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Growth Factors, Tumor Promoters, and Cancer Genes 45–56

Growth Factors, Oncogenes, and Multistage Carcinogenesis

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This paper presents evidence that the full repertoire of cellular genes involved in the carcinogenic process is several times larger than that of the known list of proto-oncogenes. Furthermore, this repertoire includes genes whose normal function is related to growth stimulation, as well as genes whose normal function is to inhibit growth or induce terminal differentiation. Multistage carcinogenesis probably results from a complex series of changes in both categories of genes. Despite this complexity, carcinogenesis can be conceived in terms of disturbances in biochemical functions that normally control the expression or function of growth factors, receptors, and pathways of signal transduction. Several protein kinases play a central role in the process of signal transduction. Our laboratory has recently isolated cDNA clones for the enzyme protein kinase C (PKC). These clones should be useful for clarifying the role of PKC in growth control and tumor promotion. Finally, the existence of genes whose normal function is to inhibit cell growth provides a rationale for new strategies of cancer prevention and treatment.

Key words: protein kinases, protein kinase C, interferon, phorbol esters, tumor promotion, signal transduction, growth factors, oncogenes, multistage carcinogenesis

The development of tumors in humans and in a number of experimental animal models occurs through an extremely complex multistep process, which can occupy over one-half of the life span of the organism. These, and other characteristics of the carcinogenic process, predict that multiple cellular genes and multiple mechanisms are involved in the conversion of normal cells to fully malignant tumor cells [1,2]. It is obvious that chemical carcinogens, in contrast to viruses, cannot introduce new genetic information into target cells. They must, therefore, call upon and distort the function of normally present cellular genes. These considerations predicted, in a sense, the existence of "proto-oncogenes." Until recently, however, it was not clear how to identify the specific genes involved. Recent studies of the acute transforming retroviruses and DNA transfection procedures have now directly identified at least 30 proto-oncogenes [3,4] and have thus revolutionized our approach to the genetic basis of multistage carcinogenesis. The increasing evidence that these proto-oncogenes

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normally code for proteins that play a role in various stages of the action of growth factors [3–5] also provides an exciting unitary theme to the underlying physiologic mechanisms.

This symposium on "Growth Factors, Tumor Promoters, and Cancer Genes" will reveal recent advances in the areas of oncogene and growth factor research. In this introductory talk I would like to broaden the scope of the current paradigm by speculating about the number of growth factors and proto-oncogenes that normally exist, and how this diversity complicates our understanding of the carcinogenic process. I will then present recent findings from our laboratory on the cloning and sequence of the gene(s) for protein kinase C (PKC), an enzyme that plays a central role in signal transduction. Finally, I will consider the subject of genes and protein factors that inhibit growth, the relevance of negative control mechanisms to carcinogenesis, and the implications of negative control with respect to the design of new strategies of cancer therapy. The latter theme will, I trust, be of particular interest to those attending the symposium "Interferons as Cell Growth Inhibitors and Antitumor Factors."

SIGNAL TRANSDUCTION

It is apparent that in a multicellular organism the behavior of individual cells must be highly coordinated with that of others. This coordination is accomplished, in part, by the endocrine system, ie, the transmission of specific hormones between tissues. It is now apparent that another level of coordination is accomplished within tissues by paracrine mechanisms, ie, mechanisms involving growth factors and differentiation factors that are synthesized within tissues and that act at short ranges on neighboring cells [5,6]. At least 20 of these factors have already been identified, and each year several new ones are discovered [5]. Most of these factors are polypeptides, although it seems likely that certain prostaglandins, and other arachidonic acid derivatives, play analogous roles [10]. Figure 1 displays in schematic form how some of these extracellular signal molecules are perceived by cellular receptors, which are often located at the cell surface, and how the occupancy of these receptors leads to a cascade of signal transduction through the cytoplasm and eventually into the nucleus, thus altering patterns of gene expression. Figure 1 also emphasizes the central role that a series of protein kinase enzymes plays in this process of signal transduction. A major theme that has emerged is that the proto-oncogenes represent a subset of genes that normally code for components in these pathways of signal transduction. Alterations in the structure and function of these proto-oncogenes can convert them to "activated" oncogenes, which cause aberrations in signal transduction and thus disrupt normal growth, differentiation, and intercellular coordination.

