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ONCOGENES

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INTRODUCTION

In the last few years the study of oncogenes has considerably advanced our understanding of the molecular mechanisms leading to cancer. Oncogenes may be defined as genes in which alterations to expression or coding potential are essential steps in neoplastic transformation. Much of the impetus for the study of oncogenes is derived from the farsightedness of those virologists who studied transforming viruses and argued that the ways in which these viruses caused transformation would mimic mechanisms in tumours where there was no viral involvement. Since the field of oncogene research has been extensively reviewed (see, e.g. Bishop, 1985; Varmus, 1984; Marshall, 1985), it is not the purpose of this review to catalogue the accumulated evidence for the role of oncogenes in tumorigenesis. I wish to discuss the viewpoint that oncogenes encode proteins that are intimately concerned in the control of cell proliferation *via* growth factors, their receptors, and mechanisms that may be involved in the transduction of signals from receptors.

That such a relationship might exist became initially apparent from observations that some oncogene proteins encoded a tyrosine kinase activity like the receptors for growth factors such as insulin, somatomedin C and platelet-derived growth factor (PDGF). More-direct links arose from the demonstrations that a viral oncogene (v-*sis*) (Doolittle *et al.* 1983; Waterfield *et al.* 1983) was derived from a normal cellular gene that encodes a chain of the platelet-derived growth factor and that a second viral oncogene (v-*erb*B) is a truncated form of the gene encoding the epidermal growth factor receptor (Downward *et al.* 1984). Subsequent evidence has shown that two more oncogenes, v-*fms* and *neu*, are probably derived from genes that encode growth factor receptors (Sherr *et al.* 1985; Schechter *et al.* 1985).

Analysis of the biochemical properties of the proteins encoded by viral oncogenes and their cellular counterparts enables them to be classified into distinct groups (Table 1). These groups are growth factors, tyrosine kinases, serine kinases, GTP binding, nuclear localized and finally those with unknown properties. While cellular oncogenes were initially recognized as being the cellular homologues of viral oncogenes, it is clear that there are also genes that could function as oncogenes that have not been associated with viral oncogenesis. These genes include those recognized by functional assays similar to those that identified the homologues of viral oncogenes, e.g. N-*ras*, *neu*. But also include those that encode growth factors and their

receptors. These molecules may be proposed as potential oncogenes on the basis of their biological properties.

GROWTH FACTORS, RECEPTORS AND ONCOGENES

Although there are now examples in which growth factors appear to be produced by the same cells on which they act (Seifert, Schwartz & Bowen-Pope, 1984; Walker, Bowen-Pope & Reidy, 1984), in other cases growth factors are produced and exported by one cell type to act on another. Clearly, one way in which cell proliferation control can be interfered with is for there to be an uncontrolled synthesis of the growth factor by the target cell itself. Such a mechanism is termed 'autocrine' and has the result that the cells no longer require a growth factor to be supplied externally (Sporn & Todaro, 1980). However, this is only one point for alterations in the pathway of growth-factor-stimulated cell proliferation. Alterations may occur in receptors so that they no longer need to bind growth factors to generate a mitogenic signal. Cells may become more sensitive to, or independent of, growth factors by either changes in the receptors or in effector molecules after the immediate ligand–receptor interaction. Finally, cells may begin to synthesize receptors that they do not normally make and thereby respond to a different set of growth factors. All of these mechanisms could result in altered proliferation signals and some specific examples will now be discussed in detail.

Table 1. *Classification of oncogene proteins*

Growth factor	*Sub-cellular localization of protein*
sis	Extracellular/membrane (PDGF)
Tyrosine kinase	
src	Plasma membrane
yes	Plasma membrane
fes/fps	Plasma membrane
ros	Plasma membrane
abl	Plasma membrane
fgr	Plasma membrane
fms	Plasma membrane (CSF-1 receptor)
*erb*B	Plasma membrane (EGF receptor)
neu	Plasma membrane
met	Plasma membrane
Serine kinase	
raf/mil	Cytoplasm
mos	Cytoplasm
GTP binding	
N, H, K-*ras*	Plasma membrane

Nuclear		*Unknown*
myc	Nuclear	*ets*
myb	Nuclear	*erb*A
fos	Nuclear	*ski*
p53	Nuclear	

