NSIGHT into normal and abnormal cell growth and differentiation may be obtained by identifying and analyzing the activity of highly specific genes, called oncogenes, whose coding sequences contain the information necessary to initiate and maintain neoplastic transformation. The identification of oncogenes and the characterization of the structure and function of these genes have progressed rapidly in the last few years. Oncogenes were first recognized in acute transforming viruses capable of mediating in vitro and in vivo cellular transformation. It was subsequently appreciated that the viral oncogenes were acquired from normal host genes (proto-oncogenes) of infected cells by genetic transduction. The host proto-oncogenes from which the viral oncogenes originated are highly conserved genes in evolution and are expressed in many different tissues. The finding of proto-oncogenes in normal cells has established the highly significant potential for malignant transformation in higher species by activation of proto-oncogenes, and subsequently led to the discovery of activated cellular oncogenes by DNA-mediated gene transfer (transfection).

CELLULAR proto-oncogenes may contribute to malignant transformation when somatic mutation leads to expression at inappropriate times, in increased amounts, or in abnormal forms, by mechanisms including gene amplification, point mutation, or transposition. These relationships are depicted in Fig 1. In this review, we will use the term viral oncogene (v-onc) to mean a transforming gene of a tumor-inducing virus and proto-oncogene to mean a normal cellular gene that, when inappropriately activated by mutations, gives rise to a cellular oncogene (c-onc) that has the potential to mediate cellular transformation and tumorigenesis.

ROLES OF ONCOGENES IN CELLULAR TRANSFORMATION

The proteins encoded by oncogenes have been difficult to identify but recently have been recognized in association with the cell membrane, the cytoskeleton, or the nucleus, suggesting that the functions served by proto-oncogenes may be involved in multistep processes in normal cellular growth and differentiation. Disruption of these processes by transformation may lead to the loss of regulation of normal cellular alignment and division.

The expression of proto-oncogenes (or c-oncogenes) has been investigated primarily by analyzing RNA transcripts in normal cells or in tumor cells. Transcription of proto-oncogenes occurs in many tissues, in all vertebrate species examined, and generally in small quantities (one to ten mRNA transcripts per cell) (summarized). The proteins encoded by proto-oncogenes generally are made in small amounts and may be very similar to the products of viral oncogenes. Thus, the product of the src proto-oncogene is a 60-kD phosphoprotein with protein tyrosine kinase activity similar to that of the viral src (v-src) gene product of the Rous sarcoma virus. They are similar in molecular weight, in immunologic reactivity, in peptide map analysis, in the serine residue at the amino-terminal end, and in the tyrosine residue at the carboxy-terminal end that is phosphorylated in vivo.

Transforming proteins are usually found at levels well in excess of their normal cellular counterparts (reviewed) and structural differences between viral transforming proteins and their cellular counterparts are also commonly encountered. For example, the 19 C-terminal amino acid residues of the protein encoded by the src proto-oncogene have been replaced by 12 unrelated amino acids in the v-src protein. Overexpression of normal ras genes is adequate for transformation but the transforming activity of ras genes in human tumors arises in association with structural mutations of the ras gene products.

Sequence analysis, functional activity, and transfection experiments have been used to classify the viral oncogenes into specific families. These include the src-related oncogenes with identified protein tyrosine kinase activity (src, abl, fps, fes, yes, fgr, ros), the sis-related oncogenes that have not yet been shown to have protein tyrosine kinase activity but have sequence homology to src (mos, mos, mht [mil], erb B, fms, rel), the ras oncogene family (has, Ha-ras, bas, kis, N-ras), the myc oncogene family (myc, N-myc), the sis oncogene family, and the src oncogene family (src, v-src).

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gene family, and unrelated oncogenes (myb, fos, erb A, efs, ski, and Blym).3

DNA sequence analysis of viral oncogenes has established remarkable homology among the protein tyrosine kinase domains of the putative transforming proteins of several tumor viruses39-41 and the catalytic subunit of the cellular protein, adenosine 3', 5'-cyclic phosphate (AMP)-dependent protein kinase.42 Tyrosine kinase activity has been shown to be related to cell growth and cell transformation. It should be noted that cells may be transformed by viruses without v-oncogenes (Fig 1). Avian leukosis viruses, which induce B cell lymphomas, lack v-oncogenes56-58 but may be integrated into the DNA of infected cells near the myc proto-oncogene, thereby promoting increased expression of myc via the viral long terminal repeat (LTR).59-64 That c-oncogenes have true transforming potential is suggested by the isolation of active cellular oncogenes (c-ras) from cells of human bladder carcinoma,65-67 by finding elevated c-myc expression in sarcomas, carcinomas, leukemias, and melanomas,21,68 and by demonstrating c-sis transcripts in high concentrations in cultures derived from sarcomas and glioblastomas.21