There is now evidence that with platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, and certain lymphokines, receptor occupancy leads to activation of a tyrosine kinase domain present in the cytoplasmic portion of the receptor [5,7]. Another mechanism of signal transduction is exemplified by the beta-adrenergic system in which occupancy of the receptor by the agonist leads to activation of the enzyme adenylate cyclase, which is coupled to the receptor through G regulatory proteins [8]. The resulting increase in cytoplasmic cAMP then activates protein kinase A, a serine and threonine kinase [9]. The role of this pathway in growth control is not clear at the present time. It is possible that certain prostaglandins

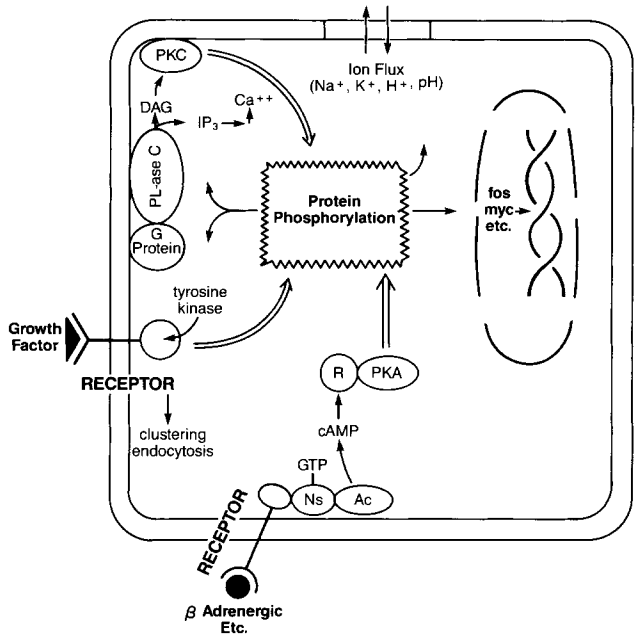

Fig. 1. A schematic diagram of a cell showing various pathways of membrane-associated receptors and signal transduction. The growth factor pathway applies to EGF, PDGF, and insulin. The beta-adrenergic pathway involves coupling via a G regulatory protein (Ns) to adenyl cyclase (Ac), and cyclic AMP (cAMP) binding to the regulatory subunit (R) of protein kinase A (PKA). Various agonists can activate phospholipase C (PL-ase C), presumably via a G protein, leading to the release of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). DAG activates protein kinase (PKC) and IP_3 causes the release of Ca^{++} from the endoplasmic reticulum. These events lead to a cascade of protein phosphorylation that alters the functions of membrane-associated receptors, ion channels, and cytoplasmic proteins. Signals (undefined) also enter the nucleus to induce the expression of various genes including *c-fos* and *c-myc* (see also Table I).

may mediate their effects via adenylate cyclase-coupled receptors [10], but this requires further study. Since in some cell systems increases in cellular cAMP can induce reversion of the transformed phenotype [11,12], the adenylate cyclase pathway may exert negative regulation (ie, inhibition) of growth. I shall return to the theme of negative regulation later. A third pathway of signal transduction involves the turnover of phosphatidylinositol and the activation of a phospholipid and Ca^{2+}-dependent serine and threonine protein kinase, designated protein kinase C (PKC) (for review, see 14). It would appear that PKC plays a central role in a variety of membrane-related signal transduction events [14]. This is because several agonists lead to the activation of a phospholipase C activity that hydrolyzes phosphatidylinositol 4,5 diphosphate to diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP_3) [13]. DAG then activates PKC [14], and IP_3 binds to receptors present on the endoplasmic reticulum (ER), causing the release of Ca^{2+} from storage sites in the ER [13,15]. The resulting increase in cytoplasmic Ca^{2+} then activates several calmodulin-dependent enzymes (protein kinases, phosphatases, phosphodiesterases), and also produces effects on the cytoskeleton. The fact that the tumor promoter 12-0-tetradecanoyl phorbol-13-acetate (TPA), and related tumor promoters, apparently act in place of DAG,

and thus usurp the function of PKC [14,16] provides a satisfying unity between the action of tumor promoters and the current conceptual framework of growth control.

