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The Action of Oncogenes in the Cytoplasm and Nucleus

Robert A. Weinberg

Summary. As many as 40 distinct oncogenes of viral and cellular origin have been identified to date. Many of these genes can be grouped into functional classes on the basis of their effects on cellular phenotype. These groupings suggest a small number of mechanisms of action of the oncogene-encoded proteins. Some data suggest that, in the cytoplasm, these proteins may regulate levels of critical second messenger molecules; in the nucleus, these proteins may modulate the activity of the cell's transcriptional machinery. Many of the gene products can also be related to a signaling pathway that determines the cell's response to growth-stimulating factors. Because some of these genes are expressed in nongrowing, differentiated cells, the encoded proteins may in certain tissues mediate functions that are unrelated to cellular growth control.

Oncogene research has changed substantially over the past several years. Initial emphasis concerned the identification of oncogenes present in tumor virus genomes and in the genomes of a number of different types of tumor cells. Together, various experimental routes have led to the characterization of at least 30 different oncogenes originating from the cellular genome and 10 or more found in the genomes of DNA tumor viruses (*1*). Having catalogued these genes and their structures, workers are now moving into a new phase in which mechanistic problems are confronted: how do oncogenes and their encoded proteins convert normal cellular metabolism to that of a tumor cell? What regulatory pathways are perturbed by oncogenes, and how can their various modes of action be interrelated?

This review attempts to synthesize much of the currently available data on these issues. It is written with the belief that much of the information about oncogenes will eventually be understandable in terms of a small number of mechanisms and that the outlines of some of these are gradually becoming apparent. Much of the present discussion concerns the modes of activation of cellular oncogenes and the effects that these genes have on cellular physiology. The implications of all this for tumorigenesis involves less discussion, because we still understand relatively little about the connections between oncogene action and the outgrowth of tumors in vivo.

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Nuclear and Cytoplasmic Oncogenes

The existence of the 40 and more oncogenes provokes an obvious question: do they represent as many as 40 distinct mechanisms of transformation, or can they be grouped into a small number of functional classes on the basis of shared functional properties? One such classification that has arisen in recent years groups oncogenes on the basis of the nuclear or cytoplasmic localization of their gene products (Table 1). Perhaps surprisingly, this crude classification scheme correlates with similarities of function within each group. The terms used to label these as "nuclear" or "cytoplasmic" oncogenes are a bit misleading, in that they imply the location of the genes rather than the site of action of their gene products. I will use the terms here nevertheless.

Among the nuclear oncogenes of special interest are several cellular genes or variants thereof (*myc*, N-*myc*, *myb*, and Ela) that exhibit some structural homology with one another (*2*, *3*). These and several other nuclear oncogenes (*p53*, polyoma large T, and SV40 large T) exhibit similarities of function, although no one of them behaves precisely the same as any other in all respects (*4*–*6*). Among the most readily measured of these traits is that of immortalizing ability—the power to convert a tissue culture cell of limited replicative potential in vitro into one that can be passaged without limit in culture.

Associated with this immortalizing ability are often other functions that may affect the altered growth properties of tumor cells in vivo. For example, a recent characterization of the *myc* oncogene (*6*) revealed that this oncogene also allows embryo fibroblasts to grow at lower serum concentrations and at lower density in monolayer culture, echoing similar results obtained with some other oncogenes of this group. These nuclear oncogenes tend to be weak in their ability to induce anchorage independence of fibroblasts, in contrast to cytoplasmic

oncogenes described below. Many of the encoded proteins are bound to nuclear structures (*7*).

A much larger group of oncogenes specifies proteins found in the cytoplasm. However, only a few of these genes have been subjected to a detailed scrutiny in which effects on cellular phenotype are studied. Among these are the three *ras* oncogenes, the *src* oncogene, and the middle T oncogene of polyoma. The cytoplasmic oncogenes are generally weak in their ability to immortalize cells (*4–6, 8*) and strong in their abillty to promote anchorage independence of fibroblasts. At least eight cytoplasmic oncogenes have been reported to induce secretion of growth factors in one or another cell type (*9, 10*), but this trait has never been associated with the effects of a nuclear oncogene. It remains unclear whether yet other members of the large group of cytoplasmic oncogenes will follow these functional patterns or exhibit their own particular pattern of function.