ROLES OF POLYPEPTIDE GROWTH FACTORS IN MALIGNANT CELL GROWTH

Evidence that growth factors might be involved in malignant transformation has recently accumulated. Historically, the platelet-derived growth factor (PDGF) was first discovered by observing that chick embryo fibroblasts grew well in media supplemented with serum, whereas plasma was without effect, indicating that a serum factor was required for cell growth. Rous sarcoma virus-infected fibroblasts grew well, however, in media supplemented with either plasma or serum, indicating that the virus-infected cells were able to bypass the serum requirement for cell growth.69,70 In retrospect, these cells may have expressed an activity that could substitute for serum. The factor in serum required for support of chick embryo fibroblast growth was subsequently shown to be released from platelets and became known as PDGF.71,72 It soon became evident that cells stimulated by growth factors and cells transformed by viruses were strikingly similar, having increased solute transport, DNA synthesis, tyrosine kinase activity, failure of contact inhibition, and continued cell division, when contrasted with nonstimulated cells (summarized).73,74 These similarities suggested common pathways of stimulation of cellular proliferation in viral-trans-
formed and growth factor-stimulated cells. Additional evidence relating growth factors and transformation arose when polypeptide-transforming growth factors were reported. These transforming growth factors were originally detected in the conditioned media of sarcoma virus-transformed cells and were shown to reversibly induce nonneoplastic cells to lose contact inhibition and to undergo anchorage-independent growth.

STRUCTURE/FUNCTION HOMOLOGY BETWEEN HUMAN PDGF AND p28V'**

The putative transforming protein of the simian sarcoma virus (SSV) is p28V'**. The simian sarcoma virus is an acute transforming retrovirus, capable of causing tumors within a short period of time, and was first isolated from a fibrosarcoma observed in a pet woolly monkey. The 5.1-kilobase (kb) SSV genome appears to have arisen by recombination of simian sarcoma-associated virus (SSAV) with a 1-kb segment (v-sis) derived from a woolly monkey cellular proto-oncogene. The nucleotide sequence of v-sis reveals a long open-reading frame that encodes for the SSV-transforming protein.

A definitive relationship between growth factor-stimulated cells and malignant cells was established by the demonstration that the amino acid sequence of a portion of human PDGF had striking homology to the predicted amino acid sequence of p28V'**. This structural relationship, schematically illustrated in Fig 2, suggested the possibility that a PDGF-like growth factor is the product of the transforming gene of SSV, and that expression of the growth factor activity might mediate the unregulated growth characteristic of SSV-transformed cells.

Such a growth factor activity has now been identified in SSV-transformed cells but not in nontransformed control cells. The mitogenic dose response curve, the reactivity in specific immunoassays, and the specific mitogenic activity of this growth factor-like activity in cell lysates of SSV-transformed cells were shown to be essentially identical to those of purified PDGF. Antisera raised against human PDGF recognized a protein in SSV-transformed cell lysates of 20 kD, identical in molecular weight to the previously identified proteolytic product of the putative transforming protein of SSV, p28V'**. The results provide direct evidence for the expression of a PDGF-like molecule encoded by the viral oncogene v-sis, which may in turn be responsible for the abnormal growth regulation of SSV-transformed cells.

SSV-transformed fibroblasts not only synthesize but also secrete a PDGF-like growth-promoting activity. The secreted PDGF-like growth-promoting activity is related to p28V'** by criteria used to establish the relatedness of activity of the PDGF-like molecule in cell lysates and PDGF. Coupled with the near identity of amino acid sequence of p28V'** and PDGF and the previously established specificity of anti-PDGF antisera in recognizing p28V'**, the results effectively establish the secreted growth-promoting activity as p28V'** or its processed product, although the possibility remains that transformation by SSV stimulates expression and secretion of the protein encoded by the sis proto-oncogene as well.

Secreted p28V'** or its processed product appears to serve as an autocrine stimulator of growth of SSV-transformed cells. Addition of anti-PDGF antisera to SSV-transformed National Institutes of Health (NIH) 3T3 cells reduced incorporation of 3H-thymidine into DNA. Furthermore, SSV-transformed NIH 3T3 cells responded to exogenous PDGF stimulation and contained high affinity binding sites for 125I-PDGF. The Kd of binding is identical to that in nontransformed cells. Transformation by SSV is associated with a significant reduction in receptor number: only 5% of PDGF receptors on nontransformed NIH 3T3 cells are found on SSV-transformed NIH 3T3 cells. PDGF stimulates autophosphorylation of a 180-kD PDGF receptor protein (a tyrosine kinase) purified from SSV-transformed cells; this receptor protein is identical in molecular weight to the previously characterized PDGF receptor in 3T3 cells. 125I-PDGF and partially purified preparations of p28V'** or its processed products compete for binding to SSV-transformed cell surface receptors. Thus, in SSV-transformed cells, p28V'** may act by being secreted into the surrounding medium where it then binds to a limited number of cell surface receptors that recognize either
PDGF or p28\textsuperscript{sis} thus stimulating \textsuperscript{3}H-thymidine incorporation and cell growth.