The protein targets that become phosphorylated by the above-described protein kinases include receptors and membrane-associated ion channels [14]. Thus, there occurs a cascade of receptor-receptor cross-talk and a highly pleiotropic series of biochemical events. A major gap in our current knowledge is the mechanism by which signals are ultimately conveyed to the nucleus and how they act at the level of the chromatin. Rapid progress is, however, being made in elucidating the genes whose expression is increased in fibroblasts undergoing a mitogenic response to growth factors or tumor promoters [17]. This mitogenic program is described in Table I. Our laboratory has recently obtained cDNA clones for genes whose expression is induced or repressed by the tumor promoter TPA [18]. The gene that is repressed is, I think, of particular interest because it is very likely that the fine tuning of growth control is achieved through both increases and decreases in the expression of specific genes.

SPECULATIONS ABOUT THE NUMBER OF PROTO-ONCOGENES

It is of importance to consider, within the above model of cellular control mechanisms, the number of proto-oncogenes that exist in higher organisms, since this may define the magnitude and complexity of our endeavor to understand the evolution of the cancer cell at the genetic level.

I will define a "proto-oncogene" as a gene that normally plays a major role in the control of growth and/or differentiation and thus a gene that has the capacity, when mutated or "activated," to become an "oncogene," ie, a gene that contributes to the abnormal behavior of tumor cells. The current number of known oncogenes totals about 30 [3,4]. These were originally discovered in the acute transforming retroviruses, or in rodent or human tumors by the DNA transfection procedure. These methods continue to reveal new oncogenes at a rate of a few per year, so it is clear that the full repertoire has not been revealed.

Based on theoretical considerations, I would predict that the genomes of higher organisms contain several hundred proto-oncogenes. Vertebrates contain at least thirty cell types (neurons, glia, hepatocytes, renal cells, bronchial cells, mammary cells, lymphocytes, etc) [19]. It is likely that the growth of each of these cell types is under the control of several growth factors, since we know, for example, that lymphocytes are controlled by at least three lymphokines [5]. It is also clear that several factors are required for the growth and maturation of myeloid progenitors [5,20]. Thus multi CSF (IL-3) stimulates proliferation of granulocyte and macrophage lineages, M-CSF stimulates cells committed to the macrophage lineage, and G-CSF stimulates cells

TABLE I. The Mitogenic Program*

1. Altered ion flux and increased cellular pH
2. Increased transport of glucose and other nutrients
3. Increased turnover of phospholipids (activation of phospholipases A_2 and C)
4. Increased activity of tyrosine kinases, protein kinase C, and protein kinase G
5. Increased mRNA for *fos*, *myc*, *actin*, ornithine decarboxylase, MRP, and various cDNA clones

*Inducers of this program in specific cell types include TPA, EGF, PDGF, serum. For review see [17].

committed to the granulocyte lineage. Erythropoetin plays a role in erythroepoesis, and thrombopoetin plays a role in platelet formation. Thus, at least six distinct factors are involved in controlling the growth and maturation of myeloid elements. It seems likely, therefore, that multiple paracrine factors are also involved in controlling the growth and maturation of specific cellular elements in other tissues. Indeed, there is evidence for the existence of a series of glia-promoting polypeptide factors (GPFs), which differ in their specificities for oligodendrocytes and astrocytes [21].

It is not unreasonable, therefore, to assume that at least three growth factors are involved in controlling the growth and differentiation of each cell type. Each growth factor, in turn occupies a specific receptor, which might also be considered a proto-oncogene. For example, the proto-oncogene *fms* apparently encodes the CSF-1 receptor [20]. It is also reasonable to assume that the pathway of signal transduction through the cytoplasm, for each receptor, is mediated by at least three additional gene products. Finally, I assume that in the nucleus there exist at least three specific transcription factors that control the expression of the responsive gene(s). Taken together, this suggests that normal cells contain about 360 proto-oncogenes.

Other considerations also suggest that the list of proto-oncogenes is large. 1) Of the approximately 50,000 genes per mammalian cell it seems reasonable that nature has committed at least 1% of these genes (ie, 500) to the control of growth. 2) New growth factors and differentiation factors continue to be discovered. 3) There is increasing evidence that some of the known proto-oncogenes belong to families; the *myc* family includes *c-myc, N-myc,* and *L-myc* [22]; the EGF receptor gene has a homolog, the *c-neu* or *c-erbB-2* gene [23]; and the *ras* family includes *H-ras*, *K-ras*, *N-ras*, and others [4,24]. Studies with the *myc* family suggest that individual members display tissue-specific expression and tissue-specific activation during carcinogenesis [22]. If all of the proto-oncogenes belong to families of at least three per family, this alone would almost triple the number of known proto-oncogenes. 4) Genes that code for receptors or components of the signal transduction pathways for some of the more conventional hormones, such as the glucocorticoids, estrogens, androgens, progesterones, prolactin, and prostaglandins, can also be considered proto-oncogenes, since aberrations in these genes could contribute to the autonomous growth of tumors. In this context it is of interest that the viral oncogene *erb A* shares homology with steroid receptors [25].