Nuclear oncogenes have been found to collaborate effectively with cytoplasmic oncogenes in malignant transformation of previously normal cells. Indeed, a nuclear or cytoplasmic oncogene, acting on its own, seems unable to induce full transformation under many conditions of culture (*4–6*). Cells that are effectively transformed by pairs of collaborating nuclear and cytoplasmic oncogenes range from rat embryo fibroblasts and chondroblasts to avian cells of the myelomonocytic and retinoblast lineage (*4, 5, 10*). The nuclear polyoma large T oncogene collaborates with the cytoplasmic middle T oncogene (*4*); the viral Ela, polyoma, and SV40 large T oncogenes and the cellular *myc*, N-*myc*, and *p53* oncogenes all collaborate with the cytoplasmic *ras* oncogene (*5, 11, 12*); and the nuclear *myc* and *myb* act synergistically with the cytoplasmic *src*, *erb*B, *fes/fps*, *yes*, *ros*, and *mil/raf* oncogenes (*10*). MH2 virus, a spontaneously arising, particularly potent retrovirus, has been found to have acquired both a nuclear (*myc*) and a cytoplasmic (*mil/raf*) oncogene from the cell genome (*13*). Use of recombinant DNA procedures has allowed construction of a hybrid avian/mammalian retrovirus also carrying these two oncogenes; the behavior of this virus also demonstrates a strong synergy between the two genes (*14*).

One apparent anomaly in this scheme is the SV40 large T oncogene, which, through the actions of a single protein, is able to induce "nuclear" functions such as immortalization and "cytoplasmic" functions such as anchorage independence (*15*). Of great interest is evidence indicating that the encoded large T antigen can be found both in the nucleus and at the plasma membrane. Mutations that inhibit the transport of T antigen into the nucleus appear to reduce its immortalizing ability while leaving intact its effects on anchorage independence and its ability to transform already immortalized cells (*16*). Consequently, this oncogene gains membership in both classes by sending its gene product to do work at two distinct cellular sites.

Table 1. Classification of oncogenes mentioned here on the basis of the nuclear or cytoplasmic localization of their gene products. (The *sis* oncogene, of cellular origin, specifies an extracellular protein.)

Nuclear	Cytoplasmic
Viral oncogenes	
SV40 large T	Polyoma middle T
Polyoma large T	
Adenovirus E1a	
Cellular oncogenes	
myc, *myb*, N-*myc*	*ras*, *src*, *erb*B, *neu*,
p53, *ski*, *fos*	*ros*, *fms*, *fes/fps*, *yes*, *mil/raf*, *mos*, *abl*

These various lines of evidence suggest that the activities of nuclear and cytoplasmic oncogenes are complementary rather than additive. One can speculate that certain cancer cell traits are more effectively induced by proteins acting in the nucleus while others are achieved more readily by gene products acting at cytoplasmic sites. There are, however, data that indicate exceptions to this. For example, certain pairs of nuclear oncogenes can collaborate with one another [for example, *fos* and polyoma large T (*17*)], and certain nuclear oncogenes can transform established, spontaneously immortalized cell types (*18*).

At greatest variance with the proposed requirement of multiple oncogenes for full transformation is the extensively documented ability of many retroviruses carrying single oncogenes to induce tumors in vivo (*19, 20*). Some recent work shows that a *ras* oncogene, acting alone, is able to convert an embryo fibroblast to the tumorigenic state (*20*). Such results show that, under the proper conditions, a single oncogene can induce all the phenotypes of transformation, not just some of them. Direct conflict exists between the two models of tumorigenesis: are two or more oncogenes required for making a cell tumorigenic, or will one suffice?

One resolution may come from consideration of another, overlooked factor in tumorigenesis—the environment of the cell that initially acquires an oncogene by mutation, infection, or transfection. Oncogene-bearing cells surrounded by normal neighbors do not grow into a large mass if they carry only a single oncogene (*4, 5, 11*). But if the normal neighbors are removed, either by killing them with a cytotoxic drug (*18, 20*) or by recruiting them into the tumor mass by viral spread in vivo (*21*), then a single oncogene often suffices. The environment of a cell may therefore strongly influence its responsiveness to an oncogene that it carries.

We know little about the identity of those cells within a tissue that are the targets of viral and of nonviral carcinogens. The nature of the cell-to-cell interactions that affect the clonal expansion of these target cells into tumors is even more obscure. Nonviral carcinogenesis appears to involve a number of distinct stages of tumor progression (*22*), and some of these stages may reflect an underlying requirement for the activation of multiple oncogenes. To date, only relatively few tumors of nonviral etiology have been found to carry multiple, independently activated oncogenes (*23*). Thus, the involvement of multiple, collaborating oncogenes in creating such tumors still requires extensive substantiation.