The results support the hypothesis that the transforming protein of the SSV is a protein with structural and functional near-identity to PDGF. It is not clear, however, what determines whether a cell is stimulated to grow normally for several cell cycles or, alternatively, is transformed by p28\textsuperscript{sis}. Transformation, as opposed to autocrine stimulation of growth, may not require secretion of the transforming protein. The SSV-transformed NPI (marmoset fibroblasts) cell line lacked detectable growth-promoting activity in conditioned media. Limitations of the sensitivity of assay may have precluded detection of small amounts of secreted p28\textsuperscript{sis} from SSV-NPI cells. It seems more likely, however, the p28\textsuperscript{sis} acts via an intracellular signal to induce cellular transformation. Such an interaction might occur when newly synthesized p28\textsuperscript{sis} enters the endoplasmic reticulum where it can bind to, and stimulate, the newly synthesized PDGF receptor protein while the receptor amino terminal binding site is being transported into the endoplasmic reticulum. Newly synthesized p28\textsuperscript{sis} may thereby stimulate activity of the receptor protein kinase domain that remains exposed to the cytoplasm. Such a mechanism, while not established, provides a framework to explain how p28\textsuperscript{sis} mediates uncontrolled DNA synthesis via PDGF receptor kinase activity while disrupting processing of the receptor protein in the endoplasmic reticulum, thereby leading to the significant reduction of high-affinity receptor sites of SSV-transformed cells. These potential roles of p28\textsuperscript{sis} in the autocrine regulation of transformed cell growth and malignant transformation are illustrated in Fig 3.

**Fig 3.** A model for the autocrine stimulation of cell growth and transformation by p28\textsuperscript{sis} (or its processed product). p28\textsuperscript{sis} may stimulate cell growth and cause transformation through interactions with both external and internal specific receptors. p28\textsuperscript{sis} and PDGF share the same cell surface receptor for mediating their functions. p28\textsuperscript{sis} is secreted from some SSV-transformed cells. Secreted p28\textsuperscript{sis} appears to stimulate cell growth as anti-PDGF antisera blocks \textsuperscript{3}H-thymidine incorporation in SSV-transformed NIH 3T3 cells. Anti-PDGF antisera has identical reactivity to p28\textsuperscript{sis} and PDGF. Because not all SSV-transformed cells secrete p28\textsuperscript{sis}, it is likely that p28\textsuperscript{sis} transforms cells via intracellular receptor interactions, perhaps in the endoplasmic reticulum or Golgi apparatus, thereby activating protein tyrosine kinase activity. The binding of p28\textsuperscript{sis} to the p28\textsuperscript{sis}/PDGF receptor results in stimulation of tyrosine kinase activity associated with the receptor (as indicated). The mechanism whereby protein tyrosine kinase activity of the receptor may transmit the signal leading to DNA synthesis is unknown.

**THE SIS PROTO-ONCOGENE ENCODES A POLYPEPTIDE PRECURSOR OF THE A CHAIN OF PDGF**

Human PDGF I and II are heterodimer glycoproteins (PDGF I, approximately 31,000 mol wt; PDGF II, approximately 28,000 mol wt) containing two polypeptide chains (A and B) linked by disulfide bonds.\textsuperscript{86} PDGF I and PDGF II have similar amino acid compositions, identical immunoreactivity, and mitogenic activity but differ in carbohydrate composition. The molecular weight of the A chain is approximately 18 kD for PDGF I and 15 kD for PDGF II.\textsuperscript{86}

The amino acid sequence of the A chain is identical to the amino acid sequence predicted for the protein product encoded by the human sis proto-oncogene over 100 amino acid residues.\textsuperscript{86,87} Overall, the predicted amino acid sequences for v-sis and the human sis proto-oncogene products are 93% homologous.\textsuperscript{86-88} These results suggest that the human sis gene encodes for the precursor of the larger polypeptide chain (A chain) of PDGF. A partial amino acid sequence of the B chain of PDGF is approximately 60% homologous to that of the A chain; the B chain, therefore, cannot be encoded for by that part of the sis gene that has already been sequenced.\textsuperscript{86,87}