Later I will discuss the evidence that there also exists a repertoire of growth inhibitor genes whose normal function is to inhibit growth and/or induce terminal differentiation. Mutations in these genes could also lead to disturbances in growth control. Furthermore, the mammalian genome contains thousands of copies of long terminal repeat (LTR)-like sequences, and alterations in the function or state of integration of these genes could also produce disturbances in growth control [2].

The evidence presented above suggesting that at least several hundred genes may play a role in growth control and differentiation, and that this multitude of genes provides a large repertoire of potential oncogenes, might cast pessimism on our ability to identify all of the oncogenes that contribute to the phenotypes of human tumors. There are, however, at least three reasons to be optimistic. The first is that, despite the apparently large repertoire of proto-oncogenes, it would appear that certain members of this repertoire (ie, *ras* and *myc*) become activated preferentially or are strongly selected for during carcinogenesis [4,26]. This might reflect the ease with which activation can occur. For example, a single base substitution in the 12th or 61st

codon of a *ras* proto-oncogene will cause its activation [26], and increased expression of *c-myc* or *N-myc* will activate these genes. In contrast activation of *c-src*, which thus far has not been found to occur in spontaneous rodent or human tumors, might require multiple changes, since its structure differs in several respects from *v-src* [3,4]. A second reason for optimism is that, although there may be a few hundred cellular proto-oncogenes, it is likely that they can be divided into a few categories (perhaps four or five) in terms of their structural homologies and mechanisms of action [3,4]. This greatly simplifies the identification of new proto-oncogenes and our understanding of the mechanisms by which they contribute to the tumor cell phenotype. It also gives rise to the hope that research in this area will provide general strategies of therapy, ie, therapy tailored to a class of oncogenes rather than to each specific oncogene.

STUDIES ON PROTEIN KINASE C (PKC)

I would now like to discuss recent data from our laboratory related to PKC. Because of the central role of PKC in signal transduction, growth control, and tumor promotion [14,16], our laboratory has recently studied the function of this enzyme and cloned the related DNA sequences. Figure 2 presents a hypothetical diagram of PKC emphasizing the fact that the enzyme has two domains: an active site, containing an ATP binding site and the region to which protein substrates bind, and a regulatory domain whose activity is controlled by lipid, Ca^{2+}, and DAG or by lipid and TPA. We hypothesize that the usual function of the regulatory domain is to inactivate the enzyme, by "closing" the catalytic site, and that the binding of appropriate factors to

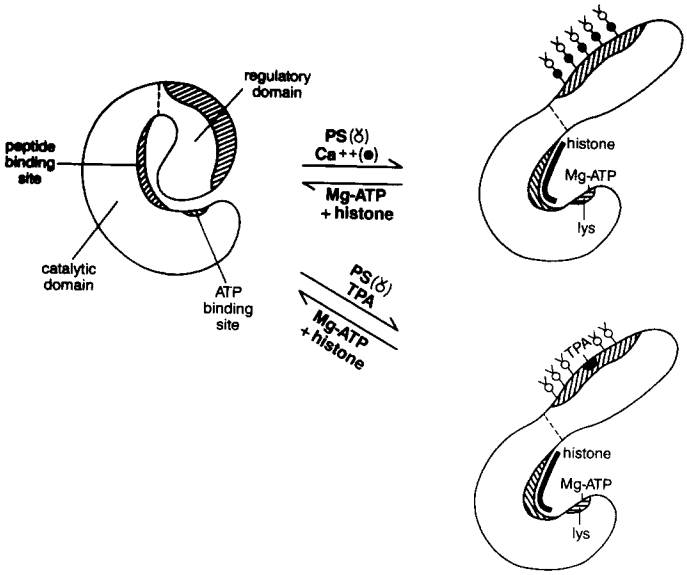

Fig. 2. A hypothetical model of protein kinase C emphasizing a catalytic domain that contains the ATP and peptide substrate binding sites and a regulatory domain that binds phosphatidylserine (PS), and Ca^{++}, or PS and TPA. We postulate that binding of PS plus Ca^{++} or PS plus TPA to the regulatory domain induces a conformational change in the enzyme that "opens" the catalytic domain, thus activating the phosphorylation of a protein substrate, for example, histone H1.

