The Approaching Era of the Tumor Suppressor Genes

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Genes that can inhibit the expression of the tumorigenic phenotype have been detected by the fusion of normal and malignant cells, the phenotypic reversion of in vitro transformants, the induction of terminal differentiation of malignant cell lineages, the loss of "recessive cancer genes," the discovery of regulatory sequences in the immediate vicinity of certain oncogenes, and the inhibition of tumor growth by normal cell products. Such tumor suppressor genes will probably turn out to be as, if not more, diversified as the oncogenes. Consideration of both kinds of genes may reveal common or interrelated functional properties.

TUMOR DEVELOPMENT AND PROGRESSION CAN BE CONSIDERED a microevolutionary process, based on sequential changes in multiple determinants (*1*). It may be viewed as the gradual emancipation of a clone of somatic cells from the complex controls that regulate its growth. Foulds (*2*) has defined tumor progression as the "independent reassortment" of multiple "unit characteristics" that influence the neoplastic phenotype. Some of these characteristics have now been defined at the genetic level. The genes whose normal or pathological function may influence the steps of tumor development in a positive or negative fashion may be provisionally classified as oncogenes, emerogenes (also designated as antioncogenes or tumor suppressor genes), and modulator genes (which can influence important but secondary malignant properties such as invasiveness, metastatic propensity, or the ability to generate an immune response).

The discovery of oncogenes stems from the search for tumor viruses at the dawn of the century, stimulated by earlier successful developments in microbiology and the epidemiology of infectious diseases. The important initial discoveries of Peyton Rous (*3*) remained conceptually latent for more than five decades. The field was revitalized in the 1950s, by the single-minded dedication of a few investigators who hoped to find the putative viral cause of most human cancers (*4*). While this expectation has remained unfulfilled, the discovery of many important cellular genes involved with the control of cell division is one of its lasting by-products; the actual discovery of a few viruses involved in the causation of some tumor types is another.

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The development of retroviral genetics and molecular biology permitted the rapid identification of the virally transduced oncogenes as cell-derived sequences, obligingly incorporated in readily analyzable retroviral particles (*5*). The serial propagation of the oncogene-carrying and usually defective retroviruses under continuous selection for tumorigenicity is essentially a laboratory artifact, distant from the realities of natural tumor development. It has nevertheless provided a wealth of information on normal growth control and the emancipation of malignant cells. Illegitimate activation of cellular oncogenes by point mutation, retroviral insertion, chromosomal translocation, or gene amplification can contribute to tumor development and progression in many systems (*6*). They can appear as major (regular) or minor (less frequent) pathways in the natural history of a given type of cancer. The chromosomal translocations that juxtapose c-*myc* with immunoglobulin (Ig) sequences in human and rodent B cell–derived tumors (*7*) and the bcr/c-*abl* translocation in Philadelphia (Ph^1)-positive chronic myelogenous leukemia (*8*) are currently the best examples for a regular, early, rate-limiting (that is, essential) oncogene activation event in spontaneous tumor development. Oncogene amplification occurs less regularly and usually at a later stage of tumor progression (*9*).

The category of genes that can suppress transformation or tumorigenicity may be as diversified as or even more diversified than the oncogenes. The constitutive activation of a "growth factor ongocene," for example, may be canceled by the loss or dysfunction of the corresponding receptor, by a roadblock elsewhere within the complex pathway of signal transmission, and by changes in the responding target. Oncogene-induced blocks to cell maturation may be overcome by strong inducers or circumvented by the use of alternative pathways. In this article I will review the fragmentary but firm evidence that shows the existence of such mechanisms.

Tumor-suppressing genes have been detected in the following systems. (i) Fusion of normal and malignant cells leads to the suppression of the tumorigenic phenotype in the majority of the combinations where the hybrid maintains a relatively complete chromosome complement. Reappearance of tumorigenicity is accompanied by chromosome losses. The loss of certain normal parent–derived chromosomes appears to be particularly important (*10–13*). (ii) Morphological and nontumorigenic revertants have been isolated from both virally and chemically induced transformants (*14, 15*). They are not necessarily generated by the loss or down-regulation of the original transforming gene. (iii) Differentiation blocks can be bypassed by the temporary down-regulation of temperature-sensitive oncogenes or by exposure to strong differentiation-inducing signals. (iv) Loss or mutational inactivation of "recessive cancer genes" plays an essential role in the genesis of retinoblastoma, Wilms' tumor, and osteosarcoma, indicating that the normal alleles of these genes can counteract neoplastic transformation in the corresponding tissues (*16*). (v) Regulatory sequences

capable of preventing illegitimate activation have been identified in the immediate vicinity of certain oncogenes (for example, c-*mos* and c-*fos*) (*17, 18*). (vi) Tumor growth can be inhibited by diffusible products released by surrounding normal cells.