*Autocrine stimulation of growth PDGF/c-*sis, TGFα, *bombesin*

Platelet-derived growth factor is a potent mitogen for connective tissue cells such as fibroblasts, smooth muscle and glial cells (Ross, Glomsett, Kariya & Harker, 1974; Scher, Shepard, Antoniades & Stiles, 1979; Heldin, Westermark & Wasteson, 1971). Other cell types do not appear to express PDGF receptors and so cannot respond to PDGF. While PDGF has to be supplied exogenously to stimulate the growth of most connective tissue cells, it now appears that PDGF is found not only in platelets but also in normal cells such as the cytotrophoblast (Goustin *et al.* 1985) and cultured aortic smooth muscle cells from neonatal rats (Seifert, Schwartz & Bowen-Pope, 1984). Thus in these situations there may be a natural autocrine stimulation of growth. The demonstration that v-*sis* encodes a molecule highly related to PDGF (Doolittle *et al.* 1983; Waterfield *et al.* 1983) was the first direct evidence of how oncogenes might be linked to growth control. Before these observations were made, Eva *et al.* (1982) had shown that connective tissue tumours such as sarcomas and glioblastomas expressed c-*sis*, whereas normal tissue did not. Thus these tumour cells appear to synthesize a mitogen to which they can respond. While it has become clear that v-*sis* is derived from a gene that encodes the B chain of PDGF (Chiu *et al.* 1984), the origin of the A chain is still not resolved.

The mechanism by which c-*sis* expression is activated in tumour cells has not been analysed. However, it is clear that when a c-*sis* cDNA is expressed in NIH-3T3 cells from a foreign promoter, it is sufficient to transform the cells morphologically and cause anchorage-independent growth (Clarke *et al.* 1984). This result presents a paradox because high levels of PDGF supplied exogenously do not result in anchorage-independent growth of NIH-3T3 cells. Thus it is the internal synthesis of PDGF-like molecules that leads to transformation. While insertion into membranes *via* a leader sequence appears to be essential for transformation (Hannink & Donoghue, 1984), in some systems secretion into the medium does not (Robbins, Leal, Pierce & Aaronson, 1985). However, in other experiments secretion of the v-*sis* PDGF-like product appears to be essential for the maintenance of the transformed phenotype because addition to the medium of antibodies against PDGF results in partial phenotypic reversion (Johnsson, Betsholtz, Heldin & Westermark, 1985). Since both the *sis* product and the PDGF receptor are synthesized in the endoplasmic reticulum and pass to the Golgi apparatus, it is possible that the critical interaction between growth factor and receptor may occur before they reach the cell membrane.

While the production of c-*sis*/PDGF by sarcomas and glioblastomas could clearly function as an autocrine loop, it is unclear why certain tumour types such as some carcinomas (Bowen-Pope, Vogel & Ross, 1984) and lymphomas should synthesize PDGF-like molecules. Epithelial and lymphoid cells do not appear to express PDGF receptors and therefore cannot respond to the growth factor they are synthesizing. Expression of c-*sis* in such tumours may reflect disturbances in gene regulation following a neoplastic transformation and have no functional significance. However, a more exciting prospect is that these tumour cells produce PDGF-like molecules in

order to influence surrounding stromal cells with PDGF receptors, perhaps to produce other growth factors necessary for tumour growth.

Although v-*sis* is the only viral oncogene that has been shown to be derived from a cellular gene encoding a growth factor, two other autocrine systems in tumours have been described in some detail. These systems are the production of transforming growth factor α (TGFα) by a number of tumour types and of bombesin in small cell carcinomas of the lung.

TGFα is a peptide that is structurally related to epidermal growth factor (EGF) and is released from virally transformed rodent fibroblasts (v-*ras*, v-*abl*, v-*mos*, v-*fes* transformants) and from human carcinomas (Todaro, Delarco & Cohen, 1976; Todaro, Fryling & Delarco, 1980). TGFα acts *via* the EGF receptor, thus only cells that express this receptor are susceptible to TGFα and a diagnostic characteristic of cells expressing high levels of TGFα is down-regulation of the EGF receptor. The mechanism by which some transformed cells express TGFα remains obscure. TGFα is not detectable in most normal adult tissues, with the possible exception of platelets (Assoian, Grotendorst, Miller & Sporn, 1984), and appears to be synthesized for only a short period of embryogenesis (Twardzik, Ranchalis & Todaro, 1982). Even in tumours the levels of TGFα mRNA expression appear to be very low (Derynck *et al.* 1984). Although the observation that rodent fibroblasts transformed with viral or cellular *ras* genes release TGFα suggests that human tumours that harbour a mutant transforming *ras* gene might express TGFα, this does not seem to be true. Two human tumour cell lines (RD, rhabdomyosarcoma; EJ, bladder carcinoma) with activated *ras* genes do not make detectable TGFα (B. Ozanne & C. J. Marshall, unpublished results), thus the presence of a *ras* oncogene in a tumour is not synonymous with TGFα production. While it has been possible to show by cell growth in serum-free medium that the release of TGFα functions as an autocrine in virally transformed rodent cells (Kaplan, Andersson & Ozanne, 1982), this has not yet been demonstrated in human tumour systems.