Mechanisms of Nuclear Oncogene Activation

Some of the nuclear oncogenes discussed here are found in the genomes of DNA tumor viruses, while others arise from alteration of normal cellular protooncogenes. Concentrating for a moment on those of cellular origin, one finds that they are all created by processes that lead to deregulation in the level of their encoded proteins.

The most well documented of these activating mechanisms concerns the deregulation of *myc* expression that results from chromosomal translocation [reviewed in (*24*)]. This genetic reshuffling often deprives the *myc* gene of its normal transcriptional promoter-enhancer regulators and replaces these with sequences from the immunoglobulin genes. Other consequences may also follow. Because the translocation often removes the initial (noncoding) exon of the gene, the resulting messenger RNA (mRNA) becomes restructured. Some have speculated that this may improve the utilization of this mRNA template (*25*), and such speculation is supported by recent work on the translation of several *myc* transcripts in vitro (*26*).

The *myc* gene may also become deregulated by the actions of a retrovirus that integrates its genome nearby in the chromosome, providing promoter-enhancer segments that override normal regulation (*27*). Other studies have demonstrated amplification of *myc*, N-*myc*, and *myb* genes in various tumor types (*3, 28*), leading in turn to increases in levels of encoded protein.

Most interesting are the alternative routes by which deregulation of the *p53* gene can be achieved. The *p53* protein is normally very labile metabolically, having a lifetime of less than 30 minutes. SV40 virus is able to increase greatly the steady-state levels of *p53* by causing its large T antigen to complex with *p53*, resulting in an increase in metabolic stability by about a factor of 50 (*29*).

The *p53* protooncogene itself can be activated experimentally by fusion with a strong promoter, resulting in greatly increased transcript and protein levels (*12*). This and analogous work on the *myc* and N-*myc* genes (*30*) provide the clearest demonstration of the importance of protein level and the lesser importance of altered protein structure. In all cases, clones of the normal cellular versions of these genes can be activated to full oncogenic potential by simple attachment to a strong, constitutive transcriptional promoter (*11, 12, 30*).

Taken together, these various lines of evidence persuade one that deregulation of the level of these nuclear oncogene proteins is sufficient to allow them to exert their effects on the cell. This deregulation does not always involve strong overexpression of the gene; instead, it may simply make the gene constitutively active and thus unresponsive to its normal regulators.

Activation of Cytoplasmic Oncogenes

Many of the well-studied cellular oncogenes of the cytoplasmic class appear to be effectively activated by mutations that affect the structure of their encoded proteins. Examples of this are now available for the *ras*, *src*, *erb*B, *abl*, *fes*/*fps*, and *neu* oncogenes. The exception to this is the *mos* oncogene, which acquires very strong oncogenic powers when linked to a constitutive promoter (*31*).

In the case of *ras*, a large number of spontaneously arising point mutations have been described in naturally occurring tumors. The point mutations affect amino-acid residues 12, 13, or 61 of the *ras*-encoded p21 protein, and they impart to this protein the ability to transform cells even when it is present in very low levels in the cytoplasm (*1, 32*).

An analogous situation pertains for the *src* gene. Structural alterations in the Rous sarcoma virus–encoded pp60 protein result in potent transforming activity. In contrast, strong overexpression of the normal gene product leaves one, at best, with a cell showing only partial transformation (*8, 33*). The *neu* gene presents a more extreme example, in which high levels of the protein encoded by the normal cell have no effect on cellular phenotype, while low levels of a structurally altered protein induce strong transformation (*34*). Yet other, analogous observations have been made on the *fes*/*fps* oncogene as well (*35*). The *erb*B gene, to which *neu* is distantly related, has been the object of intense scrutiny.

Here, once again, it appears that structural alteration, specifically truncation of the protein termini, is the favored route when attempting to activate the cloned normal gene (*36*). Indeed, when *erb*B becomes activated by retrovirus genome integration, the structure of the encoded protein is affected, in contrast to the events leading to viral activation of the nuclear protooncogene *myc* (*27, 36*), which have no effect on the structure of the *myc* protein.