Suppression of Tumorigenicity by Somatic Hybridization

A large variety of spontaneously, virally, and chemically induced tumors become low- or nontumorigenic after fusion with fibroblasts, lymphocytes, or macrophages (*10–14*). Reappearance of tumorigenicity after chromosome loss was found to occur at variable rates, depending on the stability of each hybrid combination. Unstable hybrids may generate large numbers of chromosomal segregants, giving an impression of high tumorigenicity (*19*). Suppression can only be demonstrated in relatively stable hybrids. Some highly stable hybrids do not throw off malignant segregants at all (*11, 20*). Some hybrids of a lymphoma and lymphocyte combination may represent a true exception, however, since high tumorigenicity was found in chromosomally complete hybrids (*21*).

The suppression of tumorigenicity by cell hybridization can be discussed in genetic or epigenetic terms that are not mutually exclusive. If genetic losses play an essential role in the evolution of the malignant phenotype, the normal cell genome may act by genetic complementation. In cases where the neoplastic transformation is due to a blockage of maturation, for example, by a dominantly acting oncogene, the normal partner cell may impose its own differentiation program on the hybrid. Hybrids derived from the fusion of HeLa cells with normal keratinocytes continue to grow as undifferentiated cells in vitro, but generate keratinizing pearls and stop growing after in vivo inoculation (*11, 21, 22*). Stanbridge has concluded that "the hybrid cell takes on the phenotypic signature of the normal parental cell, regardless of the origin of the malignant parental cell" (*11*, p. 91).

Some chromosome pairs of the normal parent are regularly lost from the high malignant segregants. Murine chromosome (chr) 4 has been implicated in the suppression of carcinomas, melanomas, sarcomas, and lymphomas in intraspecies hybrids (*24*). Human chr 2 played a similar role in hybrids between normal human fibroblasts and a Chinese hamster tumor (*12, 25*). The anchorage-independent, transformed phenotype of BHK cells appeared to be suppressed by some determinant on human chr 1 (*26*).

Human intraspecies hybrids were studied most extensively. Stanbridge *et al.* (*27*) found that the reexpression of tumorigenicity in hybrids of HeLa cells and normal fibroblasts was associated with the loss of one copy of chr 11 and one copy of chr 14. Klinger *et al.* (*25*) provided similar evidence for human chr 11, whereas Benedict *et al.* implicated human chr 1 and possibly chr 4 in the suppression of the HT 1080 fibrosarcoma by normal fibroblasts (*28*). This is not necessarily a contradiction. The tumorigenic phenotype may be suppressed by functionally different mechanisms, depending on the transforming gene and the phenotype of the normal partner cell. The two malignant partners of these crosses, HeLa and HT 1080, produced nontumorigenic hybrids when fused with each other (*29*), suggesting genetic complementation between cells that carry different genetic lesions. HT 1080 carries a mutationally activated N-*ras* gene on chr 1. A loss of the normal parent–derived chr 1 from the tumorigenic segregants implies the loss of the normal N-*ras* allele (*28*). Corresponding losses were found in tumors that carry mutated *ras*, including chemically induced mouse skin carcinomas (*30*), thymic lymphomas (*31*), and a variety of human tumors and derived cell lines (*32*). It is therefore conceivable that the normal *ras* may antagonize the tumorigenic effect of the mutated allele. It was particularly suggestive that the progression of chemically induced mouse skin papillomas to the carcinoma stage was accompanied by the amplification of the mutated *ras* or the loss of the normal allele or both (*30*).