However, in a second human tumour growth factor system, autocrine stimulation has clearly been demonstrated. Cutita *et al.* (1985) have shown that in small cell carcinoma of the lung where bombesin-like peptides are produced by the tumour cells, cell growth can be inhibited both *in vitro* and *in vivo* by antibodies against bombesin. Not only do these results show an autocrine stimulation of growth, but also that antibodies against growth factors or their receptors may be of use therapeutically.

Alterations to growth factor receptors

The demonstration that a growth factor gene might function as an oncogene was the first direct indication of how oncogenes might be linked to growth control. The next step was to show that a second viral oncogene, v-*erb*B, was derived from the cellular gene encoding a growth factor receptor (Downward *et al.* 1984). A number of mechanisms have now been described by which activation of a growth factor receptor could lead to that molecule functioning as a transforming protein. These

mechanisms are structural modifications of receptor protein, overexpression and possibly abnormal or ectopic expression. In different cases, such modifications may lead to independence from exogenous growth factor or increase sensitivity to growth factors.

To date, the only clear examples of a structural modification of a growth factor receptor leading to transformation are the ways in which viruses affect the *erb* B locus to cause erythroblastosis in chickens. This locus is either transduced by the two avian erythroblastosis viruses ES4 or AEV-H or disrupted by the insertion of a non-transforming virus (Nilsen *et al.* 1985). In both situations the result is the ectopic expression in erythroblasts of a modified EGF receptor truncated at the amino terminus. Truncation results in loss of the ligand binding domain and leads to constitutive activation of the biochemical activity of the EGF receptor. Why such an activated EGF receptor should function as an oncogene in erythroblasts remains unanswered. The EGF receptor is not expressed in normal erythroblasts so transformation cannot result from the cells being provided with an activated form of a receptor that they normally use. Presumably other growth-factor receptor systems in erythroblasts must generate similar biochemical signals to the EGF receptor, probably involving tyrosine kinase activity, and it is the ectopic and unregulated generation of these signals that results in transformation. Although such considerations provide an explanation of how truncated EGF receptors could cause transformation, they do not adequately account for why transformation is targeted to erythroblasts.

To date, N-terminal truncated forms of the EGF receptor similar to v-*erb*B have not been identified in human tumours. Truncated forms of the EGF receptor are found in human tumours, but here the truncation is before the transmembrane region so that cells secrete an amino-terminal EGF-binding fragment (Ullrich *et al.* 1984). Since such molecules lack a transmembrane region and cytoplasmic catalytic domain, they are unlikely to function directly as transforming proteins. Rather than truncation, overexpression of a presumably normal EGF receptor appears to be involved in human tumours. A sizeable fraction of glioblastomas (Libermann *et al.* 1985) and a majority, of perhaps all, of squamous cell carcinomas express high levels of EGF receptors (Ozanne *et al.* 1985; B. Ozanne, personal communication). Such tumour cells have between 10^6 and 15×10^6 receptors compared with 10^5 receptors in normal cells. At least in some cases overexpression of the receptors results from receptor gene amplification (Libermann *et al.* 1985; B. Ozanne, personal communication). How overexpression of the receptor acts as a transforming event remains unclear. One possibility is that when large amounts of the receptor are present, the background 'firing' of the receptor occurs at a sufficiently high level for cells to be triggered into mitosis. Alternatively, high levels of the receptor may sensitize cells to very low levels of growth factor. In this context it is intriguing that some cells with high levels of the EGF receptor produce TGFα (Derynck *et al.* 1984; B. Ozanne, personal communication). Thus these cells may be driven by an autocrine loop, but an autocrine loop that involves a sensitization to growth factor by expressing high levels of the receptor.