Very recent studies of the *abl* gene, which belongs to the cytoplasmic group, provide an equally dramatic contrast with other, earlier work on *myc*. Both the *abl* and *myc* genes become altered in human malignancies as a consequence of chromosomal translocation. As mentioned above, translocations affecting *myc*, such as those occurring during the pathogenesis of Burkitt's lymphoma, deregulate the gene but leave its protein-encoding region intact (*24*). The results with the *abl* gene, as observed in cells of chronic myelogenous leukemia, are quite different. Here, the translocations cause the *abl* protein to be altered: its amino terminus is lost and is replaced by a protein sequence encoded by the foreign partner gene that participates in the translocation event (*37*). Overexpression of the unaltered, normal *abl*-encoded protein appears to have little or no effect on cellular phenotype (*38*).

Taken together, these various lines of evidence can be used to establish a useful generalization: deregulation in the nucleus, change of protein structure in the cytoplasm. Unfortunately, the picture is clouded a bit by evidence that several of the cytoplasmic genes can contribute to transformation by simple overexpression. A *ras* gene can be activated to an oncogene by linkage to a long terminal repeat (LTR) (*39*). Moreover, both *ras* and *erb*B oncogenes have been reported to be present in greatly amplified copy numbers in certain tumors (*36, 40, 41*). While overexpression represents a relatively ineffective way of activating these two types of genes (*36, 42*), these results tell us that the distinctions between nuclear and cytoplasmic genes may not be as clearly drawn as we might like.

Significance of Activation Mechanisms

Protooncogenes play key roles in the growth control of normal cells, and the mechanisms that create cellular oncogenes surely provide us with important lessons on how they do so. Many of the normal genes and their gene products must function to pass on growth-stimulatory signals from upstream in a regulatory pathway to one or more targets downstream. The evidence provided by the activation mechanisms, as described above, provokes me to suggest that two quite different schemes for signal transduction are exhibited by the normal cytoplasmic and nuclear genes.

The cytoplasmic protooncogene products are expressed in relatively constant amounts in time. Their expression may vary somewhat depending on growth and differentiation state (*43*), but it would seem that physiological increases in levels of gene product do not per se produce a stream of growth-stimulatory signals. Instead, one can propose that the cytoplasmic protooncogene protein molecules are usually in a resting state, awaiting a direct stimulus from an upstream agonist. They then rise to an excited state, send out excitatory signals for a short period of time, and then lapse back to a state of relative inactivity.

Mechanisms must exist that limit the length of the excited state of these protein molecules to seconds or minutes. Such mechanisms would include the guanosine triphosphatase activity of *ras* proteins (see below), the internalization and kinase C–phosphorylation of receptor proteins such as that encoded by *erb*B (*44*), and other, still obscure, negative feedback mechanisms that strongly limit the length of the excited state of the proteins encoded by genes such as *src* and *abl*. By this logic, the lesions that create cytoplasmic oncogenes cause the constitutive excitation of their encoded proteins. This may happen by providing a constant, gratuitous, excitatory stimulus to the protein molecule or, perhaps more frequently, by trapping the molecule in its excited state.

The nuclear protooncogenes respond

in a quite different way to growth stimulatory signals: they increase the steady-state concentration of their encoded proteins, often by great amounts (*45*). They achieve this by changes in transcription rate, by changes in posttranscriptional processing of RNA, and perhaps by the posttranslational stabilization of protein. The responses of these nuclear genes are by necessity much slower, and once achieved they are longer lasting, being measured in many minutes and even hours (*45, 46*).

As discussed above, genetic lesions that create nuclear oncogenes lead to an apparent constitutive expression of these genes by uncoupling them from their normal regulators. The protein molecules encoded by these nuclear genes may alter cell metabolism simply by their presence in the nucleus in enhanced concentration. At present, it is difficult to formulate a rationale for these two quite different response mechanisms in the nucleus and cytoplasm.

Reversible Activation of Normal Cytoplasmic Proteins: *ras* and *src*

The most intensively studied cytoplasmic genes are those of the *ras* family and *src*. Our understanding of these proteins in their normal and oncogenic configuration has changed substantially over the past 2 years. The pioneering work of Scolnick and his colleagues on the oncogenic *ras* proteins encoded by Harvey and Kirsten sarcoma viruses revealed, among other things, two facts of great importance: the proteins (termed p21) are membrane bound, apparently in large part to the plasma membrane, and they act to bind guanosine diphosphate (GDP) or triphosphate (GTP) (*47*). These facts strongly colored subsequent thinking in that they suggested analogy with the G proteins, which are known to transduce signals from various cell-surface receptors to adenyl cyclase (*48*). Moreover, they suggested a biochemical mechanism of action quite distinct from that of the much-studied tyrosine kinases.