Our work on murine lymphomas that carry a retrovirally activated c-*myc* gene has led to analogous conclusions. In TKA, a T cell lymphoma of AKR origin that is trisomic for chr 15, two of the chr 15s contained a rearranged 33-kb c-*myc*–carrying fragment, generated by the insertion of a retrovirus upstream of the gene. The third chr 15 contained the germline fragment (*33*). TIKAUT, a ouabain- and thioguanine-resistant subline of TKA, was still trisomic for chr 15, and contained the same 33-kb rearranged fragment, but had no germline band; this indicates that the rearranged chromosome has duplicated once more, while its normal counterpart was lost. Studies on hybrids of TIKAUT and fibroblasts provided evidence that the presence of the rearranged chr 15 favors tumorigenicity in a dose-dependent fashion, whereas the normal homolog counteracts it. High tumorigenic hybrids showed further amplification of the lymphoma-derived chr 15 to five or six copies, whereas the number of its normal fibroblast parent–derived homologs decreased from two to one. Low-tumorigenic hybrids maintained the original 3:2 ratio. In a second series of studies (*34*) my colleagues and I have used MCF-B, an originally diploid AKR lymphoma that was induced by mink cell focus-inducing (MCF) virus and carried rearranged and germline *myc* in a 1:1 ratio. The majority of the tumors derived from hybrids of MCF-B and fibroblasts shifted their original 1:3 rearranged:germline ratio to 2:1 or 3:1 ratio. In similar studies on Moloney virus–induced, chr 15–trisomic T cell lymphomas, Cuypers *et al.* (*35*) found that the rearranged *myc*-carrying chr 15 was duplicated in all four tumors studied.

The functional significance of c-*myc* activation in the genesis of murine T cell lymphoma is not clear, but the clustering and the potentially functional orientation of the *myc*-juxtaposed retroviral long terminal repeats (LTRs) are consistent with the hypothesis that c-*myc* activation may contribute to lymphoma development or progression (*36*). The requirement for amplification is in contrast to the Ig/*myc* juxtaposition by chromosomal translocations in mouse plasmacytoma, Burkitt lymphoma, and rat immunocytoma [reviewed in (*7*)] where the constitutional activation of *myc* by the powerful influence of the Ig locus apparently obviates the need for chromosomal amplification, perhaps because the normal *myc*-allele is switched off. The amplification of the change and the loss of the normal chromosome indicate that the LTR-*myc* complex may be accessible to some trans-acting regulation that emanates from the normal homolog. The *myc*-associated dehancer sequence, identified by Yang *et al.* (*37*), is a potential candidate for such a role.

The suppression of tumorigenicity in hybrids between normal cells and tumor cells transformed by activated oncogenes may occur at different levels. Down-regulation of transcription has been demonstrated for v-*src* (*38*), but it is more the exception than the rule. It is more frequent that suppression acts beyond that level of oncoprotein expression. This was found in the SV40 system (*19, 39*) and particularly often in relation to *ras*-transformed cells.

Geiser *et al.* (*40*) fused the human EJ bladder carcinoma line, which carries a transforming, mutation-activated *ras* gene, with normal fibroblasts. The hybrids retained the transformed phenotype in vitro, but did not grow in nude mice. Tumorigenic segregants appeared on serial cultivation. The mutated *ras* p21 protein was present at the same level in tumorigenic and nontumorigenic hybrids. Transfection with c-H-*ras*–expressing constructs increased the amount of p21, but did not induce tumorigenicity. Suppression of transformation in the absence of changes in p21 expression was also demonstrated in a Chinese hamster (*41*) and a mouse system (*42*). In the latter study, flat revertants isolated from Kirsten

sarcoma virus–transformed murine fibroblasts still contained a functionally intact viral oncogene, as shown by rescue experiments. Their p21 level was as high as in the original transformants, but they were resistant to retransformation by activated *ras* of either cellular or viral origin. Somatic hybridization of the revertants with both nontransformed and transformed cells of the same lineage generated nontransformed hybrids. The revertants could also suppress *src, fes*, K-, H-, and N-*ras* and mutated human H-*ras* transformants, but not *mos, sis, fms, raf*, polyoma, SV40, and chemically transformed cells of the same origin.

Src and *fes* encode oncoproteins unrelated to *ras*. The common suppression pattern suggests that the dominant reversion imposes a block on a transformation pathway that converges in these three transformants. *Raf* and *mos* are believed to act at a level beyond the *ras*-dependent signaling pathway (*43*). The analysis of the suppression patterns provides a new approach towards the definition of these pathways in cells transformed by different oncogenes. The mapping of suppressor genes by the relatively cumbersome method of somatic hybridization will be probably replaced by the more direct microcell-mediated transfer of single chromosomes, as exemplified by the recent report of Weissman *et al.* (*44*) on the suppression of Wilms' tumor cells by fusion with a minicell containing chr 11 (*44, 45*).

