Advances in Viral Oncology, Volume 4,
edited by George Klein. Raven Press,
New York © 1984.

Transformation Mechanisms at the Cellular Level

Robert Pollack, Suzie Chen, *Scott Powers,
and Michael Verderame

*Department of Biological Sciences, Columbia University, New York, New York 10027; and
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11224

A major question in biology today is: What are the differences in behavior between normal cells and cells that either come from or can become tumors? A partial answer is forthcoming from somatic tissue cells grown in dishes and flasks. Even this success may be surprising, since cell cultures contain an element of artifact: insofar as they are growing, often at lower densities than found *in vivo*, they may be intentionally different from the cells in the tissue they mimic (50,109).

In the adult body, many cells are not growing; rather, they are sitting in a matrix of other cells and their mutually secreted products in assemblies called tissues. A typical tissue has epithelia and mesodermal and fibroblast cells separated by an acellular basement membrane (Fig. 1). The epithelial side is the topologic outside of the tissue. All epithelial cells are in contact with either neighboring cells or a collagen-based matrix. Blood vessels are on the mesodermal side, along with a nondividing cell called the fibroblast. Fibroblasts normally are quiescent.

GROWTH CONTROL MECHANISMS

A typical tissue in an adult is not growing in size; thus net tissue cell growth must be kept to zero. Normal cell growth in a tissue is regulated differently by cells on the two sides of the basement membrane. The first physiologic mechanism is simplest: some cells do not normally divide. The second is more complex: some cells divide regularly, but one of their two daughters is destined always to die by differentiation.

Remarkably, these two mechanisms are topologically distinct in the body. The basement membrane separates them, with the "inside" cells showing the simpler control and the "outside" ones the more complex. In the case of the epithelium, molecular biology must explain how one cell divides into two different cells, only one of which will divide again (7,39,45). In the case of the stromal cells, we must explain what signals these cells require in order to divide (4,32,49).

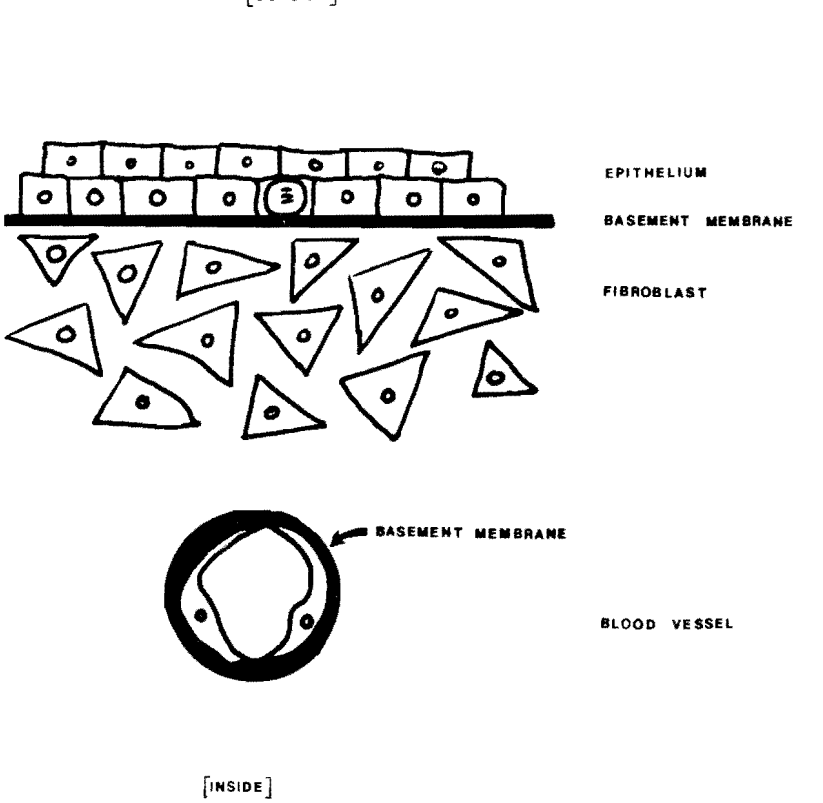


FIG. 1. This schematic diagram shows the relative positions of epithelial cells, fibroblasts, blood vessel endothelial cells, and the interposing basement membranes. Epithelial and fibroblastic cells are separated by a basement membrane.

WOUNDS AND THE GROWTH CONTROL OF FIBROBLASTS

Epithelia are the origin of the majority of tumors. Apparently, the more complex mechanism is the one that most often goes awry; more is known about the simpler case of stromal cell growth regulation. We have a good idea of what the signals are in the body for normal fibroblast growth, because we can make these cells grow by wounding any tissue. The consequence of wounding is to cause fibroblasts to grow, to secrete a collagen scar, and then to stop growing as the epithelial layer fills in over the collagen. Thus a wound provides all the signals necessary to reversibly activate the growth controls of a normal fibroblast (85).

What then are the signals provided by a wound? A wound is an opportunity for blood to exit a capillary or a larger blood vessel. The blood then clots. Unclotted blood is a suspension of lymphocytes, macrophages, erythrocytes, and platelets in plasma. Plasma is composed of fibrinogen, hormones, and a vast assortment of soluble molecules. Upon wounding, blood contacts the collagen of the cut vessel

and the collagen-based matrix in which the vessel was embedded. This leads to a string of events. First, the platelets lyse to release a mesh of actin and myosin; they also release a local high concentration of soluble, platelet-derived hormones. At the same time, the fibrinogen of the plasma is converted to an insoluble fibrin mesh by collagen activation of the plasma enzyme thrombin. These events culminate as the fibrin, actin, and myosin become a contractile clot, which squeezes out a fluid called serum. The presence of serum and a fibrin clot are the necessary and sufficient signals for fibroblasts to begin dividing and secreting large amounts of collagen in a feedback loop that reestablishes the structural integrity of a tissue.

Recently, we have learned that serum contains a set of polypeptide hormones (49), including the platelet-derived growth factor PDGF (106). PDGF stimulates fibroblasts to secrete their own mitogenic hormone, the insulin-like growth factor somatomedin C (19). Somatomedin C, also known as insulin-like growth factor-I (IGF-I), is the final hormone in the hypothalamic loop stimulating normal body growth through growth hormone (Fig. 2). At the same time as IGF-I appears, the clot with collagen provides an anchoring structure on which a fibroblast can spread and organize its cytoskeleton into large stress fibers containing intracellular actomyosin (42,71,126). Receptors for such hormones as IGF, PDGF, epidermal growth factor (EGF), and insulin are integral membrane proteins (63) (Fig. 3), and the insulin and EGF receptors have an associated tyrosine kinase activity (3,13,54,60). Tyrosine kinase activity is associated with tumor virus transforming gene products (9). Evidence has accumulated that hormone binding is followed by clustering of these hormone receptors (59), and that such lateral, directed receptor movement requires a well-organized cytoskeleton (6,31).

Cell culture techniques thus permit the growth of tissue fibroblasts by providing necessary growth signals. These come in the form of serum and a dish on which the fibroblasts can anchor and spread. Given these signals, tissue fibroblasts in a dish will proliferate for a few dozen divisions, as if filling in a giant wound. Eventually, they will cease dividing, dying in a crisis (50) that mimics aging. If crisis were inevitable, there would be little hope of doing reproducible molecular biology with the cultures. Often, however, a process called establishment (2,44,123) occurs, to provide populations of cells in culture with an abnormal but desirable extra degree of autonomy.

ESTABLISHMENT

Experimental protocols, especially those requiring recovery of variant cell types, depend on the capacity of cells to form separate colonies on a dish (47). Colony formation demands that tissue fibroblasts show a novel degree of autonomy, initiating colonies at a distance from one another that exceeds by many orders of magnitude the distance wound healing cells get from their nearest neighbors in any tissue (44,109,130). Most tissue cells cannot form colonies, and these colonies are even less often capable of throwing off colonies in turn. Variants that survive crisis and readily form colonies thereafter are called established (123). Although they

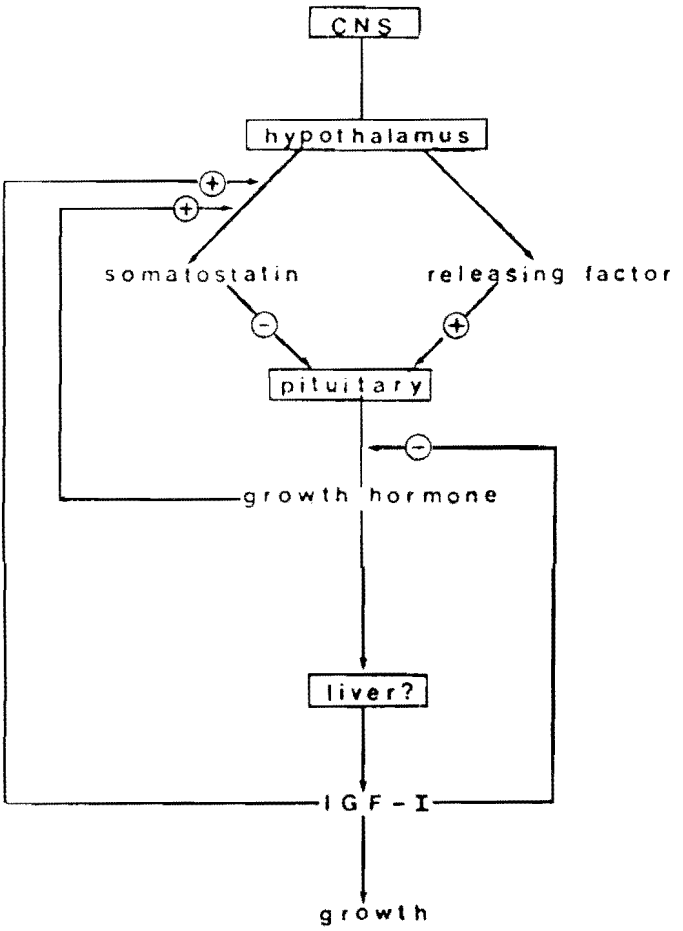


FIG. 2. Feedback inhibition in the regulation of IGF-I levels. Although the liver is currently the suspected site of IGF-I production, this issue has never been conclusively settled. The existence of a releasing factor has recently been discovered (11). +, positive stimulation; −, negative stimulation. (Adapted from ref. 8.)

are often not abnormal enough to make tumors, they have clearly become genetically different from normal tissue fibroblasts.