The tyrosine kinases use their bound adenosine triphosphate (ATP) as a phosphate donor for modifying the tyrosine residues of target proteins. The GTP bound by the G proteins plays a quite different role: its presence indicates that the protein binding it has been raised to an excited state. The hydrolysis of this bound GTP by an activity intrinsic to the G protein signals a relaxation to a ground state and in this way can serve to limit the period of excitation.

Recent results allow us to rationalize

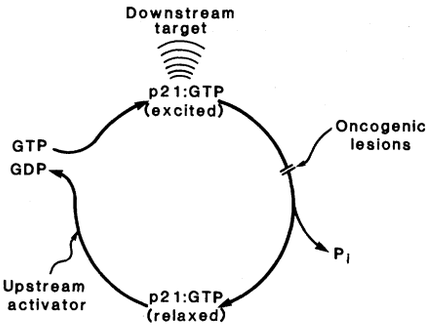

Fig. 1. A scheme of alternating excited and relaxed states of the *ras*-encoded p21 protein.

the oncogenic activation of the *ras* genes in terms of such a scheme of alternating excited and relaxed states. Enzymology performed on the p21 proteins has revealed that the p21 protein encoded by the normal allele of the H-*ras* gene exhibits GTP binding ability and has the power to hydrolyze this GTP to GDP. The p21 protein encoded by an oncogenic allele and bearing an amino-acid substitution at residue 12 continues to bind GTP effectively, but its powers of hydrolysis are reduced by a factor of about 10 (*49*). This fact may explain the molecular mechanisms of *ras* oncogene activation (Fig. 1). The inability to hydrolyze GTP effectively would seem to trap the protein in its excited state by blocking the route normally responsible for relaxation.

An understanding of the regulation of the other well-studied cytoplasmic oncogene protein, pp60*src*, remains more elusive. This protein also seems to enjoy only transitory periods of activation, during which its tyrosine kinase activity is manifest (*50*), but in this instance no GDP-GTP cycle can be invoked to explain its control. The *src* oncogene has not been found to date in any spontaneously arising tumors, and investigators are consequently not provided with useful mutant alleles that might aid in understanding normal regulation.

One important clue may come from analysis of the normal cellular *src* protein as it functions in polyoma virus–transformed cells. It is now apparent that the middle T antigen encoded by polyoma virus binds tightly to the cellular *src* proteins in the cytoplasm of infected cells (*51*). The complexed pp50*src* exhibits a tyrosine kinase specific activity that may be as much as 30 to 50 times higher than that of the free protein (*52*). This viral middle T may be mimicking an analogously functioning, endogenous cellular regulatory subunit that is responsible for the reversible activation of the normal pp60*src* protein.

Effector Functions of Cytoplasmic Oncogenes

The results described here provide examples of how three intensively studied protooncogenes can become activated: the *myc* gene loses transcriptional control; the *ras* gene protein loses guanosine triphosphatase activity; and the *src* gene protein acquires a foreign, physiologically unresponsive regulatory subunit. However, none of these insights into regulation provides useful information on the effector functions of these proteins. How do the oncogene proteins elicit responses from the cell? Any answer to this question must consider the pleiotropic action of oncogenes, that is, their ability to evoke multiple changes and, by implication, to affect multiple molecular targets within the cell.

The first lessons on oncogene effector functions came from the discovery of Erikson and colleagues, who showed that pp60*src* carries tyrosine kinase activity (*53*). Mutations in the *src* gene that inactivate the kinase activity invariably eliminate the transforming potential of the protein (*54*), indicating that this activity is not an adventitious element in the transformation process.

Paradoxically, this work provided limited insight into mechanisms of cellular transformation. While a number of cellular proteins were found to be modified by the kinase activity, the modification of none of these proteins could be tied directly to malignant conversion (*55*). Indeed, some target proteins were found to be phosphorylated even in the absence of observable transformation (*56*).

One important clue may be provided by studies that connect the activity of the *src* protein with changes in the pathway that involves the phosphorylation and degradation of the membrane constituent phosphatidyl inositol (PI). Several experiments have shown that the *src* protein and the tyrosine kinase encoded by the related *ros* oncogene are associated with a lipid kinase activity that is able to convert PI to mono- and diphosphorylated forms (*57*). Work with polyoma-infected cells, in which the *src* protein has been activated, also indicates a shift in PI metabolism (*58*). A remaining question is whether these tyrosine kinase proteins function directly as lipid kinases. Alternatively, they may be copurified with or regulate other proteins having lipid kinase activity (*59*).