Reversion

The term "progression" was originally coined by Peyton Rous to designate "the process whereby tumors go from bad to worse" (*46*). The natural history of most cancers, their clinical course, and the serial transplantation of experimental tumors reinforce the image of a "one-way street." This image is biased by selective pressure, however, and falls short of reflecting the total spectrum of potentially relevant cellular events. Each regulatory or structural change that pushes the cell forward along the pathway of progression must have a counterpart that would cause reversion. Reversion can only be detected at the population level if the growth of the original malignant cell is inhibited, however. This requires special techniques.

The first revertants were isolated by negative selection with toxic agents that preferentially killed transformed cells in confluent cultures (where transformants alone were still able to grow) (*47*). More recently, revertants have been isolated by positive selection, on the basis of their increased resistance to ouabain (*42*), methionine (*48*), or paraquat (*49*). Bassin and Noda (*14*) have subdivided revertants into an oncoprotein-related and a target-related category. The former arise by the loss or inactivation of a transforming gene, whereas the latter continue to express the transforming protein, but are phenotypically normal or quasi-normal.

Revertants with a defective oncoprotein are relatively trivial. They usually arise in cultures of virally transformed cells and are susceptible to retransformation by the same agent. Target-related revertants are resistant to retransformation. Noda *et al.* (*42*) increased the probability of isolating such revertants by starting with doubly infected cells that carried two copies of the viral v-K-*ras* gene. *N'*-methyl-*N*-nitrosoguanidine (MNNG)–mutagenized cultures contained approximately 10^{-7} revertants that were more flattened, cloned less well in agarose, lacked tumorigenicity, and had an increased chromosome number. They contained the same two v-K-*ras* copies as the transformant, grew equally well in low serum, and produced the same high amounts of the p21 *ras* protein and transforming growth factor–alpha (TGF-α). It was suggested that they had arisen by a change in the transformation pathway, occurring at some point beyond the interaction of TGF with its receptor. A possible clue about the nature of this change was provided by the finding that the characteristically reduced tropomyosin content of the *ras*-transformed cells was restored to the control level in the revertants (*50*). As a first approach towards the molecular definition of the suppressor, Noda *et al.* have succeeded in isolating flat revertants from v-K-*ras*–transformed NIH 3T3 cells transfected with a human complementary DNA (cDNA) expression library (*51*).

Another study by the same group (*52*) has shown that one gene can act in a transforming or a suppressing capacity, depending on the target cell. Activated *ras* and v-*src* genes can transform fibroblasts, but suppress growth of PC12 cells, which were derived from a rat pheochromocytoma. PC12 cells can multiply indefinitely in growth medium, but differentiate into sympathetic neurons after exposure to nerve growth factor. The two viral oncogenes mimic the activities of nerve growth factor. It was suggested that they may induce the same intracellular signals in both kinds of cells, but elicit different responses, depending on the properties of the target cell.

Bypass of Differentiation Blocks

The unidirectional tendency of progression has created the impression that tumor cells become less and less differentiated as they evolve toward increased autonomy. The early concept of "dedifferentiation" was gradually replaced by the idea that tumor cells resembled their normal progenitor cells phenotypically, but were blocked at specific stages of maturation. This is an important distinction. Dedifferentiation and subsequent redifferentiation can be exemplified by the regeneration of a higher plant from a single somatic cell. Reversion of highly specialized cells to a multipotent state has not been observed in animal cells.

Tumor cells tend to remain faithful to their lineage, even after having been blocked by a pathologically activated oncogene over many cell divisions. Rous sarcoma virus that carried a temperature-sensitive *src* gene (ts–v-*src*) transformed myogenic cells into permanently growing sarcoma cells at the permissive temperature. Differentiated myotubes appeared after the transformed cells had been incubated for even a short time at the nonpermissive temperature, indicating that the cells have become committed to terminal differentiation (*53*). Thus, the virally transduced oncogene has failed to cancel the myogenic commitment of the cells; at the permissive temperature it retained them in the cycling compartment, froze them at the same stage of differentiation, and blocked their further maturation. Subsequent reexpression of the transforming protein could no longer stop or revert the maturation process. The appearance of chondrocyte and melanoblast differentiation markers was also prevented by v-*src*, and the eclipsed phenotype appeared readily after a short exposure to the nonpermissive temperature (*54*). These experiments also showed that activated oncogenes can only transform cells if they are permitted to act on their target within a specific differentiation window, at a point where they can prevent further maturation.