Obviously, ignoring the difference between established cells and tissue cells introduces artifact; nevertheless, the choice to do so provides a great boon. Colonies are the descendents of single cells, while tissues are a complex architecture of many cell types. Homogeneity of material is a prerequisite to the molecular analysis of mechanism.

TUMOR CELLS AND TRANSFORMATION

A sarcoma is a tumor of fibroblast origin. It will grow whether or not a wound or clot is present; it is no longer in need of these signals. The loss of a need for

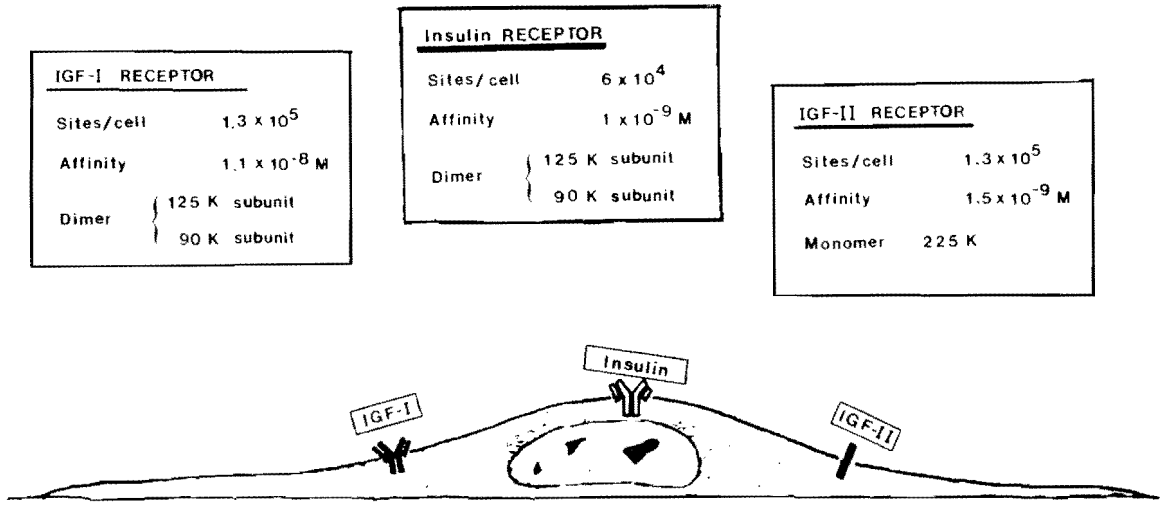


FIG. 3. Insulin-hormone family of receptors on 3T3 cells. Each hormone has a distinct receptor. Note that the receptors for insulin and IGF-I are multiple polypeptide chains held together by disulfide bonds, while the IGF-II receptor is a single polypeptide chain (M. Czech, *personal communication*).

one or more of the signals involving hormones or anchorage defines the loss of growth control in the cells of this form of tumor (33,57). Established normal cell lines can be converted by a number of agents into a state in which they can form tumors upon injection into an appropriate normal tissue (35,61,75,119,122,124). Thus cell culture provides two different kinds of transformed cells: from the tumor and from the transformation (Fig. 4).

Once a culture is established from a single cell, it can be grown into a large amount of genetically and biochemically homogeneous material for further study. This material, whether from a tumor, a transformant, or a normal cell culture, will be suitable for the tracking of biochemical differences in a manner not possible using biopsy material.

What then are the biochemical and phenotypic differences between normal and tumor cells, and how do they occur? Because it is inappropriate to approach the question of mechanism by the accumulation of differences (Table 1), we concentrate only on these differences that are characteristic of both cultured tumor cells and transformed cells (Fig. 5). In this light, three phenotypic changes are common to both sarcomas and transformed fibroblasts: (a) the ability to grow without anchorage (28,32–34,75,104,115), (b) the inability to organize the actomyosin cytoskeleton properly (1,51,52,65,80,89,116,126), and (c) the abnormal capacity to secrete proteases (72,87,100,102,103).

Selections designed to separate out the parts of this complex of phenotypic changes have generated a set of cell lines that permit linkage of these phenotypes to tumorigenicity (4,75,104,122,124). The wound teaches us that normal fibroblasts must receive at least two different signals before they can divide. These are, again, the hormones found in serum and the substrate on which to spread. Tumor cells in culture often grow equally well in the presence or absence of these signals (4,25,28,40,49,53). Since both kinds of cells grow well in culture, we can construct assay conditions that directly select for the cells that no longer need these signals (Fig. 6). In such an assay, cells are intentionally deprived of one or more of the signals necessary for growth. They remain in suspended animation. A transforming agent (e.g., virus or chemical) may convert some of the cells so that they can grow despite the absence of the signal. When the conversion is genetically stable, a clone

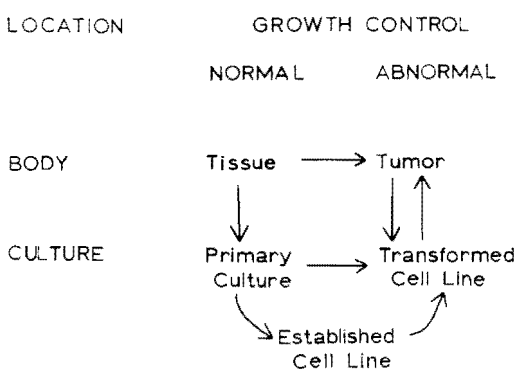


FIG. 4. Origins of cultured transformed cells.

TABLE 1. *Properties of cells transformed by SV40 or polyoma virus*[a,b]

Growth
High or indefinite saturation density[c]
Different, usually reduced serum requirement[c]
Growth in agar or Methocel suspension, anchorage independence[c]
Tumor formation upon injection into susceptible animals
Not susceptible to contact inhibition of movement
Growth in a less oriented manner[c]
Growth on monolayers of normal cells[c]
Surface
Increased agglutinability by plant lectins[c]
Changes in composition of glycoproteins and glycolipids
Tight junctions missing
Fetal antigens revealed
Virus-specific transplantation antigen
Different staining properties
Increased rate of transport of nutrients
Increased secretion of proteases or activators[c]
Intracellular
Disruption of the cytoskeleton
Changed amounts of cyclic nucleotides
Evidence of virus
Virus-specific antigenic proteins detectable
Viral DNA sequences detected
Viral mRNA present
Virus can be rescued in some cases

[a]From ref. 125.
[b]Transformed cells show many, if not all, of these properties, which are not shared by untransformed parental cells.
[c]Several of these properties have formed the basis of selection procedures for isolating transformants.

of transformed cells will grow out against a background of nongrowing normal cells.

Surprisingly, the transformants isolated in these different assays are not the same (104,105,127,128). Each selection generates a cell type insensitive to the growth signal that is absent in the selective assay; for example, serum transformants arise from the serum-starvation assay and anchorage transformants from the anchorage-deprivation assay. However, other transformation phenotypes accompanying the one that served as a basis for selection usually occur in an ordered way (Fig. 7). Serum-transformed clones lack the capacity to grow in the anchorage-deprivation assay, while tumorigenic anchorage transformants always also grow in low serum (34). Also, cells of tumors arising from a normal hamster line after chemical mutagenesis are both serum and anchorage independent, even though the transformants themselves are initially only anchorage independent (83). Apparently, while both the serum and the anchorage requirements must be lost before tumorigenicity is possible, the anchorage assay has a much higher probability of generating tumorigenic transformants (90,126). In biochemical terms, the concomitants of the anchorage requirement are likely to be most directly related to tumorigenicity (90).

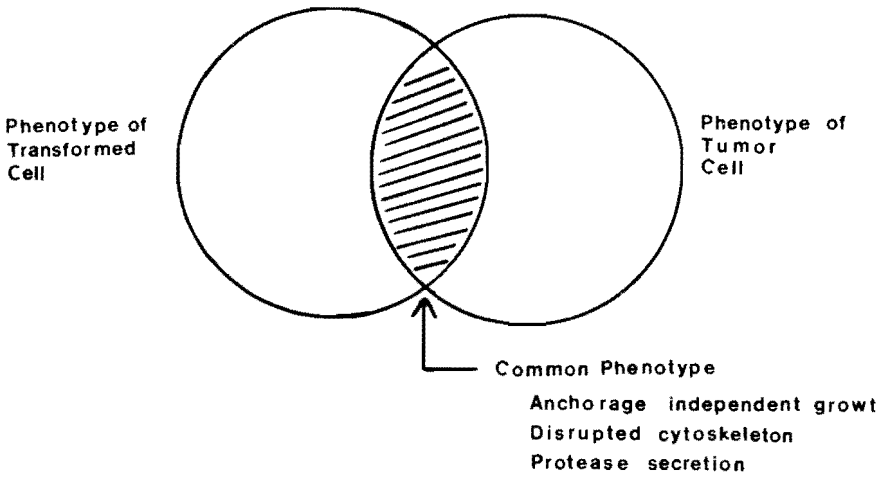


FIG. 5. Overlap of the phenotypic differences in transformation and in tumor cell growth. Each circle in this Venn diagram represents the complete set of phenotypes for the transformed cell **(left)** or the tumor cell **(right)**. A common strategy of modern research is to focus on those properties common to both sets (here represented by the hatched area).