This work presents an attractive hypothesis that explains tyrosine kinase effector function. By activating lipid kinases, these oncogene proteins may be able to induce formation of inositol poly-

phosphates, which in turn yield the pharmacologically potent "second messengers" diacylglycerol and inositol trisphosphate (*60*). These in turn activate protein kinase C and mobilize intracellular calcium, thereby effecting many of the pleiotropic changes that are associated with oncogene action.

This theme of oncogene-mediated regulation of second messenger molecules has been echoed by recent studies on the *ras* protein. The finding of *ras* homologs in the yeast genome (*61*) catalyzed a major and highly productive effort to analyze yeast *ras* function (*62*). This work has exploited the elegant genetic manipulations that are possible when working with yeast and are not available to those working with mammalian cells. Results to date have shown that the analogies between the *ras* proteins and the adenyl cyclase G proteins are more than superficial, at least in yeast. The yeast *ras* protein functions as a strong positive regulator of adenyl cyclase; it may regulate other yeast functions as well (*63*). Its inactivation leads to defects in spore germination that can be cured by introduction of the homologous mammalian *ras* gene (*62*).

These are striking findings that demonstrate the power and elegance that results from the convergence of biochemistry and the molecular genetics of yeast. The immediate transferability of the adenyl cyclase results to mammalian systems remains in question, however, because there are often discordances between cellular cyclic adenosine monophosphate levels and malignant growth. Oncogenic transformation of mammalian cells would not seem to be explainable simply by derangement in adenyl cyclase. Nevertheless, the strong homologies between the yeast and mammalian *ras* systems, as evidenced by the interchangeability of some components, together with the powers of the yeast system, may soon reveal the elements that are indeed critical to *ras*-mediated malignant transformation in mammalian cells.

Effector Functions of Nuclear Oncogenes

We may also have a clue concerning the effector functions of the *myc* and analogously acting gene products (*64*). In this instance, one deals with a different locus of action, the nucleus, and with an entirely different mode of action. These insights were stimulated by studies of the Ela oncogene of human adenovirus, which showed an ability of its encoded gene product to be a *trans*-acting regulator of the transcription of other viral genes (*65*). The initial findings have been extended in several directions. First, the Ela oncogene has been found to stimulate expression of cellular genes (*65*). Second, observation of cells transfected with *myc* oncogenes indicates a greatly increased ability of the cell to promote expression of resident cellular genes as well as introduced genes, such as a heat-shock protein gene (*66*).

These results appear to provide important clues to the functioning of the nuclear oncogenes of both viral and cellular origin. They suggest that the *myc* protein perturbs the activity or specificity of the cellular transcription apparatus and in this way mobilizes the expression of a bank of cellular genes whose products are critical to growth and differentiation. The normal cell genome carries multiple protooncogenes of this type (*myc*, N-*myc*, *myb*, *fos*, *p53*, and *ski*), and the abilities of most of these to affect transcription remain to be demonstrated. One might speculate that each of the proteins encoded by these genes may address the activation of a slightly different constituency of cellular genes.

The *myc* and Ela genes are not the only examples of oncogenes encoding nuclear proteins that serve on the one hand as *trans*-activators of transcription and on the other as agents of cellular immortalization. The same can be said of the large T oncogenes of polyomavirus and SV40 virus (*64, 67*). This provokes the question of whether the transcriptional activators of human T-cell leukemia virus type I (HTLV-I), HTLV-III, and pseudorabies virus (*68*) can also express an immortalizing function and will stimulate some to pursue the apparent mechanistic connection between cellular immortalization and *trans*-activation of transcription.