Other oncogenes may act in an analogous fashion. Avian erythroleukemia cells induced by temperature-sensitive avian erythroleukemia virus (AEV) make no hemoglobin (*55*). The mutant-transformed erythroblasts are indistinguishable from wild-type transformants at the permissive temperature. Their globin genes lack the hypomethylated and deoxyribonuclease I–hypersensitive sites characteristic for erythroid cells (*56*). Such sites appear promptly at the nonpermissive temperature, followed by the synthesis of hemoglobin–messenger RNA and terminal differentiation. The temperature-induced differentiation is essentially synchronous and resembles the

maturation of normal erythroid precursors with respect to many morphological, antigenic, and biochemical markers.

With the exception of the multipotential teratomas and some oligopotential, stem cell–derived tumors of the hematopoietic system, transformed cells only express the markers of a single lineage, although their phenotype may be aberrant (*57*). If the synthesis of a certain differentiation product (such as Ig) is compatible with cell proliferation, illegitimately activated oncogenes, like an Ig-juxtaposed c-*myc*, can drive the cell to proliferate without interfering with the synthesis of the product. Production of hemoglobin or certain specialized proteins of the higher nervous system are incompatible with proliferation, and their potential producer cells can only be transformed by oncogenes that can prevent their appearance. It is unlikely that the oncoproteins interact directly with the corresponding structural gene. Broadly acting oncogenes like v-*src* or v-*myc* can transform a wide range of cell types, perhaps by interfering with some pleiotropic switch mechanism. Membrane-associated oncoproteins may act by interfering with receptor-ligand systems. Nuclear oncoproteins may stimulate DNA replication more directly or may prevent chromatin condensation at a time when the cell is normally programmed to pass from the cycling to the resting compartment.

Before the development of the oncogene field, it was already well known that certain malignancies like erythroleukemia, myeloid leukemia, neuroblastoma, and histiocytoma could be induced to differentiate terminally in vitro (*58*). Some murine myeloid leukemia lines, designated D+, could be induced by normal differentiation factors to become normal macrophages or granulocytes, whereas others (D−) responded only to chemical triggering (*15*). Teratomas differentiated normally in the appropriate early embryonic environment, and could then participate in generating the full range of normal mouse tissues (*59*). Strong differentiation-inducing signals can thus override the maturation block of certain tumors. This was even shown to occur in cells that carry a highly amplified oncogene, as in the promyelocytic leukemia line HL60 that contains 40 to 60 copies of c-*myc* (*60*). Granulocytic differentiation induced by retinoic acid, and macrophage differentiation induced by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) were accompanied by the prompt down-regulation of the amplified *myc* genes (*61*). Promyelocytic leukemia cells do not readily grow in vitro. The amplification of c-*myc* may have increased the growth potential of the HL60 cell, at the expense of its propensity to differentiate.

Activated *myc*-constructs can inhibit differentiation (*62*) and may act in a tumorigenic capacity (*63–65*). The juxtaposition of c-*myc* to the immediate vicinity of constitutively active Ig sequences can play a rate-limiting role in the causation of B cell–derived tumors in humans, mice, and rats [for review see (*7*)]. Unlike HL60, where the amplified *myc* genes are located within their normal flanking regions and can readily respond to regulatory signals, the Ig/*myc* translocation leads to a state of permanent deregulation. We have suggested that the translocation may act by preventing antigenically stimulated B cells from entering the long-lived, noncycling, memory cell compartment, after the cessation of the antigenic stimulus (*66*). Normally, c-*myc* is highly expressed in proliferating tissues but is down-regulated when the cells enter the resting phase. This is particularly obvious in the case of resting cells with condensed chromatin, such as small lymphocytes, granulocytes, and spermatozoa (*67*). The illegitimate blocking of the normal down-regulation of *myc* may antagonize the chromatin condensation process. Alternatively, or in addition, the *myc* protein may also stimulate DNA replication (*68*).

If the constitutive expression of the Ig-juxtaposed *myc* is due to the cis-acting, constitutively activated Ig sequences, it should be possible to down-regulate the transfected *myc* gene by suppressing Ig expression. This was shown to occur in Burkitt lymphoma/mouse fibroblast hybrids where Ig synthesis is always eclipsed (*69*).