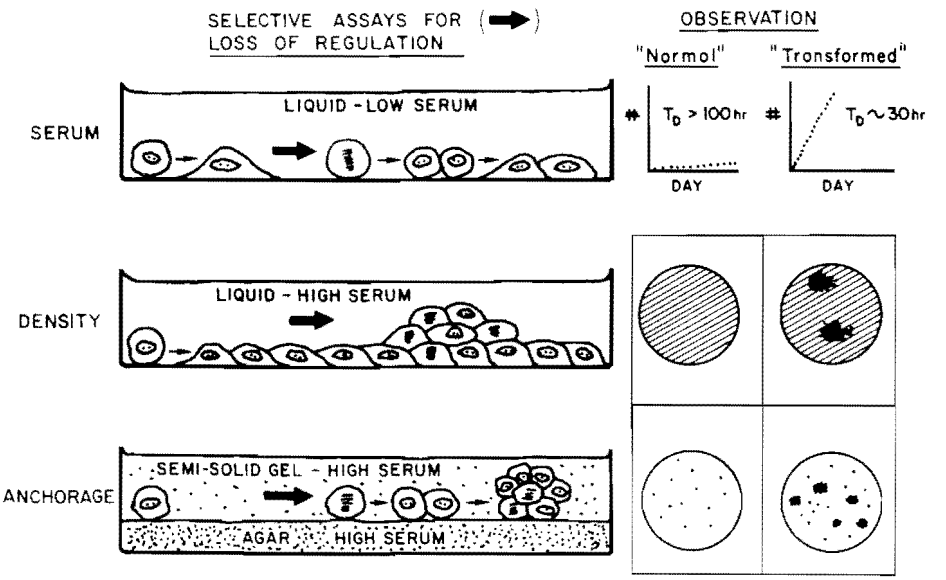


FIG. 6. Three selective transformation assays. In each assay, transformed cells and their descendents grow, whereas untransformed cells, due to one or another restrictive environmental signals, remain alive but do not increase in number. *Heavy arrow*, "transformation." Note that only in the anchorage assay can the transformant continuously traverse the cell cycle without ever spreading out. (Adapted from ref. 90.) Other transformation assays include hormone deprivation (94) and calcium reduction (79).

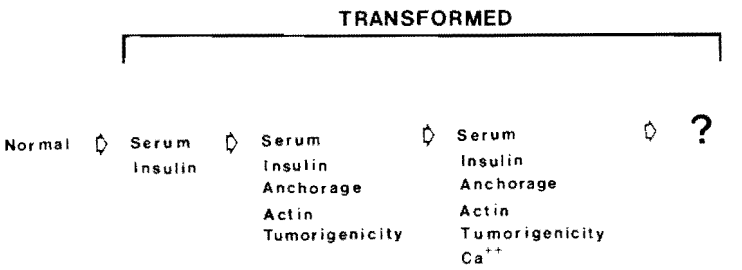


FIG. 7. Transformations occur in an ordered series. Cells that are serum transformed are also transformed with respect to the hormone insulin or IGF-I. Cells that are transformed with respect to anchorage are also transformed with respect to serum and the other phenotypes indicated; also, they usually are tumorigenic. Transformants selected for their ability to grow in low calcium (79) are transformed with respect to all the other phenotypes indicated.

INSULIN TRANSFORMATION

We recently carried the serum assay one step further toward molecular resolution. By growing cells in the absence of serum, differences in hormone requirement between normal and transformed cells became apparent (94,95). Figure 8 shows the growth requirement curves for insulin and IGF-I of a normal, established cell line. Insulin inefficiently mimics IGF-I (62); in fact, the physiologic IGF-I requirement is one of the major hormone requirements for established normal fibroblasts (118). Insulin itself is unlikely to have any direct growth-promoting activity through its own receptors on these cells (62).

Figure 9 shows the curves for a serum-transformed derivative line. Note that the requirements for both hormones are shifted to the left by at least a factor of 10. Apparently, serum transformation involves the reduction of the IGF-I requirement.

ANCHORAGE TRANSFORMATION

Because anchorage transformation most strongly correlates with the capacity to grow as a tumor in a susceptible animal (34,90), we concentrated on asking which biochemical events are most closely correlated with anchorage independence in fibroblasts. First, anchorage-transformed cells elaborate proteases, in particular the protease that activates the clot-dissolving plasma proenzyme plasminogen to plasmin (88,101) (Table 2). Second, we found that anchorage-transformed cells specifically lack the capacity to organize their cytoskeleton into large, actin-containing stress fibers, and that plasmin can efficiently disrupt the cytoskeleton of the normal cell (87,89,126). The cytoskeletal change is most easily seen by comparing the organization of normal and transformed cell cytoskeletons with a dye, such as phalloidin, that specifically stains the actin of the cell (104) (Fig. 10A). The cables run to the edge of the spread cell and are large and many in number. In most anchorage-transformed cells, but not serum-transformed cells, this organization is replaced by a markedly diffuse one (Fig. 10B). The red nucleus is stained at the same time with antibodies that pick out the presence of a transforming virus protein, SV40

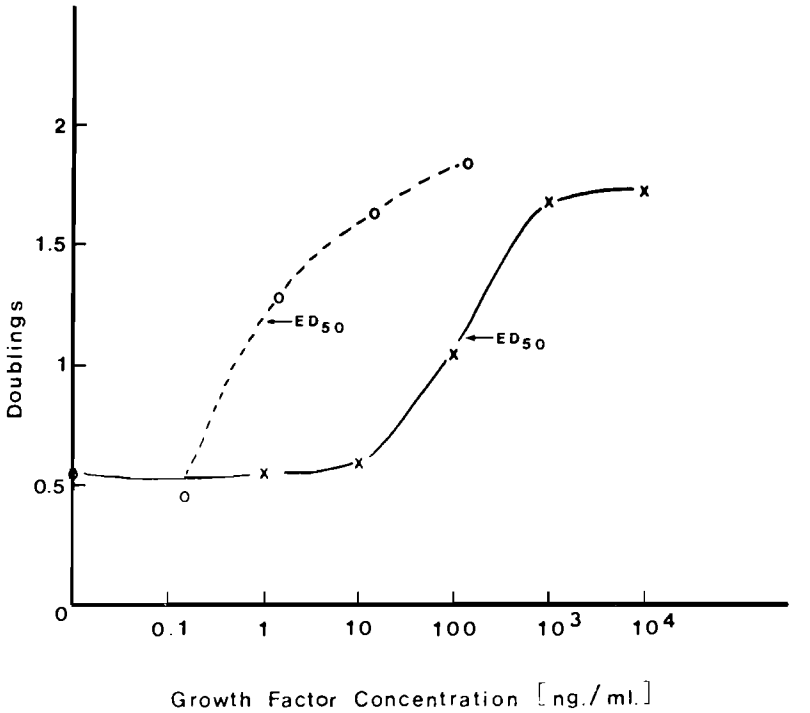


FIG. 8. Dose response curves of 3T3 with insulin and IGF-I. Cells were grown in serum-free medium, including either insulin (x) or IGF-I (o). Without either of the hormones, normal 3T3 cells do not even complete one doubling in 4 days. Note the shifted response curve for IGF-I as compared to insulin (94).

T antigen. Figure 10C is a serum-transformed, anchorage-requiring line. Note the correlation of cytoskeletal retention with the maintenance of the anchorage requirement in this cell, despite the presence of the SV40 T antigen (126).

EPITHELIAL CELLS AND THE TRANSFORMED PHENOTYPE

The majority of *in vitro* analyses concerning cellular growth control have been conducted with fibroblasts because of the relative ease with which these cells grow out in culture from any tissue biopsy. Recent progress in plating epithelial cells in culture allows short-term survival of these cells (30,38,82,96,97,99,114,131). Given the advantages of *in vitro* growth outlined above, studies with these populations should lead to significant observations concerning the mechanisms of both growth and differentiation—two intimately related phenomena in tissues that give rise to the majority of human malignant neoplasms.

The long-standing observation that tumorigenic fibroblasts have a disrupted actin cytoskeleton (89,126) provides a starting point for the examination of colonies of epithelial cells derived from colon biopsies of patients with frank adenocarcinomas,

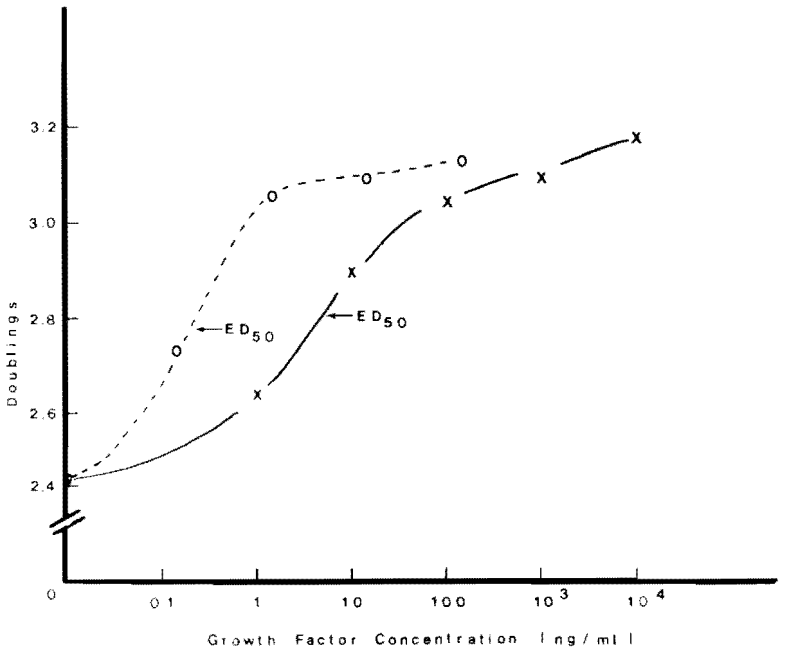


FIG. 9. Dose response curves of SV101 with insulin and IGF-I. These transformed cells were grown in serum-free medium, including insulin (x) or IGF-I (o). Note that even without added insulin or IGF-I, SV101 cells grow considerably (~2.5 doublings in a 4-day assay). Also note the shifted response curve for IGF-I as compared to insulin (94).