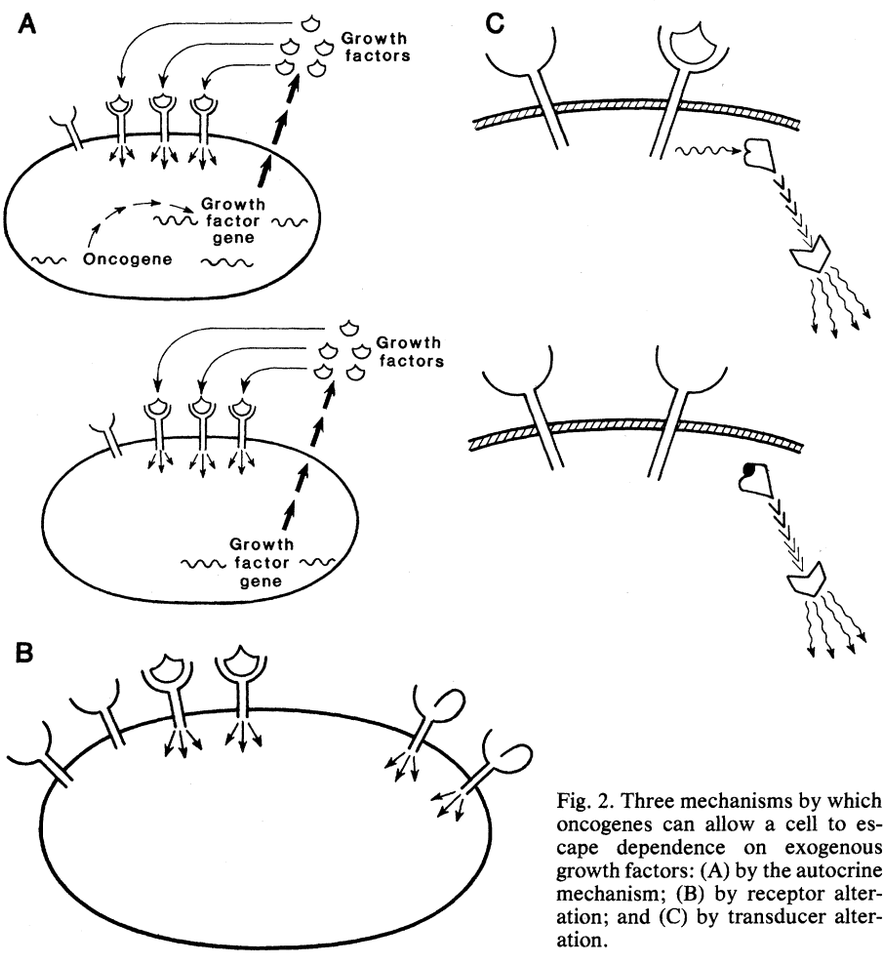

Fig. 2. Three mechanisms by which oncogenes can allow a cell to escape dependence on exogenous growth factors: (A) by the autocrine mechanism; (B) by receptor alteration; and (C) by transducer alteration.

Oncogenes and Growth-Factor Autonomy

Grouping oncogenes into nuclear and cytoplasmic classes represents only one way to conceptualize the interrelations between these various genes. Other results from recent work provide a quite different way to view the mechanisms by which protooncogenes and their oncogenic alleles regulate growth.

This view stems from observations of many workers that a fundamental trait of tumor cells is a decreased dependence on growth factors (GF's) for the promotion of their growth (*69*). Following this view, normal metazoan cells, with the

possible exception of certain embryonic cell types, may never proliferate unless prompted to do so by one or more types of GF present in their surroundings. Tumor cells, in contrast, acquire partial or complete autonomy that permits proliferation, even in the absence of any encountered GF's.

Accepting this, one then confronts the question of how oncogenes can confer such GF autonomy on the cell (*70*). Four different mechanisms come to mind, three of which are depicted in Fig. 2 (*70*). The first mechanism, termed autocrine, was proposed some years ago by Sporn and Todaro (*71*) and depends on the ability of a tumor cell to manufacture GF's. These can be secreted into the medium and then adsorbed to appropriate GF receptors present on the same cell that has just released them, thereby creating an uncontrolled autostimulation (Fig. 2A).

Oncogene-stimulated GF secretion can occur in two different ways. Cells transformed by certain cytoplasmic oncogenes (*ras*, *src*, middle T, *mos*, *fes*, *abl*, *fps*, *erb*B, *yes*, and *mil/raf*) release growth-stimulatory factors into their culture medium (*10, 72*). It is now clear that these oncogenes do not themselves encode the GF's. Instead, the released GF's are encoded by distinct genes whose expression is indirectly stimulated by these cytoplasmic oncogenes. The mechanism of this stimulation and its purpose in normal cell and tissue physiology remain unexplained.

A more direct autocrine route was indicated by the finding that a gene encoding the structure of a GF can itself become deregulated and converted to the status of an active oncogene. Thus, the oncogene *sis* was found to be an altered, deregulated version of the normal cellular gene specifying platelet-derived growth factor (PDGF) (*73*).

A generalization of this finding states that any cellular gene encoding a GF may be considered a protooncogene. If appropriately activated, it can force the constitutive elaboration of a GF. Should the cell in which this occurs also happen to display the cognate GF receptor, then a closed, positive feedback loop becomes established, providing the cell with a steady stream of growth-stimulatory signals and freeing the cell from its previous dependence on GF imported from elsewhere in the tissue or organism. Curiously, although many GF's have been identified, only the gene for PDGF has been found to date in the form of an active oncogene.