In conclusion, many and perhaps most tumor cells can be induced to mature by natural or artificial inducers. Their responsiveness may decrease during tumor progression, because of sequential oncogene activation events (including amplification) or the loss of suppressor genes that belong to the category of differentiation regulators or both. Part of the latter may relate to the dominant genes that can suppress the transformed and/or tumorigenic phenotype after translation of the oncogene in the revertants and in the somatic hybrids discussed in the preceding sections.

Recessive Cancer Genes—a Special Category of Tumor Suppressor Genes?

Molecular analysis has fully confirmed the ingenious theory of Knudson that retinoblastoma arises by the loss of both alleles at the same locus (RB-1). The gene is localized at 13q14 on the human chromosome map [for review see (*70*)]. In familial retinoblastoma, a defective RB-1 allele is transmitted through the germline. It may be associated with a deletion at 13q14.2, but is more frequently invisible at the cytogenic level. The second change occurs during somatic development. It may arise by the loss of one chr 13 with or without the duplication of the other, or, less frequently, by somatic crossing over or by interstitial deletion (*71*). Most retinoblastomas express N-*myc* at a high level suggesting that the loss of RB-1 may act through the up-regulation of N-*myc* or that a high N-*myc* level is merely an incidental marker of the growing retinoblast (*72*). The latter alternative appears more likely. Loss of both copies of RB-1 or a closely linked gene appears to be involved in the genesis of osteosarcoma as well (*73*).

Recently, a cDNA fragment has been cloned that corresponds to a gene that spans over at least 70 kb of human chr 13q14. The gene was sequenced and identified as the retinoblastoma susceptibility gene (*74*). It is expressed in many tumor cells and also in fetal retina, but not at all or only in a truncated form in retinoblastomas and osteosarcomas. It remains to be shown whether this gene is capable of reverting some of the malignant properties of retinoblastoma and osteosarcoma, when introduced in an appropriately active form.

A gene localized at 11p13 appears to play a similar role in Wilms' tumor (*75*) and perhaps in hepatoblastoma and embryonal rhabdomyosarcoma as well (*76*). Nisen *et al.* (*77*) found a greatly enhanced N-*myc* expression in 12 of 13 Wilms' tumors. Similar genetic losses may be involved in some solid tumors in adults. The 3p14 region is frequently deleted in renal carcinoma and in small cell carcinoma of the lung (*78*). A recessive locus on chr 13 may be involved in the genesis of ductal breast carcinomas and the loss of an allele on chr 5 may occur in colonic carcinomas (*79*).

How can gene losses lead to tumor development? Comings (*80*) has suggested that every cell contains structural "transforming" genes, active during embryogenesis but suppressed during differentiation by dominant "suppressor" or "regulatory" genes. Loss of both copies of the latter may lift the suppression, with continuous expression of the transforming gene and tumor development as the result. Comings's theory is essentially consistent with the modern development, at least for retinoblastoma. Normally, the retinoblast differentiates into a retinocyte that has irreversibly lost the ability to divide. Children who inherit the deletion of one RB-1 allele from one of their parents run the risk of developing retinoblastoma only during their first years of life. If the second allele is not lost by a somatic change by the age of five, all retinocytes will have differentiated terminally. It is therefore likely that the wild-type RB-1 allele is

essential for the terminal step, in a structural or a regulatory capacity.

Current evidence (*74*) strongly suggests that osteosarcoma is also due to the double loss of the RB-1 gene, rather than to the loss of a very closely linked gene. It is therefore necessary to consider the possible existence of pleiotropic suppressor genes, required for the normal maturation of several cell types. It is intriguing to speculate that the same putative genes may serve as the targets for the maturation blocks imposed by the broadly acting dominant oncogenes.