the regulatory domain induces a conformational change that "opens" the catalytic site and thus activates enzyme function. Consistent with this scheme is evidence that limited proteolysis of the enzyme yields a fragment of about 66 kD that is active in the absence of lipid and cofactors [14]. In addition, there exist inhibitors of PKC that appear to act preferentially on the regulatory domain or the catalytic domain [16]. The development of pharmacologic agents that specifically inhibit PKC could provide a novel and nonmutagenic strategy of cancer chemoprevention and cancer chemotherapy.

Molecular studies on PKC would be tremendously enhanced by having available the cloned genes for this enzyme. We have, therefore, purified the enzyme from rat brain by ammonium sulphate precipitation and a series of column purification steps. As a final step in the purification we allowed the partially purified enzyme to undergo autophosphorylation and then purified the ^{32}P-labeled protein by gel electrophoresis. The homogenous protein obtained from the gel (82 kD) was then reduced, carboxymethylated, and cleaved with the protease endolyse C to yield a series of polypeptides. These were separated by high-pressure liquid chromatography, and the amino acid sequences of a few of these polypeptides were then determined by microsequencing techniques. A peptide, designated P2, has the sequence Ser-Val-Asp-Trp Trp-Ala-Phe-Gly-Val-Leu-Leu-Tyr-Glu-Met-Leu-Ala-Gly-Glin. It was particularly useful since it is 18 amino acids long and contains two adjacent tryptophan residues. A 53-base pair oligonucleotide probe whose sequence corresponds to the predicted coding sequence for P2 (based on mammalian codon usage frequencies and codon degeneracy) was then synthesized and used to probe a rat brain cDNA library. Several homologous clones have been obtained, and we are now determining their complete nucleotide sequences [27].

The sequences that we have obtained display rather striking homologies to other protein kinase genes. Thus the sequence of the P2 peptide shares strong homology with a region present in the catalytic domain of protein kinase A. We have also identified an ATP binding site sequence, as well as several other sequences, that are homologous to sequences found in several protein kinases. As expected, in each of these regions the PKC clones show more homology to the serine and threonine kinases than to the tyrosine kinases. In a recent review [7], Dr. Tony Hunter has stressed the evolutionary relationship between several protein kinases. Our studies with PKC extend this theme. In several protein kinases, the catalytic domain is located in the carboxyterminal end of the molecule, whereas the regulatory domain is at the amino terminal end or is a separate protein subunit. Thus it would appear that during evolution the amino terminal end of these proteins has diverged, and in some cases has become a separate polypeptide chain, so as to provide regulation of protein kinase activities by diverse agonists. Depending on the particular kinase, the regulatory domain is responsive to cAMP in the case of protein kinase A, to cGMP in the case of protein kinase G, to Ca^{2+} in the case of the myosin light-chain kinase (which is regulated by calmodulin), and to EGF in the case of the EGF receptor. We plan studies to determine whether deletion of the regulatory domain of PKC by recombinant DNA methods will, following transfection into mammalian cells, produce a protein that is autonomous and, therefore, cause disturbances in growth control. These studies will test the possibility that PKC can function as a proto-oncogene during carcinogenesis.

We have used our cDNA PKC clones from rat brain as probes to analyze the poly A^+ RNAs obtained from rat brain, heart, and liver, using Northern blot

hybridizations. The highest levels of PKC mRNA were found in brain, with much lower levels in heart and liver. This reflects the relative levels of PKC activity observed in these tissues [14]. It is of interest that one of our PKC clones hybridizes to a single RNA species that is 7.5 kb, whereas a second clone hybridizes to two RNA species, one of 3.5 kb and the other of about 9 kb. We are currently comparing the nucleotide sequences of these two clones and find that there are subtle differences. These results suggest that there may be more than one gene for PKC and multiple forms of PKC enzyme, but this requires further study. Consistent with this possibility is the fact that our purified PKC protein displays a doublet profile on gel electrophoresis, and other laboratories have also obtained evidence for multiple species of PKC [28,29] (T. Hunter et al, personal communication). If subsequent studies verify the existence of more than one gene for PKC, then different forms of the enzyme could account for some of the tissue-specific and pleiotropic effects of this enzyme.