TABLE 2. *Correlation among* in vitro *growth properties and tumorigenicity of Syrian hamster cell lines*[a]

Growth parameter	Coefficient of rank correlation
Cloning efficiency, semisolid agar	0.986[b]
Fibrinolytic activity	0.754[c]
Generation time, 1% serum	0.754[c]
Cloning efficiency, liquid medium	0.638[c]
Organization of intracellular actin	0.570[c]
Saturation density, 10% serum	0.246
Generation time, 10% serum	0.062

[a]From ref. 5.
[b]Significant at the 95% confidence level.
[c]Significant at the 90% confidence level.

premalignant conditions of various states, or apparently normal tissue. Unlike fibroblasts, which have an elaborate stress fiber network in normal cells that is lost upon transformation to the tumorigenic state, primary epithelial colonies cultured for a short time *in vitro* exhibit several distinct classes of organization differing from fibroblasts (37).

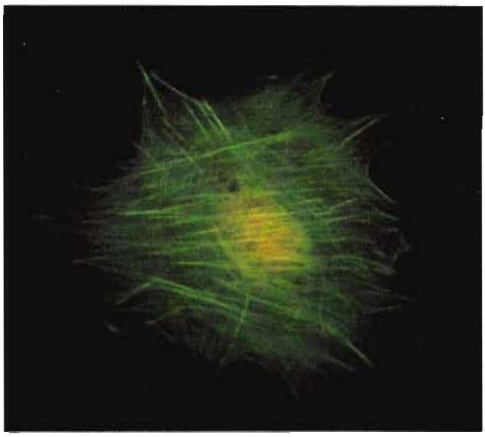
In normal colon, the epithelium is organized into finger-like indentations called crypts of Lieberkuhn. Only the cells at the base of the crypt are dividing (78). Cells *in vitro* derived from normal epithelial tissue are well spread. Electron micrographs have shown that the normal cells overlap one another like roofing shingles (36). In some colonies, the cells are well separated from their neighbors (i.e., not touching one another). In all cases, normal biopsy colonic epithelial cells contain some actin cables, but not as abundant an amount as found in fibroblasts. Instead, there is some weak staining at the perimeter of the cells (37) (Fig. 11A, B).

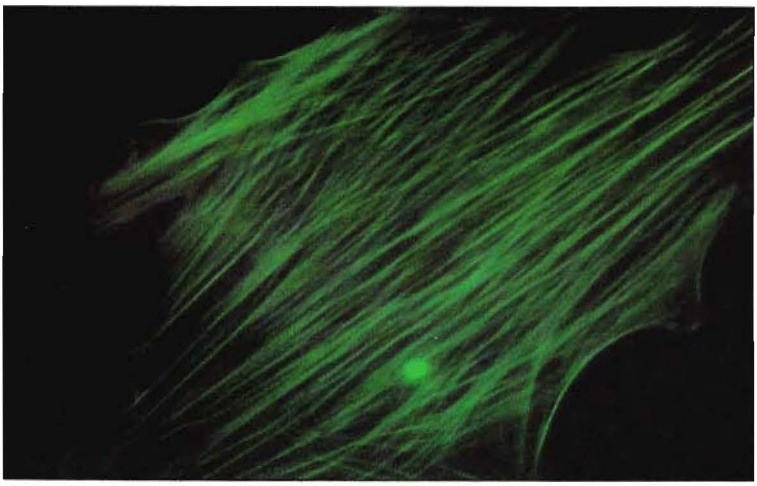
Premalignant adenomas *in situ* are characterized by a breakdown in the normal organization of the crypts usually seen *in vivo* (78). Dividing cells can be found all the way up the crypt wall, not confined to the bottom as in normal tissue (74). This elongates the crypt, until it forms a polyp which extends into the lumen of the gut. Colonies derived from early stage (tubular) adenomous tissue show a remarkable alteration in their *in vitro* phenotype as well (37). The cells are always organized into sheets of well-spread cells, in which each cell is in intimate contact with its neighbor along its entire perimeter (Fig. 11C, D). There is usually an intense staining lining the plasma membrane of each cell. Actin stress fibers can be seen scattered throughout some of the cells. A small number of colonies from premalignant adenomas exhibit an elaborate system of stress fibers, which seem to extend intercellularly, essentially blanketing the colony.

Late stage (villous) adenomas show a similar organization to early stage adenomas (37). Cells from late stage adenomas can be distinguished from early stage adenomas by their response to the potent tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA). In addition to increasing the secretion of the protease plasminogen activator (36,72), TPA causes the late stage adenomas to round up, separate from one another, and completely lose all actin cables (37). In contrast, early stage adenomas are completely unaffected by this treatment.

Adenocarcinomas are characterized by a loss of tissue organization and inappropriate cellular proliferation (78). Biopsies containing these cells are placed in culture, and the cells are stained for actin; the cells are readily distinguished from either normal or premalignant cells by their rounded appearance. They can occur either patches of cells touching each other or as single cells, but in neither case

FIG. 10. Cells stained for F-actin and for SV40 large T-antigen. These cells have been double stained with the fluorescent compound Fl-phalloidin and with antibodies to SV40 large T-antigen (126). **Top:** A normal cell with its characteristic numerous F-actin bundles, shown here in green. **Middle:** A typical transformed cell. Note the presence of SV40 T-antigen (indicated by the red nucleus) and the absence of large actin bundles. **Bottom:** A typical anchorage revertant. Despite the continued expression of the SV40 T-antigen, the F-actin bundles are obvious, demonstrating the correlation of anchorage-dependent growth and an organized cytoskeleton.





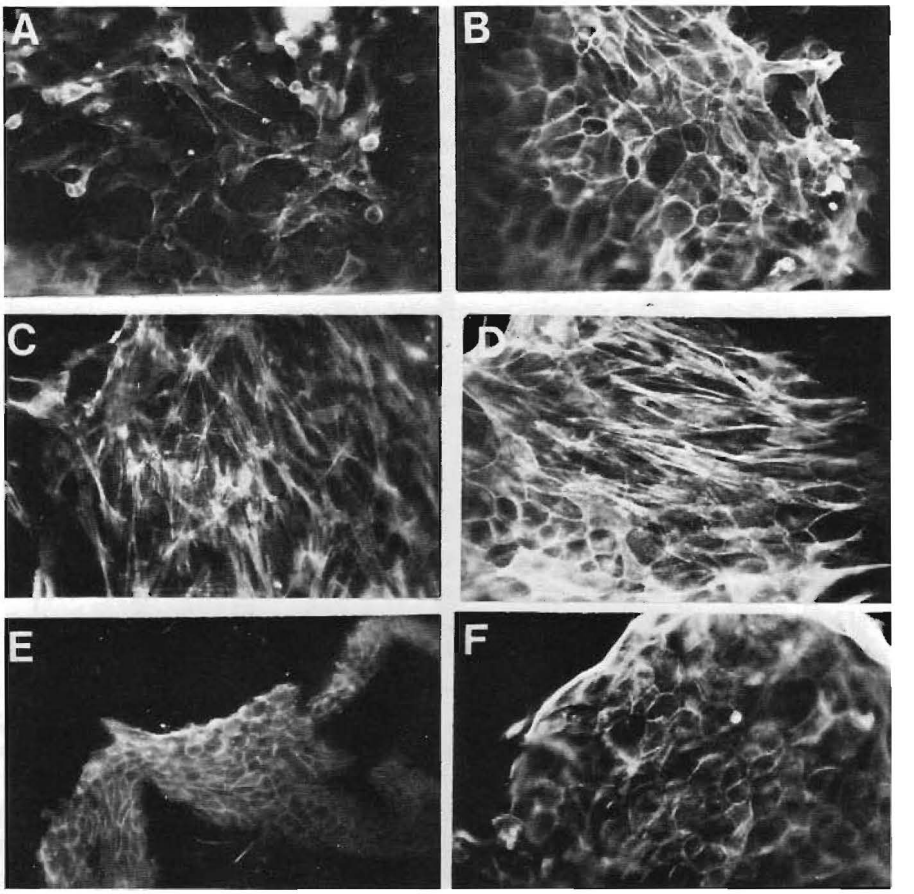


FIG. 11. Epithelial cells derived from colon tissue and stained for F-actin. Primary epithelial cells from an explant were cultured for 1 to 4 days and stained for F-actin with the compound Fl-phalloidin (37). **A** and **B:** Typical examples of cells from normal tissue. **C** and **D:** Typical examples of cells from benign adenomas. **E** and **F:** Examples of cells derived from adenocarcinomas. There is only a moderate amount of actin bundles in the normal cells **(A,B)**. The cells from the adenomas, on the other hand, are blanketed by actin cables **(C,D)**. Note the complete absence of detectable cables in the cells from the adenocarcinomas **(E,F)**.

do they exhibit any stress fibers (Fig. 11E, F) (37). These invasive populations usually display actin at their periphery, but they are easily distinguished from adenoma cells, which are much more angular at their edges. Interestingly, late stage adenomas that have been treated with TPA in culture are similar in appearance to adenocarcinoma cells, perhaps because either plasminogen activator or plasmin is disrupting their cytoskeletons (36,103). Because TPA can disrupt the cytoskeleton even in serum-free media, it is possible that plasminogen activator is directly responsible for the effect (36).