**EXPRESSION OF THE SIS GENE IN MALIGNANT CELLS**

The sis gene may be expressed in transformed cells also, although any contribution of its expression to initiation and maintenance of transformation has not been proven. Using anti-PDGF antisera to seek proteins with structural similarities to PDGF, PDGF-like molecules have been identified in conditioned media from cells derived from human osteosarcomas and human glioblastomas\textsuperscript{89-91} and from an SV-40 transformed BHK cell line.\textsuperscript{92-94} Cell lines not known to be transformed by viruses but derived from human glioblastomas and sarcomas contained a 4.1-kb mRNA transcript that hybridized with v-sis--containing probes, whereas normal fibroblasts and melanoma- and carcinoma-derived cell lines lacked this transcript.\textsuperscript{21} The HUT-102 cell line,\textsuperscript{53,96} derived from a cutaneous T cell lymphoma and infected with human T
cell leukemia virus, expresses several proto-oncogenes, including sis.\textsuperscript{97,98} This is unusual because hemopoietic cells in general do not express the sis gene.\textsuperscript{21} A cDNA clone of sis mRNA obtained from HUT-102 cells has been shown to be capable of transforming NIH 3T3 cells.\textsuperscript{99} In the absence of sequence analysis of the cloned gene, however, it has not been established with certainty that the cloned gene was free of mutation. These several lines of evidence do, however, suggest that abnormal expression of the sis gene may contribute to neoplasia perhaps by mechanisms analogous to those demonstrated for the v-sis gene product in viral-infected cells (Fig 3).

**GENES ENCODING FOR PROTEINS THAT MEDIATE THE CELLULAR RESPONSE TO GROWTH FACTORS MAY BE POTENTIAL ONCOGENES**

The binding of growth factors is mediated through specific cell surface receptors which, in turn, initiate a cascade of biochemical events leading to DNA synthesis and to cellular proliferation.\textsuperscript{71} It follows that a receptor itself, as opposed to the growth factor, may contain the requisite activity or sites necessary to initiate the proliferation response in target cells. Such a possibility is suggested by the tyrosine kinase activity identified with epidermal growth factor (EGF)\textsuperscript{100} and PDGF\textsuperscript{85,101,102} receptors, an activity also identified with the transforming proteins of the src-related oncogenes.\textsuperscript{5} A further confirmation of the close relationship between growth factor receptors and the transforming proteins of oncogenes has been provided recently by the finding of marked homology between the EGF receptor and the protein encoded by v-erb-B.\textsuperscript{55} Six peptides derived from the human EGF receptor match closely part of the deduced sequence of the v-erb-B--transforming protein of the avian erythroblastosis virus (AEV): 74 of 83 amino acids are shared. The AEV progenitor may have acquired the cellular sequences that encode the transmembrane domain and a domain requisite for stimulation of cellular proliferation but not the sequences that encode the EGF-binding domain. Thus, transformation of cells by AEV may result in part from inappropriate acquisition of a truncated EGF receptor encoded by the v-erb-B gene. These observations suggest an additional mechanism whereby mutations in specific cellular genes might alter the protein products involved in response to growth factors and thereby lead to unregulated growth characteristic of cancer cells.

Data to be discussed next month in a review by R. Weinberg\textsuperscript{103} suggest a mechanism by which the products of c-ras found in human tumor cells may lead to growth factor-independent cellular proliferation. A general model emerges whereby several proteins involved in the biochemical cascade stimulated by various growth factors may be products of proto-oncogenes (Fig 4). Possible products might include other growth factor receptors, other kinases including serine and threonine, kinases such as protein kinase C,\textsuperscript{104} and the myc gene product.\textsuperscript{105}

The problem of neoplastic transformation is not yet solved. Greater detail and understanding of growth factors and of their receptors are required at both the functional and structural levels. For example, the relationship of the A and B chains of PDGF to each other and to stimulation of cell growth activity needs to be clarified; PDGF is a heterodimer whereas p28\textsuperscript{116} is a homodimer, perhaps providing a clue as to the growth-stimulating, as opposed to the transforming, activities of the respective proteins. The presumed intracellular locus of action for transformation of p28\textsuperscript{116} needs to be defined and the biological activities and sites of action of the PDGF receptor protein likewise require elucidation. The substrates and biological functions of receptor/protein tyrosine kinases in general remain to be established and the steps subsequent to growth factor-receptor interactions leading to cell proliferation are clear foci for future investigation. Such studies, in conjunction with the advancing understanding of oncogenes and their regulation, should delineate additional mechanisms of transformation and, hopefully, provide insights into ways to control neoplastic growth. Control of malignancy will not come easily, however; transformation to the malignant state is complex and is known to involve multiple steps.\textsuperscript{112} Multiple proteins and multiple interactions among many oncogenes may be required for tumorigenesis and clonal evolution.\textsuperscript{20}

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Roles of growth factor activities in oncogenesis

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