Although the truncation of the EGF receptor appears to generate a viral oncogene in the case of v-*erb*B, in a second example of a viral oncogene, v-*fms*, constitutive activation of the receptor by truncation may not be involved. The c-*fms* gene appears to encode the receptor for CSF-1, the growth factor for mononuclear phagocytic cells (Sherr *et al.* 1985). Structural studies suggest that v-*fms*, unlike v-*erb*B, retains an extensive N-terminal external domain and therefore may retain the ligand binding domain. However, the fact that fibroblasts, the target cell for v-*fms* transformation, produce CSF-1 suggests that the mechanism of transformation may be for cells to be provided with the receptor for a growth factor that the cells normally make but do not respond to. This would imply that v-*fms*-expressing cells need to be exposed to CSF-1 to be transformed.

A third example of how oncogenes may bypass growth factor requirements is provided by v-*abl*. Infection of IL3-dependent mast cells (Pierce *et al.* 1985) or an IL3, GM-CSF-dependent myeloid cell line (Cook *et al.* 1985) results in factor-independent cells. This factor independence does not appear to arise from an autocrine stimulation of growth resulting from the v-*abl* gene stimulating the cells to produce factors. Neither is factor independence the consequence of an increased level of receptors. While it has not yet been demonstrated that the c-*abl* protein is part or the whole of a receptor, transformation by v-*abl*, like v-*erb*B, may involve a constitutionally activated tyrosine kinase activity. Thus growth factor independence appears to result from the v-*abl* protein mimicking a growth factor signal.

Secondary messenger systems after growth factor stimulation: the role of nuclear oncoproteins

Four viral oncogenes (v-*myc*, v-*myb*, v-*fos* and v-*ski*) and their cellular homologues encode proteins that are localized in the nucleus (Bishop, 1985). A fifth protein, p53, which was initially recognized because it complexed with the simian virus 40 (SV40) large T antigen (Rotter & Wolf, 1985) is also nuclear and appears to be able to function as an oncogene (Jenkins, Rudge & Currie, 1984; Parada *et al.* 1984; Eliyahu *et al.* 1984). In three of these genes that have been studied, c-*fos*, c-*myc* and p53, expression as judged by RNA levels appears to be elevated following the stimulation of quiescent fibroblasts (Kelly, Cochran, Stiles & Leder, 1983; Greenberg, & Ziff, 1984; Kruijer, Cooper, Hunter & Verma, 1984; Muller, Bravo, Burkhardt & Curran, 1984; Reich & Levine, 1984). However, the kinetics of induction vary for the three genes. The induction of c-*fos* is very rapid, reaching a peak within 30 min and then rapidly falling away (Greenberg & Ziff, 1984; Kruijer *et al.* 1984; Muller *et al.* 1984); c-*myc* reaches a peak level of RNA expression at around 2 h post-stimulation and then falls away after 4 h (Kelly *et al.* 1983), while p53 expression reaches a peak 18–24 h after stimulation (Reich & Levine, 1984). Furthermore, there appear to be differences in the way these levels of changed RNA expression are achieved. For c-*fos* the control appears to be transcriptional (Greenberg & Ziff, 1984), while for c-*myc* there may be both a transcriptional and a post-transcriptional component at the level of mRNA stability (Blanchard *et al.* 1985).

While the kinetics and mechanism of induction may vary, both the mRNAs and proteins encoded for c-*fos*, c-*myc* and p53, are all characterized by having short half-lives of the order of 20–25 min (Ramsay, Evan & Bishop, 1984; Reich & Levine, 1984; Klempanauer, Symonds, Evan & Bishop, 1984; Dani *et al.* 1984). The induction kinetics and the short half-lives have been used to argue that the nuclear proteins function as effectors to link events at the cell surface to nuclear events, such as changes in gene transcription and the initiation of DNA synthesis. The constitutive expression of c-*myc* relieves, at least partially, the requirement for growth factors (Armelin *et al.* 1984; Rapp *et al.* 1985) or sensitizes cells to growth factors (Roberts *et al.* 1985). Furthermore, it has been shown in some cases that the relaxation of growth factor requirements is not a result of the cells producing growth factors but is bypassing the need for growth factor stimulation (Rapp *et al.* 1985). At present there is no clear indication of how the nuclear proteins act as a link between nucleus and membrane, but one obvious mechanism is that they somehow affect the expression of other genes.