We have tentatively concluded that cultured epithelial cells from the colon lose their organized cytoskeleton upon becoming tumorigenic. Compared to normal skin fibroblasts, however, normal colonic epithelial cells apparently have only a moderately organized skeleton (Fig. 11A, B) (37). The cytoskeleton is dramatically more prominent in premalignant colonic cells and is completely lost upon the transition to malignant tissue. The molecular mechanism underlying cytoskeletal changes in both fibroblast and epithelial cells remains a mystery; nevertheless, models exist.

MODELS OF MECHANISM

Three distinct models of mechanism have been proposed to link growth signals, cytoskeletal changes, and protease activations to each other in a biochemical pathway (85,86,91,92,101).

Automimicry of an External Signal

The postulate is that the transformed cell is secreting the equivalent of a growth hormone or serum, or clustering the receptors for that hormone as if the hormone had been present and bound to those receptors. Secretion of IGF is indeed a normal response by fibroblasts to PDGF or growth hormone. Clearly, either stimulation of PDGF receptors or inherited loss of a requirement for that hormone would lock in repeated cell division. In the first case, this would happen by a cell stimulating itself into repeated divisions; in the second case, it would occur without any further hormone requirement.

Clustering of hormone receptors through protease disruption of the cytoskeleton would be a simple way for the cell to accomplish this bypass. Such a model links cytoskeletal changes with reduced hormone requirements and is consistent with the presence in culture fluid from transformed cells of growth-stimulating factors (85).

Regulatory Mutation Derepressing a Cellular Gene That is Normally Activated Only by a Growth Signal

The cell is presumed to bypass the need for environmental signals completely by making a cellular gene product that is normally never made except as a consequence of full hormone and anchorage signaling in a normal growing tissue. This model predicts that due to gene activation, a transformed cell will express intracellular or surface antigens common to rapidly growing normal cells. If such gene activation were sufficient to generate tumorigenic potential in a cell, certain hierarchically ancient genes should be found expressed both in embyros and in a disparate collection of tumors. Monoclonal antibodies are indeed turning up such antigens.

A major example is a cellular phosphoprotein of 54,000 (54K) daltons. This protein was detected almost simultaneously by workers in two separate fields. Searchers for tumor antigens reported that mice with methylcholanthrene-induced

tumors had antibodies to this cellular protein (23). Virologists working with the DNA tumor virus SV40 reported that the 54K cellular protein could be immunoprecipitated with sera obtained from animals with tumors induced by SV40-transformed cells (68,73). The same 54K cellular protein subsequently was found in cells transformed by Abelson murine leukemia virus and adenovirus, in spontaneous teratocarcinomas, and in a variety of other tumor-derived cell lines of murine, human, and other species (58,108). Small amounts of this protein have been detected in normal mouse tissues and cell cultures (46,73) and larger amounts in early mouse embryos (14).

Recently, we have used inbred mouse strains to examine endogenous cellular 54K protein in the absence of any transforming agent (18). We have found that the amount of endogenous cellular 54K protein in early precrisis mouse cells varies in a strain-specific manner. Strains that can produce a high level of endogenous 54K protein in late passage do not further increase 54K expression after SV40 transformation (Table 3).

Structural Mutation Generating an Abnormal Gene Product Involved in Hormone and Anchorage Signaling

A cell would bypass the environment by making an abnormal gene product whose activity is no longer subject to normal cell cycle regulation. This would be the intracellular equivalent of surface PDGF receptor clustering. Examples of such host proteins with altered activity are not yet available. However, transcriptionally active variant alleles of human oncogenes are found in cultured cells of many tumors, and genes for these variant alleles are capable of transfecting anchorage independence (12,120; M. Wigler, *personal communication*).

TUMOR VIRUSES AND MECHANISMS OF TRANSFORMATION

These three mechanisms need not be mutually exclusive, and any is likely to be at best only partly correct. Because they are small, viruses offer the possibility of a resolution of the problem of mechanism. The smallest ones encode only one protein, which by itself can overcome all the growth requirements of a fibroblast and convert it into a sarcomagenic transformed line (125). The transforming gene product of the RNA retroviruses is typically a protein with a hydrophobic C-terminus that places it in the plasma membrane of the cell (67). This protein usually has kinase activity, transferring phosphate to tyrosine residues of a small number of cellular proteins (20,22,55). A close similarity to hormone receptors and their immediate substrates at the inner membrane probably is not coincidental (6,26,31,59,60,107).

The transforming proteins of the DNA tumor viruses are of two sorts: small proteins, which are claimed to have the capacity to mimic growth hormones (125), and large phosphoproteins, which bind to viral and cellular DNA (48) and the 54K cellular phosphoprotein (70). Recently, we have found that SV40 transformation

TABLE 3. *Quantitation of [^{35}S]-methionine-labeled host 54K protein in primary mouse cells*[a]

Cells	Passage	Amounts of 54K protein immunoprecipitated with monoclonal 122 antibody[b]
C57L	p3	0.27
C57L	p12	0.30
SVC57L	[c]	5.75
NFS	p3	0.30
NFS	p12	6.75
SVNFS	[c]	6.38
A[d]	p2	0.36
A	p12	2.70
BALB/C	p2	0.81
BALB/C	p12	2.92
SVBALB/C[d]	[c]	5.73
C3H	p2	0.26
C3H	p12	0.20
SVC3H	[c]	5.58
(C57LxNFS)F1	p2	0.38
(C57LxNFS)F1	p12	4.25

[a]Adapted from ref. 18.

[b]Total trichloracetic acid precipitable radioactivity ([^{35}S]-methionine) was determined in each cell extract, and immunoprecipitation was done using equal amounts of protein. Amounts of 54K protein were determined from fluorograms by scanning on a Gilford 250 Spectrophotometer equipped with a Gilford 2520 Gel Scanner (arbitrary units).

[c]After SV40 transformation, phenotypes are passage independent.

[d]Female mice only.

frequency varies coordinately with endogenous 54K expression (Table 4). This suggests that the genes regulating 54K expression may predispose mice to have a greater or lesser chance of developing at least some forms of cancer.

With tumor viruses and cellular transformation assays, it should be possible to pose the molecular question: How does this one gene product work, and by interaction with which cellular molecules, to overthrow the requirement of the cell for growth signals? A major limitation of the tumor virus strategy so far lies in the use of established normal cells. We have already pointed out that normal tissue cells can be passaged in culture as autonomous microorganisms. Upon passage, the cell population changes, since passage itself selects for the most autonomous of the cells in the biopsied tissue.

Presumably, a set of structural gene mutations must accompany stable establishment. For example, expression of the 54K phosphoprotein is increased in established lines, since this protein is found in many "normal" established lines as well as in many tumors (73); tumor viruses in most laboratories are expected to transform abnormal but anchorage-requiring cells into anchorage-transformed lines. There-

TABLE 4. *Covariation of endogenous 54K protein and susceptibility to SV40 transformation in various strains of mice*

Amount of 54K protein	SV40 transformation frequency	
	High	Low
High	BALB/C NFS (C57L x NFS) A Female (late passage)	None
Low	None	C57L C3H A Female (early passage)

fore, viral gene products are usually interacting with a set of cellular gene products that are different from those found in a normal tissue cell. This is confirmed by a recent study of the polyoma gene products necessary to transform cells. Polyoma middle T was sufficient to anchorage-transform the established rat line FR3T3. However, both polyoma middle T and large T were necessary to anchorage-transform precrisis fibroblasts (98).

The strategy of transforming already abnormal cells may generate information only about a necessary but not sufficient part of oncogenic transformation. Direct approaches will also be necessary to map and understand the mechanism by which a normal cell elaborates genetically stable established variants. Indeed, it is possible that the changes from normal to established cell are part of the changes that must occur before a tissue cell becomes oncogenic. If so, then this second set of changes will be of equal importance to anchorage independence in our understanding of the origins of cancer (10,18,29,56,93).

TRANSFORMATION BY GENES FROM SPONTANEOUS TUMORS

Recently, a new strategy has permitted an old question to be asked about transformation: Which genes in a spontaneously transformed cell or putatively nonviral tumor are capable of transforming a normal cell?

In the past 10 years, a new technology has grown for the direct manipulation of specific DNA sequences. The enzymatic and biochemical tools are now available to isolate, amplify, and sequence a gene and to insert it into a cell, where it will integrate and express in that cell and its descendents (21,43,64,129). Furthermore, the sequence transforming a cell can be found at a later time in the cellular genome and recovered for another cycle of gene sequencing, site-specific mutagenesis, and reimplantation in the genome of another recipient cell. With this set of tools, the search has begun in tumors for the cellular genes that can transform normal cell lines. These are called oncogenes.

The protocol is simple. DNA from a transformed cell is transfected into normal recipient cells. The normal cell cultures are then treated as if they were infected with any other transforming agent. Transformants for anchorage or density are

recovered, and the causative sequences of DNA from the donor are tracked and isolated (21,66,69,84,111–113). These studies have led to two remarkable results: First, the genes that have been followed out in detail are homologous to the transforming genes found in Kirsten and Harvey sarcoma viruses, even though the donor DNA may come from a cell line derived from a "spontaneous" human tumor (16,17,81). Second, these tumor oncogenes differ only slightly from ones found in a conserved way in every mammalian species. Expression from the "normal" alleles of these genes is tightly down-regulated in normal cells (12,120). For example, the normal *ras* gene and the tumor *ras* gene can both anchorage transform NIH3T3. However, these transformants showed significant difference in p21 *ras* production. Much more normal *ras* protein than tumor *ras* protein was made in the transformants (121). Apparently, a small amount of tumor p21 *ras* is as effective as a normal amount of normal p21 *ras*.

HYPOTHESES TO EXPLAIN ONCOGENES

Model 1: Tumor oncogenes are oncogenic because they are mutant forms of necessary growth regulatory genes, which play a role in rapid cell growth, perhaps during embryogenesis.

