not only bind PDGF receptors but are capable of triggering their autophosphorylation on tyrosine residues.

The above findings establish that v-*sis* translational products have the known functional activities of PDGF, including the ability to bind PDGF receptors, to stimulate PDGF receptor autophosphorylation, and to induce DNA synthesis of quiescent fibroblasts. If transformation by SSV were directly mediated by the interaction of v-*sis* products with cellular PDGF receptors, one should expect to observe a strict correlation between target cells susceptible to SSV transformation and cell types having PDGF receptors. To address this question, we investigated the ability of SSV to transform a variety of cells in culture. We analyzed cell types expected to have PDGF receptors—including fibroblasts and smooth muscle cells—as well as cultures derived from epithelial or endothelial tissues, which are expected to lack PDGF receptors. Table 3 compares the binding of ^{125}I-labeled PDGF to each cell line, experimentally indicating those that have or lack PDGF receptors.

Table 2. Inhibition of ^{125}I-labeled PDGF ([^{125}I]PDGF) binding by crude membrane extracts of SSV-transformed cells. ^{125}I-Labeled PDGF (0.05 n*M*) was incubated with 3T3 cell membrane preparations in the presence of boiled and clarified crude membranes (100 μg) of HF/SSV cells. Specific ^{125}I-labeled PDGF binding was determined as described (*9*). Binding in the absence of HF/SSV cell membrane protein was between 40 and 90 fmol per milligram of protein (100 percent). Each value represents the mean of triplicate determinations from three separate experiments.

Treatment*	Inhibition of specific [^{125}I]PDGF binding (percent of maximum)
None	100
sis-N Antibody + *sis*-N	54
	90
sis-C Antibody + *sis*-C	17
	80

*Additions to crude membrane extract of HF/SSV cells.

The host range of replication-defective acute transforming retroviruses is determined by their associated helper virus. Thus, as a control, we compared the transforming activity of SSV with that of another acute transforming retrovirus, Kirsten-murine sarcoma virus (Ki-MSV), which was propagated in association with the same helper virus. Each virus stock was standardized for focus-forming activity on NIH 3T3 fibroblasts (Table 3). Transformation of smooth muscle cells was difficult to visualize after infection with either virus because these cells grow to high density. Therefore, we also assayed for transforming activity by colony formation in semisolid agar.

Kirsten-MSV efficiently transformed each of the target cells analyzed (Table 3). In contrast, SSV showed a more restricted pattern. We observed SSV-transforming activity for fibroblasts and smooth muscle cells at high titer, but there was no discernible morphologic or detectable growth alteration of either epithelial or endothelial cells in response to SSV infection. As an independent means of establishing that SSV had indeed infected the epithelial and endothelial cells, we performed infectious center assays using NIH 3T3 cells as a standard for susceptibility to SSV transformation. More than 1 percent of SSV-infected mink lung epithelial (MvlLu) cells or bovine aorta–derived endothelial cells registered as SSV-positive by infectious center assay. This was an ample level of infection for detection of SSV-altered cells had they been capable of registering either in the direct focus or agar colony-forming assays. The complete correlation between those assay cells susceptible to SSV transformation and those having PDGF receptors strongly implies that SSV-transforming activity is mediated by the obligatory interaction of its *sis* gene product with the PDGF receptor.

The high degree of relatedness between PDGF and the v-*sis* gene product (*1, 2*) provided early experimental evidence that transforming genes act to perturb normal cellular pathways by which growth factors and their receptors induce cellular proliferation. More recent findings of close homology between

Table 3. Susceptibility to SSV transformation of cell types having or lacking PDGF receptors. ^{125}I-labeled PDGF (specific activity, 20,000 count/min per nanogram) was incubated with live cell suspensions in a binding assay as described (*9*). Nonspecific binding was estimated in the presence of 10 n*M* unlabeled PDGF. The density of binding sites was calculated from Scatchard analysis as described (*9*). Biologic activity of rescued transforming virus was determined by direct focus (*15*) or soft agar colony-forming (*16*) assays. Foci or colonies were scored 14 to 21 days after infection. Transforming activities of SSV or Kirsten-MSV (Ki-MSV) are reported in focus-forming units (FFU) or colony-forming units (CFU) per milliliter. N.T., not tested.