As I mentioned earlier, a major gap in our knowledge of the pathway of signal transduction is the question, how are signals conveyed from the cytoplasm to the nucleus? In the case of PKC it seems unlikely that the enzyme itself is translocated to the nucleus because attempts to demonstrate significant levels of the enzyme in the nucleus, using either TPA tagged with a fluorescent dansyl residue [30] or immunofluorescence with PKC antibody [31], have been negative. Another unsolved problem is how, during the process of tumor promotion, the imprint of a tumor promoter such as TPA eventually becomes fixed, so that cells remain abnormal even when the tumor promoter is no longer applied. If tumor promoters such as TPA act simply through activation of PKC, then one would expect cells to revert to their previous state once the promoter is removed.

We have found that when mouse [32] or rat fibroblast [33] cell lines are transfected with an activated H-*ras* oncogene and are grown in the presence of TPA, the tumor promoter markedly enhances the yield of transformed foci. This finding has been confirmed and extended to early passage rodent cells [34]. Since the cells obtained from these foci remain transformed even in the absence of TPA, this system may be useful for analyzing the mechanism underlying stable effects of TPA. A recent study [35] indicating that PKC selectively phosphorylates the DNA methyltransferase enzyme raises the intriguing possibility that the imprinting may be produced via an alteration in patterns of DNA methylation that influence gene expression. This would assume that PKC or a catalytically active fraction of the enzyme does enter the nucleus, or that the methyltransferase is phosphorylated by PKC in the cytoplasm before the methyltransferase enters the nucleus. Alternatively, prolonged activation of PKC might activate pathways related to the production of activated forms of oxygen [36] or alter cellular levels of poly ADP ribose [37], and thus produce stable effects on DNA or chromatin structure.

GROWTH INHIBITOR GENES AND NEW STRATEGIES OF CANCER THERAPY

Finally, I want to discuss the likelihood that, in addition to genes that code for stimulatory growth factors (such as EGF and PDGF) and activated oncogenes that cause aberrant cell growth, there exists a reciprocal set of genes whose products inhibit cell growth and/or induce cells to undergo terminal differentiation. I shall refer to these genes as "growth inhibitor genes" although there is evidence that the

products of specific genes can also induce terminal differentiation [38]. A priori, it seems likely that nature employs both growth stimulator and growth inhibitor genes to achieve the complex control that is required for normal growth, development, and differentiation. In the case of growth stimulator genes (or proto-oncogenes), mutations that result in activated oncogenes exert a dominant effect on growth. On the other hand, mutations that *inactivate* growth inhibitor or differentiation genes (by, for example, deletions) would lead to growth stimulation, and/or failure of terminal differentiation. Such mutations would be recessive since it would be necessary to inactivate the function of both alleles for tumors to occur, and replacement of the normal gene product would correct the defect. Tumors could result from either mechanism or, as seems most likely in terms of a multistep-multigene model of carcinogenesis, from a distortion in the net balance in function of both types of genes. Cell-cell hybridization studies lend support to the latter formulation [38,39].

Genetic studies provide strong evidence for the existence of genes that inhibit growth and modulate differentiation. In *Drosophila*, recessive mutations in at least 24 different genes can produce tumors of various types [for review, see 40]. It is of interest that all of these genes normally play a role in development. When the mutant allele is homozygous it is often lethal, because development is arrested at a specific stage. Thus, in flies homozygous for the mutation *giant larvae* there is an arrest in the development of the presumptive optic centers of the larval brain so that ganglion precursor cells continue to proliferate, eventually giving rise to neuroblastomas. The hereditary forms of human retinoblastoma and Wilm's tumor provide evidence for similar genes in humans [41,42]. Table II lists other evidence for growth inhibitor genes. Other authors [41–46] have emphasized this theme and refer to this category of genes as suppressor genes or "anti-oncogenes" [41–46], although it is not clear that these genes act simply by directly inhibiting the function of proto-oncogenes.