The elevated level of expression of c-*myc* following mitogenic stimulation of quiescent cells led to the idea that c-*myc* expression might be regulated during the cell cycle (Kelly *et al.* 1983). It was also argued that alterations to the expression of c-*myc* in the cell cycle might be a common consequence of the varied changes to the c-*myc* gene that result from specific chromosome translocations in B-lymphoid cells. Subsequent experiments, however, have convincingly shown that in exponentially growing cells, the level of c-*myc* expression at both the RNA and protein levels remains constant throughout the cell cycle (Hann, Thompson & Eisenman, 1985; Thompson, Challoner, Neiman & Groudine, 1985). These observations in the cell cycle of growing cells raise the question of why there is a pulse of elevated *myc* RNA following stimulation of quiescent cells. Although this question is not fully resolved, some experiments do suggest that the level of *myc* expression is somewhat lower in quiescent cells (Keath, Kelakar & Cole, 1984*a*). However, the rise in c-*myc* RNA following stimulation appears to be much higher (up to 40-fold) than the few-fold difference in levels between quiescent and growing cells. One explanation of the elevated RNA levels is that following stimulation there is a requirement for a more rapid turnover of c-*myc* protein, perhaps because c-*myc* protein functions as a nuclear message system. As far as the other nuclear proteins, c-*myb*, c-*fos* and p53, are concerned, there is at present no strong indication as to whether their expression is regulated in the cell cycle.

Nuclear oncoproteins and cell immortilization

The observation that some malignant cell lines with alterations to c-*myc* genes also contained mutant transforming *ras* genes (Murray *et al.* 1983) led to the idea that there might be an interaction between the two oncogenes. Experimental support for this idea was obtained when it was shown that non-established rat embryo cell strains could be converted into established tumorigenic cell lines if they were transfected with both a *ras* and a *myc* oncogene (Land, Parada & Weinberg, 1983). Transfection with *myc* or *ras* on its own was insufficient to produce a tumorigenic cell line,

although it was shown that a *ras* oncogene on its own could result in morphological transformation, but the cells still retained a finite lifespan (Land *et al.* 1983; Newbold & Overell, 1983). It was later shown that transfection with a *myc* oncogene on its own could lead to immortalization without morphological transformation or tumorigenicity. These experiments led to the idea that there were two classes of oncogenes, exemplified by *myc* and *ras*, that cooperated to produce a fully malignant cell. Subsequent experiments have shown that both the *myc* and p53 genes can immortalize embryo fibroblasts and cooperate with *ras* genes to produce a transformed cell (Parada *et al.* 1984; Jenkins *et al.* 1984; Eliyahu, Michalovitz & Oren, 1985). Thus the oncogenes that code for nuclear proteins appear to be immortalizing genes and to cooperate with *ras*. The mechanism by which these gene products lead to immortalization and cooperation remains unclear, although one possible explanation is that the nuclear proteins may sensitize cells to the effects of growth factors (Balk, Riley, Gunther & Morris, 1985; Roberts *et al.* 1985). In spite of the clear-cut evidence for cooperation between oncogenes, recent evidence argues that there may not be a functional division between the effects of the transforming *ras* genes and those of the genes encoding nuclear transforming proteins. Such arguments rest on the observation that under appropriate circumstances of high-level expression *ras* genes can immortalize cells (Spandidos & Wilkie, 1984), a *fos* oncogene can both immortalize and make cells tumorigenic (Jenuwein, Muller, Curran & Muller, 1985), and the introduction of *myc* or p53 genes into established non-tumorigenic cell lines leads to the cells becoming tumorigenic (Keath, Caimi & Cole, 1984*b*; Eliyahu *et al.* 1985). Thus, while two oncogenes may be better than one, in the right conditions one oncogene may be sufficient to render cells tumorigenic.

WHAT IS THE ROLE OF THE *ras* ONCOGENES?