Model 2: Tumor oncogenes are normal genes; that is, the oncogene of the donor tumor may be a member of a polyallelic family of growth regulatory genes. In this family of genes, all alleles would yield normal growth control in tissue, while some would generate the transformed state against the changed genetic background of an established cell. The donor tumor itself would then have arisen as a result of the establishment of its parental cell, perhaps by mutation of the sort that converts normal tissue cells to established cell lines. Upon transfection into a recipient cultured cell that is also established, such a variant allele of a growth regulatory gene would again be able to generate the full phenotype of transformation.

If model 1 is the case, then these mutant cellular oncogenes should make products whose functions are sufficient to generate a tumor from a tissue cell. If model 2 is true, then certain alleles of these host oncogenes may be markers for a higher than normal cellular propensity to develop a tumor. This would imply that the oncogene set may persist in any one of a set of multiple allelic forms in the human genome without inevitably generating a tumor. If a tumor arises, it would be the result of a combination of an inherited "bad" allele of an oncogene in all cells of the body and somatic mutation leading to establishment in a single cell. This second mutation is a good candidate for environmental mutagenesis.

According to model 2, environmental mutation would be responsible for the conversion of cells from normal to established phenotypes in the tissue. Such mutated established cells would not produce tumors unless they arose in a host carrying the sort of bad allele now detected as a "transforming gene" in gene transfer assay. In the presence of a bad allele, however, environmental mutagenesis would result in a fully transformed phenotype, and the cell would grow into a tumor.

One of the oncogenes cloned from a human bladder tumor cell line is reported to be closely related to the oncogenes of the rat-derived Harvey sarcoma virus, as

well as a cellular homolog present in normal human DNA (15,24,41,81,110). This particular oncogene is distinguished from its normal cellular counterpart by the loss of a specific restriction endonuclease cleavage site and a single amino acid difference at that site. However, only the normal allele of c-ras_1^{Ha} has been reported to be present in at least one bladder tumor (77).

At least some tumor oncogenes are mutated in one or more amino acid, compared with their normal homologs. Enhanced transcription of c-onc sequences in colon carcinoma (27), however, and similar examples of regulatory changes in oncogenes need not be the result of oncogene mutation. Indeed, changing the local microenvironment of early mouse embryo cells by placing them under the testes capsule of an adult male mouse is sufficient to generate a highly malignant, undifferentiated population of teratocarcinoma cells (117). Even after 8 years of being maintained as a transplantable tumor, teratocarcinoma cells can be entrained to normality by a subsequent change in the microenvironment to the inner cell mass of a normal early mouse embryo (56,76).

Time will tell whether current human sequences called oncogenes are actually agents of carcinogenesis or markers of individual susceptibility to environmental carcinogenesis, or both, or neither.

REFERENCES

1. Albrecht-Buehler, G. (1977): Phagokinetic tracks of 3T3 cells: Parallels between the orientation of track segments and of cellular structures which contain actin or tubulin. *Cell*, 12:333–339.
2. Augusti-Tocco, G., and Sato, G. (1969): Establishment of functional clonal lines of neurons from mouse neuroblastoma. *Proc. Natl. Acad. Sci. USA*, 64:311–315.
3. Baldwin, G. S., Knesel, J., and Monckton, J. M. (1983): Phosphorylation of gastrin-17 by epidermal growth factor-stimulated tyrosine kinase. *Nature*, 301:435–437.
4. Balk, S. D., Whitfield, J. F., Youdale, T., and Braun, A. (1973): Roles of calcium, serum, plasma and folic acid in the control of proliferation of normal and rous sarcoma virus-infected chicken fibroblasts. *Proc. Natl. Acad. Sci. USA*, 70:675–679.
5. Barrett, C. J., Crawford, B. D., Mixter, L. O., et al. (1979): Correlation of in vitro growth properties and tumorigenicity of Syrian hamster cell lines. *Cancer Res.*, 39:1504–1510.
6. Ben-Ze'ev, A., Duerr, A., Solomon, F., and Penman, S. (1979): The outer boundary of the cytoskeleton: A lamina derived from plasma membrane proteins. *Cell*, 17:859–865.
7. Bennett, D. C. (1980): Morphogenesis of branching tubules in cultures of cloned mammary epithelial cells. *Nature*, 285:657–659.
8. Berelowitz, M., Szabo, M., Frohman, L. A., Firestone, S., Chu, L., and Hintz, R. L. (1981): Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science*, 212:1279–1281.
9. Bishop, J. M., and Varmus, H. (1982): Functions and origins of retroviral transforming genes. In: *RNA Tumor Viruses*, edited by R. Weiss, N. Teich, H. Varmus, and J. Coffin, ch. 9., pp. 999–1108. Cold Spring Harbor Laboratories, Cold Spring Harbor.
10. Boone, C. W. (1975): Malignant hemangioendotheliomas produced by subcutaneous inoculation of Balb/3T3 cells attached to glass beads. *Science*, 188:68–70.
11. Brazeau, P., Ling, N., Bohlen, P., Esch, F., Ying, S. Y., and Guillemin, R. (1982): Growth hormone releasing factor, somatocrinin, releases pituitary growth hormone *in vitro*. *Proc. Natl. Acad. Sci. USA*, 79:7907–7913.
12. Capon, D. J., Chen, E. Y., Levinson, A. D., Seeburg, P. H., and Goedel, D. V. (1983): Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. *Nature*, 302:33–37.
13. Carpenter, G., King, L., and Cohen, S. (1978): Epidermal growth factor stimulates phosphorylation in membrane preparations in vitro. *Nature*, 276:409–410.

14. Chandrasekaran, K., McFarland, V. W., Simmons, D., Dziadek, M., Gurney, E., and Mora, P. T. (1981): Quantitation and characterization of a species specific and embryo stage-dependent 55 kilodalton phosoprotein also present in cells transformed by simian virus 40. *Proc. Natl. Acad. Sci. USA*, 78:6953–6957.
15. Chang, E. H., Gonda, M. A., Ellis, R. W., Scolnick, E. M., and Lowy, A. R. (1982): Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. *Proceedings of the National Academy of Sciences (79)*, 4848–4852.
16. Chang, E., Furth, M. E., Scolnick, E. M., and Lowy, D. R. (1982): Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus. *Nature*, 297:479–483.
17. Chang, E. H., Gonda, M. A., Ellis, R. W., Scolnick, E. M., and Lowy, D. R. (1982): Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. *Proc. Natl. Acad. Sci. USA*, 79:4848–4852.
18. Chen, S., Blanck, G., and Pollack, R. (1983): Early passage of pre-crisis mouse cells shows strain specific covariation in expression of 54k phosphoprotein and in susceptibility to transformation by SV40. *Proc. Natl. Acad. Sci. USA*, 80:5670–5674.
19. Clemmons, D. R., Underwood, L. E., and Van Wyk, J. J. (1981): Hormonal control of immunoreactive somatomedin production by human fibroblasts. *J. Clin. Invest.*, 67:10–17.
20. Collett, M., and Erikson, R. (1978): Protein kinase activity associated with the avian sarcoma virus *src* gene product. *Proc. Natl. Acad. Sci. USA*, 75:2021–2024.
21. Cooper, G. M., Okenquist, S., and Silverman, L. (1980): Transforming activity of DNA of chemically transformed and normal cells. *Nature*, 284:418–421.
22. Courtreidge, S. A., Levinson, A. D., and Bishop, J. M. (1980): The protein encoded by the transforming gene of avian sarcoma virus ($pp60^{src}$) and a homologous protein in normal cells ($pp60^{proto\text{-}src}$) are associated with the plasma membrane. *Proc. Natl. Acad. Sci. USA*, 77:3783–3787.
23. DeLeo, A. B., Appella, E., DuBois, G. C., Law, L. W., and Old, L. J. (1979): Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc. Natl. Acad. Sci. USA*, 76:2420–2424.
24. Der, C. J., Krontivis, J. G., and Cooper, G. M. (1982): Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proc. Natl. Acad. Sci. USA*, 79:3637–3640.
25. Eagle, H. (1955): Nutritional needs of mammalian cells in tissue culture. *Science*, 122:501–504.
26. Eckhart, W., Dulbecco, R., and Burger, M. (1971): Temperature-dependent surface changes in cells infected or transformed by a thermosensitive mutant of polyoma virus. *Proc. Natl. Acad. Sci. USA*, 68:283–286.
27. Eva, A., Robbins, K. C., Andersen, P. R., Srinivasan, A., Tronick, S. R., Reddy, E. P., Ellmore, N. W., Galen, A. T., Lautenberger, J. A., Papas, T. S., Westin, E. H., Wong-Staal, F., Gallo, R. C., and Aaronson, S. A. (1982): Cellular genes analogous to retroviral *onc* genes are transcribed in human tumour cells. *Nature*, 295:116–119.
28. Farmer, S. R., Ben-Ze'ev, A., Benecke, B., and Penman, S. (1978): Altered translatability of messenger RNA from suspended anchorage-dependent fibroblasts: Reversal upon cell attachment to a surface. *Cell*, 15:627–637.
29. Fidler, I. J., and Kripke, M. L. (1977): Metastasis results from preexisting variant cells within a malignant tumor. *Science*, 197:893–895.
30. Fischer, S. M., Viaje, A., Harris, K. L., Miller, D. R., Bohrman, J. S., and Slaga, T. J. (1980): Improved conditions for murine epidermal cell culture. *In Vitro*, 16:180–188.
31. Flanagan, J., and Koch, G. L. E. (1978): Cross-linked surface Ig attaches to actin. *Nature*, 273:278–281.
32. Folkman, J., and Moscona, A. (1978): Role of cell shape in growth control. *Nature*, 273:345–349.
33. Freedman, V., and Shin, S. (1974): Cellular tumorigenicity in *nude* mice: Correlation with cell growth in semi-solid medium. *Cell*, 3:355–359.
34. Freedman, V., Shin, S., Risser, R., and Pollack, R. (1975): Tumorigenicity of virus transformed cells in nude mice is correlated specifically with anchorage independent growth *in vitro*. *Proc. Natl. Acad. Sci. USA*, 72:4335–4339.