Tissue and cell line	PDGF receptors per cell (number × 10^3)	Transforming activity			
		SSV		Ki-MSV	
		FFU	CFU	FFU	CFU
Fibroblast					
Mouse (NIH 3T3)	30 to 50	$10^{5.1}$	$10^{5.1}$	$10^{5.7}$	$10^{5.8}$
Human skin	50	$10^{3.2}$	N.T.	$10^{3.0}$	N.T.
Smooth muscle					
Bovine	80	N.T.	$10^{3.1}$	N.T.	$10^{2.8}$
Endothelial					
Bovine aorta	<10	$<10^0$	$<10^0$	$10^{4.7}$	$10^{5.0}$
Epithelial					
Mink (MvlLu)	<10	$<10^0$	$<10^0$	$10^{5.2}$	$10^{5.1}$

the predicted v-*erb*-B product and the EGF receptor (*11*), as well as possible links between other oncogene products and growth factor–hormone receptors that share tyrosine kinase activity, have provided further support for this concept.

This study has shown that v-*sis* translational products synthesized by and associated with SSV-transformed cells specifically bind PDGF receptors, stimulate tyrosine phosphorylation of the PDGF receptor, and induce DNA synthesis in quiescent fibroblasts. In each case, we were able to establish the v-*sis*–coded nature of these activities by specific inhibition with antibodies to different regions of the v-*sis* gene product. These findings demonstrate that the SSV-transforming protein is functionally equivalent to PDGF.

PDGF provides a proliferative stimulus only to those cells that have specific receptors for the growth factor. We observed a strict correlation between those cell types with PDGF receptors and those susceptible to transformation by SSV. Although there are undoubtedly other differences between fibroblasts and endothelial or epithelial cells in addition to their PDGF receptor status, our findings strongly suggest that the v-*sis* gene product must interact with the PDGF receptor to manifest its transforming activity. If so, the PDGF-like activities of the v-*sis* translational product are those functions responsible for its transforming ability.

After synthesis on membrane-bound ribosomes, the $p28^{sis}$ monomer has been shown to form rapidly a $p56^{sis}$ homodimer in the endoplasmic reticulum. This homodimer then travels through the Golgi apparatus toward the cell periphery, where it is further processed at both termini (*6*). Recent evidence indicates that the low-density lipoprotein receptor travels along a similar intracellular pathway (*12*). Moreover, sequence analysis of the EGF receptor predicts a protein whose receptor domain is translocated across the endoplasmic reticulum with its kinase domain occupying a cytoplasmic orientation (*13*). If the PDGF receptor were processed in an analogous manner, its ligand binding domain might proceed along the same intracellular route as newly synthesized *sis*/PDGF-2–like dimers. Our study establishes that several forms of the *sis*/PDGF-2 product, including the $p56^{sis}$ homodimer, have potent mitogenic activity. Thus, our results are consistent with the possibility that the critical transforming interaction of the *sis* product with its receptor target occurs during their transport along the same intracellular route toward the cell periphery.

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Leukotrienes as Mediators in Tissue Trauma

Abstract. *A significant increase in the production of cysteinyl leukotrienes was observed after mechanical or thermal trauma in the anesthesized rat. The amount of biliary* N*-acetyl-leukotriene* E_4*, which represents a suitable indicator for blood plasma leukotrienes, was used as a measure of leukotriene generation. Cysteinyl leukotrienes were rapidly eliminated from blood plasma into bile where* N*-acetyl-leukotriene* E_4 *was the major metabolite. Leukotrienes were at a much lower concentration in blood plasma than in bile and differed in the pattern of metabolites. The detected amounts of leukotrienes were sufficient to induce known phenomena associated with trauma, such as tissue edema and circulatory and respiratory dysfunction. Increased leukotriene generation appears to play an important role in the pathophysiology of tissue trauma.*

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In the mammalian organism, severe tissue trauma is followed by a local inflammatory reaction and a systemic shock-like reaction. Knowledge of the biochemical events underlying these reactions is limited despite the frequent occurrence of trauma-induced lesions.

There are many similarities between the symptoms associated with trauma and the effects of injected cysteinyl leukotrienes. Leukotriene C_4 (LTC_4) and LTD_4 induce tonus changes in small vessels, tissue edema caused by extravasation of plasma, myocardial depression, bronchoconstriction, and lethal shock under conditions of impaired cardiovascular compensation (*1–5*). The dihydroxylated leukotriene LTB_4, in contrast, seems to exert its main effects on local leukocyte immigration and activation (*3–5*). Additional indications of a role for leukotrienes in the pathophysiology of tissue injury come from pharmacological studies demonstrating beneficial effects of inhibitors of leukotriene biosynthesis or action in the treatment of trauma (*6*).

Cysteinyl leukotrienes are formed by conjugation of the 5-lipoxygenase product LTA_4 with glutathione, yielding LTC_4. This peptide leukotriene is con-