Although it is clear how genes encoding growth factors, their receptors and perhaps secondary nuclear message systems after growth factor–receptor interaction could function as oncogenes, it is not clear how the major class of oncogenes detected in human tumours fits into such a scheme. The *ras* genes form a family of at least three (Shimizu *et al.* 1983; Chang *et al.* 1982; Hall, Marshall, Spurr & Weiss, 1983) and maybe more (Madaule & Axel, 1985) genes that are altered in about 10–20% of most human tumour types (see Marshall, 1985). Five sites, amino acids 12, 13, 59, 61 and 63, in the p21–188/189 amino acid *ras* protein molecules are known to be positions at which single amino acid substitutions can lead to transforming activity (Fasano *et al.* 1984). Mutations at three of these sites, amino acids 12, 13 and 61 (Tabin *et al.* 1982; Yuasa *et al.* 1983; Bos *et al.* 1985), have been identified in human malignancies. However, the mechanism by which these mutations result in neoplastic activity are unclear. The p21 *ras* proteins do not appear to be either growth factors or growth factor receptors, but it is known that the p21 *ras* proteins are located on the inner surface of the cell membrane, bind GTP and have a GTPase activity. Localization at the inner surface of the cell membrane is dependent on

palmitation at the C terminus and is essential for transformation (Willumsen *et al.* 1984). Mutations of either Gly12 to valine, or Ala59 to threonine, both of which lead to transforming activity, result in a reduction of GTPase activity (Gibbs, Sigal, Poe & Scolnick, 1984; McGrath, Capon, Goeddel & Levinson, 1984; Sweet *et al.* 1984). Together with significant sequence homology to G proteins (Hurley *et al.* 1984; Tanabe *et al.* 1985), these observations suggest that the p21 *ras* proteins may be part of a family of G proteins. The known G proteins are involved in mediating interactions between cell surface receptors and effector enzymes (Gilman, 1984). These effector enzymes may be adenylate cyclase (Gilman, 1984), cyclic GMP phosphodiesterase (Tanabe *et al.* 1985), or possibly phosphodiesterases involved in the breakdown of phosphoinositols (Cockcroft & Gomperts, 1985). Furthermore, different G proteins may exert stimulating or inhibitory effects on effector enzymes. Although the idea that p21 *ras* proteins function in a homologous way to G proteins is attractive, this hypothesis lacks experimental support. First, the receptors with which *ras* interacts have not been identified, although there is some evidence that there may be an interaction with the EGF receptor (Kamata & Feramisco, 1984). Second, the effector enzymes have not been identified. Finally, the G proteins have a three-subunit structure with 41, 26 and $10\times10^3 M_r$ components, which does not correspond to the single-subunit structure of p21 *ras*. Although evidence from yeast suggests that in this organism *ras* is part of the adenylate cyclase system (Broek *et al.* 1985; Toda *et al.* 1985), there is some evidence to suggest that this does not appear to be the case in vertebrate cells (Beckner, Hatton & Shih, 1985). At present the clearest evidence that p21 *ras* may be involved in mediating growth factor signals is provided by experiments in which antibodies against the *ras* proteins have been injected into cells. If antibodies are injected into quiescent cells following serum stimulation, the stimulated cells do not make DNA (Mulcahy, Smith & Stacey, 1985). In addition these experiments suggest that p21 *ras* is involved in an event that occurs up until late G_1 and commits the cell to S phase. We are therefore left in a situation in which there are strong circumstantial arguments that the p21 *ras* molecules interact with growth factor receptors, but there is no direct evidence that this is the case.

CONCLUSIONS

In this review I have emphasized the way in which alterations to genes encoding growth factors or their receptors leads these genes to function as oncogenes and to maintain the transformed phenotype. Although such observations have given us a central theme for investigating the role of oncogenes, much remains to be answered. The major question is to understand the role of these oncogene products for which we have no known function. This includes both the serine kinases and tyrosine kinases, which have not been attributed to a receptor function. In spite of some promising leads, the role of p21 *ras* proteins remains ill-defined. But perhaps most intractable at present is the role of those oncogenes whose products are nuclear. The shared characteristics of these proteins, such as short half-lives, induction after

quiescence and ability to immortalize, suggest that they all do something similar; however, we have no clear evidence of what that is. It is likely that much of the understanding of the functions of oncogenes will come from areas that a short time ago would not have seemed closely connected to transforming genes. These areas include the metabolism of phosphoinositols, protein kinase C, cyclic AMP metabolism, ion fluxes and many other aspects of cell physiology. In addition, it can be argued that the mechanism of oncogenesis by the production of a transforming protein that has to be present to transform the cell is only part of the picture of oncogenesis. There is evidence suggesting that there is another class of transformation events that operate in a different mode by deleting some functions essential to normal cell behaviour (Murphree & Benedict, 1984; see Harris, 1986, this volume). How such mechanisms operate and possibly interact with the products of transforming proteins remains to be elucidated.

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