Except for the *Drosophila* gene *giant larvae* [47], none of these growth inhibitor genes has been cloned. A major challenge is to isolate these genes and elucidate their mechanisms of action. Until this is achieved, studies on proto-oncogenes and oncogenes will provide only a portion of the full repertoire of genes that underlie the carcinogenic process. Within the context of the signal transduction model displayed in Figure 1, what might be the biochemical functions of the proteins encoded by these putative growth inhibitor genes? I suggest that these might include: 1) protein phosphatases; 2) protein kinases that have effects that oppose those of PKC and the tyrosine protein kinases, for example, protein kinase A; 3) phospholipase inhibitors, for example, lipocortin or related inhibitors that inhibit specific phospholipases; 4) transcription control factors that suppress rather than enhance the transcription of specific genes involved in cell proliferation, for example, oncogenes; 5) translation control factors that inhibit the translation of specific mRNAs; and 6) genes that induce

TABLE II. Evidence for Growth Inhibitor Genes ("Anti-Oncogenes")*

1. Fusion of normal with malignant cells suppresses malignancy
2. Certain hereditary human tumors (Retinoblastoma and Wilm's tumor) are associated with chromosomal *deletions* and loss of both alleles in the tumor
3. In *Drosophila* there exist recessive mutations in over 20 loci that predispose to developmental abnormalities and malignancy
4. In fish hybrids the absence of a gene that controls melanocyte differentiation causes melanomas

*For review see [41–46].

terminal differentiation by mechanisms that are not well understood at the present time. Presumably growth inhibitor factors, such as TGF-beta or specific interferons (see below), act through one or more of these biochemical mechanisms. Some of these factors may be lipids rather than polypeptides since there is evidence that specific prostaglandins can inhibit the growth of tumor cells or induce differentiation [10]. TGF-beta provides an example of a factor that can either inhibit or stimulate cell growth depending on the cell type [5]. Obviously, decreased production of a growth inhibitor or aberrations in its receptor could lead to abnormal cell proliferation and/or failure of normal development and differentiation.

The existence of growth inhibitory genes suggests, of course, new strategies for cancer prevention and treatment, by employing agents that mimic the action of such genes or actually induce their expression. There already exist several agents that appear to have such effects since they can, in appropriate cell systems, inhibit the growth of tumor cells and/or induce such cells to differentiate. These agents include: 1) retinoids, 2) vitamin D derivatives, 3) glucocorticoids, 4) dimethylsulfoxide (DMSO) and hexamethylene bis acetamide (HMBA), 5) cyclic AMP, 6) butyrate, 7) TPA and mezerein, 8) TGF-beta, and 9) specific interferons [for review see 5,38,48]. The growth inhibitory effects of the latter two substances will be discussed in considerable detail at these meetings.

My colleagues and I have been intrigued with the ability of the interferons to inhibit the growth and induce the differentiation of certain human tumor cell lines [48]. We have also found that these effects are markedly enhanced by the combination of interferon with either retinoids or mezerein [48]. It has been demonstrated that the growth suppression induced by treatment with interferon is associated with decreased expression of ornithine decarboxylase (ODC), *c-myc*, and *c-fos*, as well as increased expression of the 2′5′-oligoA synthetase [49]. Curiously, these events are then followed by increased production by these leukemic cells of an endogenous interferon. It is possible, therefore, that the normal role of specific interferons is to inhibit cell growth and/or modulate cellular differentiation. In this sense, specific interferon genes might normally function as "anti-oncogenes."

Obviously much more work remains to be done, but I am confident that the exciting papers that will be presented during the coming week at the parallel conferences on "Growth Factors, Tumor Promoters, and Cancer Genes" and "Interferons as Cell Growth Inhibitors and Antitumor Factors" will contribute insights into the multigenic basis of cancer as well as suggest new strategies for cancer prevention and treatment.

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NOTE ADDED IN PROOF

Since the preparation of this manuscript four other groups have also reported the isolation and analysis of cDNAs encoding protein kinase C (PKC) (Ono Y, et al: FEBS Lett 203:111–115, 1986; Knopf JL, et al: Cell 46:491–502, 1986; Parker PJ: Science 233:853–858, 1986; Coussens L: Science 233:859–866, 1986). Their results and additional data from our laboratory (Housey MD, et al: Proc Natl Acad Sci USA, in press) provide convincing evidence that PKC belongs to a multigene family. On the subject of recessive genes involved in cancer, a recent publication describes a human DNA segment that appears to correspond to the gene that predisposes to retinoblastoma and osteosarcoma (Friend SH, et al: Nature 323:643–645, 1986).