35. Freeman, A. E., Black, P. H., Wolford, R., and Huebner, R. (1967): Adenovirus type 12-rat embryo transformation system. *J. Virol.*, 1:362–367.
36. Freidman, E. A. (1981): Differential response of premalignant epithelial cell classes to phorbol ester tumor promoters and to deoxycholic acid. *Cancer Res.*, 41:4588–4599.
37. Friedman, E., Verderame, M., and Pollack, R. (1983): Loss of actin cytoskeletal organization accompanies the benign to malignant tumor transition in colonic epithelium examined *in vitro*. *Can. Res. (submitted)*.
38. Friedman, E. A., Higgins, P. J., Lipkin, M., Shinya, H., and Gelb, A. M. (1981): Tissue culture of human epithelial cells from benign colonic tumors. *In Vitro*, 17:632–644.
39. Friend, C., Scher, W., Holland, J. G., and Sato, T. (1971): Hemoglobin synthesis in murine virus-induced leukemic cells *in vitro*: Stimulation of erythroid differentiation by dimethyl sulfoxide. *Proc. Natl. Acad. Sci. USA*, 68:378–382.
40. Gey, G. O., Coffman, W. D., and Kubicek, M. T. (1952): Tissue culture studies of the proliferative capacity of cervical carcinoma in normal epithelium. *Cancer Res.*, 12:264–265.
41. Goldfarb, M., Shimizu, C., Perucho, M., and Wigler, M. (1982): Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. *Nature*, 296:404–409.
42. Goldman, R. D., and Follett, E. A. C. (1969): The structure of the major cell processes of isolated BHK21 fibroblasts. *Exp. Cell Res.*, 57:263–276.
43. Graessman, M., Graessman, A., and Mueller, C. (1977): The biological activity of different early SV40 DNA fragments. *INSERM*, 69:233–240.
44. Green, H., and Meuth, M. (1974): An established pre-adipose cell line and its differentiation in culture. *Cell*, 3:127–133.
45. Green, H., Rheinwald, J. G., and Sun, T. (1977): *Properties of an Epithelial Cell Type in Culture: The Epidermal Keratinocyte and its Dependence on Products of the Fibroblast*. Liss, New York.
46. Gurney, E., Harrison, R. O., and Fenno, J. (1980): Monoclonal antibodies against simian virus 40 T antigens: Evidence for distinct subclasses of large T antigen and for similarities among nonviral T antigens. *J. Virol.*, 34:752–763.
47. Hamilton, W. G., and Ham, R. (1977): Clonal growth of Chinese hamster cell lines in protein-free media. *In Vitro*, 13:537–547.
48. Hand, R. (1981): Functions of T antigens of SV40 and polyoma virus. *Biochim. Biophys. Acta*, 651:1–24.
49. Hayashi, I., and Sato, G. (1976): Replacement of serum by hormones permits growth of cells in a defined media. *Nature*, 259:132–134.
50. Hayflick, L., and Moorehead, P. S. (1961): The serial cultivation of human diploid cell strains. *Exp. Cell Res.*, 25:585–621.
51. Heggeness, M., Wang, K., and Singer, S. J. (1977): Intracellular distributions of mechanochemical proteins in cultured fibroblasts. *Proc. Natl. Acad. Sci. USA*, 74:3883–3887.
52. Heuser, J. E., and Kirscher, M. W. (1980): Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. *J. Cell Biol.*, 86:212–234.
53. Kohler, G., and Milstein, C. (1975): Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, 256:495–497.
54. Hunter, T., and Cooper, J. A. (1981): Epidermal growth factor induces rapid tyrosine phosphorylation of proteins in A431 human tumor cells. *Cell*, 24:741–752.
55. Hunter, T., and Sefton, B. M. (1980): The transforming gene product of rous sarcoma virus phosphorylates tyrosine. *Proc. Natl. Acad. Sci. USA*, 77:1311–1315.
56. Illmensee, I., and Mintz, B. (1976): Totipotency and normal differentiation of single teratocarcinoma cells cloned by injection into blastocyts. *Proc. Natl. Acad. Sci. USA*, 73:549–553.
57. Israel, M., Chan, H. W., Martin, M. A., and Rowe, W. P. (1979): Molecular cloning of polyoma virus DNA in *Escherichia coli*: Oncogenicity testing in hamsters. *Science*, 205:1140–1142.
58. Luka, J., Jornvall, H., and Klein, G. (1979): Purification and biochemical characterization of the Epstein-Barr virus determined nuclear antigen and an associated protein with a 53,000 dalton. *J. Virol.*, 35:592–602.
59. Kahn, C. R., Baird, K., Jarrett, D., and Flier, J. (1978): Direct demonstration that receptor crosslinking of aggregation is important in insulin action. *Proc. Natl. Acad. Sci. USA*, 75:4209–4213.
60. Kasuga, M., Zick, Y., Blithe, D. L., Crettaz, M., and Kahn, C. R. (1982): Insulin stimulates tyrosine phosphorylation of the insulin receptor in a cell-free system. *Nature*, 298:667–669.
61. Kennedy, A. R., Fox, M., Murphy, G., and Little, J. (1980): Relationship between X-ray exposure and malignant transformation in C3h 10T1/2 cells. *Proc. Natl. Acad. Sci. USA*, 77:7262–7266.

62. King, G., and Kahn, C. (1981): Non-parallel evolution of metabolic and growth-promoting functions of insulin. *Nature*, 292:644–646.
63. King, L. E., Carpenter, G., and Cohen, S. (1980): Characterization by electrophoresis of epidermal growth factor stimulated phosphorylation using A431 membranes. *Biochemistry*, 19:1524–1528.
64. Klobutcher, L. A., and Ruddle, F. H. (1979): Phenotype stabilization and integration of transferred material in chromosome-mediated gene transfer. *Nature*, 280:657–660.
65. Kopelovich, L., Blattner, W. A., Fraumeni, J. F., Lipkin, M., and Pollack, R. (1980): Organization of cytoskeletal actin in cultured skin fibroblast from patients at high risk of colon cancer. *Int. J. Cancer*, 26:301–308.
66. Krontiris, T. G., and Cooper, G. M. (1981); Transforming activity of human tumor DNAs. *Proc. Natl. Acad. Sci. USA*, 78:1181–1184.
67. Krueger, J. G., Wang, E., and Goldberg, A. R. (1980): Evidence that the *src* gene product of rous sarcoma virus is membrane associated. *Virology*, 101:25–40.
68. Lane, D. P., and Crawford, L. V. (1979): T antigen is bound to a host protein in SV40 transformed cells. *Nature*, 278:261–263.
69. Lane, M., Sainten, A., and Cooper, G. M. (1981): Activation of related transforming genes in mouse and human mammary carcinomas. *Proc. Natl. Acad. Sci. USA*, 78:5185–5189.
70. Lane, D., and Crawford, L. (1979): T antigen is bound to a host protein in SV40 transformed cells. *Nature*, 278:261–263.
71. Lazarides, E., and Weber, K. (1974): Actin antibody: The specific visualization of actin filaments in non-muscle cells. *Proc. Natl. Acad. Sci. USA*, 71:2268–2272.
72. Lee, L., and Weinstein, I. B. (1978): Epidermal growth factor, like phorbol esters, induces plasminogen activator in HeLa cells. *Nature*, 274:696–697.
73. Linzer, D. I. H., and Levine, A. J. (1979): Characterization of a 54K dalton cellular SV40 tumor-antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell*, 17:43–52.
74. Lipkin, M., and Deschner, E. (1976): Early proliferative changes in intestinal cells. *Cancer Res.*, 36:2665–2668.
75. Macpherson, I., and Montagnier, L. (1964): Agar suspension culture for the selective assay of cells transformed by polyoma virus. *Virology*, 23:291–294.
76. Mintz, B., and Illmensee, K. (1975): Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proc. Natl. Acad. Sci. USA*, 72:3585–3589.
77. Muschel, R. J., Kouhry, G., Lebowitz, P., Koller, R., and Dahr, R. (1983): Retraction of data on the human oncogene c-ras_1^H. *Science*, 220.
78. Muto, T., Bussey, H. J. R., and Morson, B. C. (1975): The evolution of cancer of the colon and rectum. *Cancer*, 36:2251–2270.
79. Nicholson, N. B., and Pollack, R. (1983): SV40 induces a reduced calcium requirement in mouse cells. *Mol. Cell Biol., (submitted)*.
80. Nicholson, N., Verderame, M., Lipkin, M., and Pollack, R. (1981): F-actin patterns quantitated with Fl-phalloidin in skin fibroblasts of individuals genetically predisposed to colon cancer. In: *International Cell Biology 1981–82*, edited by H. Schweiger, pp. 331–335. Springer-Verlag, Berlin.
81. Parada, L. F., Tabin, C. J., Shih, C., and Weinberg, R. A. (1982): Human EJ bladder carcinoma oncogene is homologue of harvey sarcoma virus *ras* gene. *Nature*, 297:474–478.
82. Peehl, D. M., and Ham, R. G. (1980): Growth and differentiation of human keratinocytes without a feeder layer or conditioned medium. *In Vitro*, 16:516–525.
83. Perez-Rodriguez, R., Chambard, J. C., Van Obberghen-Schilling, E., Franchi, A., and Pouyssegur, J. (1981): Emergence of hamster fibroblast tumors in nude mice—evidence for in vivo selection leading to loss of growth factor requirement. *J. Cell. Physiol.*, 109:387–396.
84. Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J., and Wigler, M. (1981): Human tumor-derived cell lines contain common and different transforming genes. *Cell*, 27:467–476.
85. Pollack, R. (1981): *Hormones, Anchorage and Oncogenic Cell Growth*. Grune & Stratton, New York.
86. Pollack, R., and Hough, P. V. C. (1974): The cell surface and malignant transformation. *Annu. Rev. Med.*, 25:431–446.
87. Pollack, R., and Rifkin, D. (1975): Actin-containing cables within anchorage-dependent rat embryo cells are dissociated by plasmin and trypsin. *Cell*, 6:495–506.
88. Pollack, R., and Rifkin, D. (1976): Maintenance of mammalian cell shape: Redistribution of

intracellular actin by SV40, proteases, cytochalasin B and dimethylsulfoxide. In: *Cell Motility*, edited by J. Rosenbaum, T. Pollard, and R. Goldman, pp. 389–401. Cold Spring Harbor, New York.