Oncogenes can confer GF autonomy in a second way, this one involving the receptors that are displayed on the cell surface and used by cells to recognize the presence of GF's in the extracellular space (Fig. 2B). In this instance, the receptors themselves become changed in a fashion that allows them to bombard continually the cell with growth stimulatory signals, even in the absence of any encountered GF. The cell is thus deluded by its malfunctioning receptor, being informed of high GF concentration when little or none is in fact present. In this way the GF receptor can assume the role of oncogene protein, and the sequences encoding it can assume the role of oncogene.

Three examples of this have now been reported. The first and precedent-setting example came from the work on the epidermal growth factor (EGF) receptor (*74*). Sequencing of a portion of this receptor demonstrated near identity with the protein specified by the *erb*B oncogene, known from its presence in the avian erythroblastosis virus genome. This showed clearly that a gene encoding a GF receptor should be considered as a protooncogene, capable of participating in cellular transformation when appropriately altered.

A second oncogene, termed *neu*, has been discovered in rat neuroblastomas and glioblastomas. This gene, related distantly to *erb*B, encodes a protein that has all the structural characteristics of a GF receptor; a ligand has not yet been identified (*75*). Most recently, the oncogene *fms*, originally found in a feline sarcoma virus, has been shown by elegant detective work to be an altered version of the normal cellular gene specifying the mononuclear phagocyte (CSF-1) GF receptor (*76*). Other tyrosine kinase oncogene proteins may eventually be associated with known GF receptors (*77*).

The third means by which oncogenes can confer GF autonomy involves proteins that lie within the cell and transduce signals from the GF receptors to targets farther downstream in the signaling pathway (Fig. 2C). Here once again the analogy with the G protein of the adenyl cyclase system becomes useful. Altered forms of these transducing proteins may acquire an autonomy that enables them to send out signals, even without prior prompting by a GF receptor. As before, the presence of the GF is not required for growth.

The *ras* proteins stand as good candidates for a role in the transduction of signals from cell surface receptors to intracellular targets. There are at least four *ras* p21 proteins (one H-*ras*, two K-*ras*, and one N-*ras*), and their distinctive carboxyl-terminal tails may permit each to interact with its own set of GF receptors. The remaining portion of these proteins is almost identical among the four, and this may indicate a common downstream effector function. To date, only one piece of evidence supports such a role for *ras* proteins—a report that the EGF receptor stimulates the nucleotide binding of the H-*ras*–encoded p21 (*78*).

The fourth and final way to achieve GF autonomy is associated with the nuclear oncogenes. We do not understand all the mechanisms that govern their expression and the levels of their encoded proteins. Certainly one very important mediator of nuclear protooncogene regulation is the GF's, which can strongly stimulate *myc*, *fos*, and p53 protooncogene expression (*45*). The transcriptional deregulation of these genes, described earlier, frees them from GF dependence. This may in turn relieve much of the normal GF requirement that must be satisfied in order for the cell to undertake a growth program.

Effects of Protooncogenes on Functions Other Than Growth

Because cellular oncogenes mediate abnormal cellular growth regulation, it follows that the corresponding protooncogenes must regulate normal growth patterns. Indeed, this notion has permeated much of the discussion in this article. Biological reality may, however, prove to be much more interesting: protooncogenes may be involved in a variety of cellular functions that are quite unrelated to growth and its regulation.

This idea stems from observations over the past several years that the cellular *src* gene is expressed in much higher levels in cells of the nervous system than in other tissues. Such observations have been made in chick, rat, and *Drosophila* cells (*79*). In many cases, *src* expression is high in fully differentiated cells, such as neurons that have no prospect of undertaking a growth program.

Because of these observations, it seems that the *src* protooncogene may be involved in some aspect of neuronal function that is unrelated in any way to growth control. It is even possible that the *src* gene, in its normal form, is never involved in any aspect of cellular growth regulation; its association with growth deregulation may be a consequence of the rare accident that caused its activation by a transducing retrovirus.

Perhaps the control circuits that were initially developed to regulate protozoan growth have been exploited repeatedly during the evolution of metazoa to regulate a variety of differentiated functions, such as neuronal signaling and exocyto-

sis. Perhaps oncogenes and protooncogenes will provide useful insights into many more problems than just that of cancer.

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