89. Pollack, R., Osborn, M., and Weber, K. (1975): Patterns of organization of actin and myosin in normal and transformed cells. *Proc. Natl. Acad. Sci. USA*, 72:994–998.
90. Pollack, R., Risser, R., Conlon, S., Freedman, V., Rifkin, D., and Pollack, R. (1975): Production of plasminogen activator and colonial growth in semisolid medium are *in vitro* correlates of tumorigenicity. In: *Proteases and Biological Control*, edited by E. Reich, D. Rifkin, and E. Shaw, pp. 885–899. Cold Spring Harbor, New York.
91. Pollack, R., Prives, C., and Manley, J. (1983): Do variant SV40 sequences have a role in the maintenance of the oncogenic transformed phenotype? In: *Genes and Proteins in Oncogenesis*, edited by H. Vogel and I. B. Weinstein, pp. 295–302. Academic Press, New York.
92. Pollack, R. (1977): Viral and cellular contributions to expression of transformed state. In: *Neoplastic Transformation*, edited by H. Kaprowski, pp. 169–179. Dahlem Konferenzen, Berlin.
93. Pollack, R. (1983): Letter. *Science*, 218:4577.
94. Powers, R. S., Chen, S., and Pollack, R. (1983): SV40 transformation in hormonally defined medium: Direct isolation of insulin-independent transformed lines. *Science (submitted)*.
95. Powers, S., Alcorta, D., Nicholson, N., and Pollack, R. (1982): Effects of calcium and hormones on the cytoskeleton and cell proliferation. In: *Cold Spring Harbor Conferences on Cell Proliferation*, pp. 243–258. Cold Spring Harbor, New York.
96. Price, F. M., Camalier, R. F., Grant, R., Taylor, W. G., Smith, H. G., and Sanford, K. K. (1980): A new culture medium for human skin epithelial cells. *In Vitro*, 16:147–158.
97. Quaroni, A., Wands, J., Trelstad, R. L., and Isselbacher, K. I. (1979): Epitheloid cell cultures from the small intestine. Characterization by morphological and immunological criteria. *J. Cell Biol.*, 80:248–265.
98. Rassoulzadegan, M., Cowie, A., Carr, A., Glaichenhaus, N., Kamen, R., and Cuzin, F. (1982): The roles of individual polyoma virus early proteins in oncogenic transformation. *Nature*, 300:713–718.
99. Rheinwald, J. G., and Green, H. (1975): Serial cultivation of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell*, 6:331–334.
100. Rifkin, D., and Pollack, R. (1977): The production of plasminogen activator by established cell lines of mouse origin. *J. Cell Biol.*, 73:47–55.
101. Rifkin, D., and Pollack, R. (1976): Proteases produced by normal and malignant cells in culture. In: *Proteases and Physiological Regulation*, edited by D. W. Ribbons and K. Brew, pp. 263–285. Academic Press, New York.
102. Rifkin, D. B., Beal, L. P., and Reich, E. (1975): Macromolecular determinants of plasminogen activator synthesis. In: *Proteases and Biological Control, Cold Spring Harbor Conferences on Cell Proliferation*, edited by E. Reich, D. B. Rifkin, and E. Shaw, pp. 841–847. Cold Spring Harbor, New York.
103. Rifkin, D., Crowe, R., and Pollack, R. (1979): Tumor promoters induce changes in cytoskeletal organization in chick embryo fibroblasts. *Cell*, 18:1504–1510.
104. Risser, R., and Pollack, R. (1974): A nonselective analysis of SV40 transformation of mouse 3T3 cells. *Virology*, 59:477–489.
105. Risser, R., and Pollack, R. (1974): Biological analysis of clones of SV40-infected mouse 3T3 cells. In: *Control of Proliferation in Animal Cells*, edited by B. Clarkson and R. Baserga, pp. 125–138. Cold Spring Harbor, New York.
106. Ross, R., and Vogel, A. (1978): The platelet-derived growth factor. *Cell*, 14:203–210.
107. Roth, R., and Cassell, D. (1983): Insulin receptor: Evidence that it is a protein kinase. *Science*, 219:299–301.
108. Rotter, V., Witte, O., Coffman, R., and Baltimore, D. (1980): Abelson murine leukemia virus-induced tumors elicit antibodies against a host cell protein P50. *J. Virol.*, 36:547–555.
109. Rous, P., and Jones, F. S. (1916): A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells. *J. Exp. Med.*, 23:549–555.
110. Shih, C., and Weinberg, R. A. (1982): Isolation of a transforming sequence from a human bladder carcinoma cell line. *Cell*, 29:161–169.
111. Shih, C., Shilo, B., Goldfarb, M. P., Dannenberg, A., and Weinberg, R. A. (1979): Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proc. Natl. Acad. Sci. USA*, 76:5714–5718.

112. Shih, C., Padhy, L. C., Murray, M., and Weinberg, R. A. (1981): Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. *Nature*, 290:261–264.
113. Shilo, B., and Weinberg, R. A. (1981): Unique transforming gene in carcinogen-transformed mouse cells. *Nature*, 289:607.
114. Smith, H. S., Lan, S., Ceriani, R., Hackett, A. J., and Stampfer, M. R. (1981): Clonal proliferation of cultured non-malignant and malignant human breast epithelium. *Cancer Res.*, 41:4637–4643.
115. Steinberg, B. M., Rifkin, D., Shin, S., Boone, C., and Pollack, R. (1979): Tumorigenicity of revertants from an SV40 transformed line. *J. Supramol. Struct.*, 11:539–546.
116. Steinberg, B., Smith, K., and Pollack, R. (1980): Viral transformation diminishes cellular contractivity in native collagen gels. *J. Cell Biol.*, 87:304–308.
117. Stevens, L. C. (1970): The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. *Dev. Biol.*, 21:364–382.
118. Stiles, C. D., Capone, G. T., Scher, C. D., Antoniades, H. N., Van Wyk, J. J., and Pledger, W. J. (1979): Dual control of cell growth by somatomedins and platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA*, 76:1279–1283.
119. Sutherland, B. M., Cimino, J. S., Delihas, N., Shih, A. G., and Oliver, R. P. (1980): Ultraviolet light-induced transformation of human cells to anchorage-independent growth. *Cancer Res.*, 40:1934–1939.
120. Tabin, C. J., Bradley, S. M., Bargmann, C. I., Weinberg, R. A., Papageorge, A. G., Scolnick, E. M., Dahr, R., Lowy, D. R., and Chang, E. H. (1982): Mechanism of activation of a human oncogene. *Nature*, 300:143–149.
121. Taparowski, E., Suard, Y., Fasano, O., Shimizu, K., Goldfarb, M., and Wigler, M. (1982): Activation of the T24 bladder carcinoma gene is linked to a single amino acid change. *Nature*, 300:762–764.
122. Temin, H. M., and Rubin, H. (1958): Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. *Virology*, 6:669–688.
123. Todaro, G., and Green, H. (1963): Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *J. Cell Biol.*, 17:299–313.
124. Todaro, G., and Green, H. (1964): An assay for cellular transformation by SV40. *Virology*, 23:117–119.
125. Topp, W., Lane, D., and Pollack, R. (1980): Transformation by polyoma and SV40. In: *DNA Tumor Viruses*, edited by J. Tooze, pp. 205–296. Cold Spring Harbor, New York.
126. Verderame, M., Alcorta, D., Egnor, M., Smith, K., and Pollack, R. (1980): Cytoskeletal F-actin patterns quantitated with fluorescein-isothiocynate phalloidin in normal and transformed cells. *Proc. Natl. Acad. Sci. USA*, 77:6624–6628.
127. Vogel, A., and Pollack, R. (1974): Isolation and characterization of revertant cell lines. VI. Susceptibility of revertants to retransformation by simian virus 40 and murine sarcoma virus. *J. Virol.* 14:1404–1412.
128. Vogel, A., Oey, J., and Pollack, R. (1974): Two classes of revertants isolated from SV40 transformed 3T3 mouse cells. In: *Control of Proliferation in Aminal Cells*, edited by B. Clarkson and R. Baserga, pp. 125–138. Cold Spring Harbor, New York.
129. Wigler, M., Pellicer, A., Silverstein, S., and Axel, R. (1978): Biochemical transfer of single-copy eukaryotic genes using total cellular DNA as donor. *Cell*, 14:725–731.
130. Yaffe, D. (1968): Retention of differentiation potentialities during prolonged cultivation of myogenic cells. *Proc. Natl. Acad. Sci. USA*, 61:477–483.
131. Yang, J., Richards, J., Gluzman, R., Imagawa, W., and Nandi, S. (1980): Sustained growth in primary culture of normal mammary epithelial cells embedded in collagen gels. *Proc. Natl. Acad. Sci. USA*, 77:2088–2092.