Experimental systems that deserve to be explored to gain more information about suppressor genes include the transgenic mice that carry enhancer-linked oncogene constructs. Such mice develop monoclonal tumors in spite of the broad polyclonal activation of the transgene. Activation of additional oncogenes or the loss of suppressor genes or both may be required before progressive growth can occur. For example, introduction of MMTV-enhancer–c-*myc* constructs into mouse zygotes has led to the development of mammary carcinoma in a significant proportion of the transgenic female offspring (*81*). The transgene was transcribed at a high constitutive level in the entire mammary gland, but the tumors were monoclonal, indicating that at least one additional rate-limiting event has occurred. This is not surprising, because mouse mammary tumors are known to develop in several steps, even in the most highly susceptible mouse strains (*82*). The nearly uniform association of the Ig/*myc* translocations with Burkitt lymphoma, mouse plasmacytoma, and rat immunocytoma (*7*) appeared as a more likely candidate for a possible single-step mechanism but facsimile experiments have given similar answers as for mammary carcinoma. The lymphoid tissues of transgenic mice implanted with IgH-enhancer–c-*myc* constructs contained only blast-transformed but no resting B lymphocytes; nevertheless, the animals developed oligo- or monoclonal B or pre-B lymphomas (*64, 83*). The construct was activated by the IgH-enhancer at an early stage of B cell differentiation as expected, but the vast majority of the *myc*-activated blasts remained under some type of host control. The additional change or changes required for tumorigenesis may involve the activation of other oncogenes or the loss of suppressor genes, or both.

Another facsimile was created by the introduction of a retrovirally activated v-*myc* construct (J3) into pristane oil–treated, plasmacytoma-susceptible BALB/c mice (*63*). Plasmacytomas appeared earlier and in a higher frequency than in the pristane oil–exposed controls. Ten of 12 karyotyped plasmacytomas expressed the avian v-*myc*. They lacked the usual murine plasmacytoma–associated Ig/*myc* translocations, in contrast to the remaining two tumors that did not express v-*myc* and carried the typical 12;15 translocation. This is consistent with the postulated rate-limiting role of *myc*-activation in the genesis of murine plasmacytomas (*7*). However, the fact that the construct failed to induce murine plasmacytomas in the absence of pristane oil indicates the need for an additional change or changes. The mineral oil may act by increasing the frequency of chromosomal aberrations (*84*) or by stimulating the secretion of conditioning growth factors, or both, as required even by established plasmacytomas (*85*).

The great majority of endemic Burkitt lymphomas carry Epstein-Barr virus (EBV), and they all contain Ig/*myc* translocations. The order in which EBV versus *myc* activation acts during the tumorigenic process is controversial (*86, 87*). Experimental models have led to contradictory conclusions. EBV-transformed B-blasts are immortalized but nontumorigenic in vivo (*88*). They can be converted to tumorigenicity by transfection with activated *myc* constructs (*89*). Conversely, the B cell–derived low malignant BJAB lymphoma that has a low *myc* transcription rate can be converted to high *myc* expression and tumorigenicity by EBV (*90*).

Negative Control Can Prevent Illegitimate Activation of Oncogenes

Several cellular oncogenes are linked to regulatory sequences that may serve as safeguards against illegitimate activation, for example, by the insertion of retroviral enhancers. Such sequences were first identified near c-*mos*, a proto-oncogene that is not transcribed in adult somatic tissues. After activation by a retroviral LTR, a small number of *mos* protein molecules can transform NIH 3T3 cells. Vande Woude *et al.* (*17*) identified a sequence, UMF, approximately 1500 bp upstream of the murine c-*mos*, that can inhibit the activation of the gene by a downstream LTR. If inserted between the viral promoter and the ATG of the v-*mos* gene, UMF acts as a transcription terminator. Similarly powerful regulatory sequences have been found in the neighborhood of c-*fos* that appear to play a dynamic role in cell growth and differentiation. Verma *et al.* (*18*) and Meijlink *et al.* (*91*) showed that a stretch of 67 nucleotides, located downstream of the coding domain of c-*fos*, must be removed to confer transforming activity on the gene. They have suggested that the c-*fos* protein may regulate its own synthesis, perhaps by interacting with the 67-bp region or the carboxyl-terminal coding sequences, or both. Transcription of the human c-*fos* gene was modulated by both negatively and positively acting cellular factors that interacted with an upstream regulatory region (*92*). Serum-starved fibroblasts produced a repressor molecule that could block c-*fos* transcription. These are only the first examples in a rapidly developing field. It is already clear that the correct expression of the cellular proto-oncogenes is under strict control of multiple regulatory factors. They may act both at the transcriptional and the posttranscriptional level (*93*).

Suppression by Diffusible Products of Normal Cells

Paul (*94*) has recently summarized evidence indicating that small molecules, produced by normal cells, can diffuse in solid tissues through gap junctions and exert a damping effect on tumor cell precursors that contain activated oncogenes. If so, a second event may involve a reduction of the damping effect by modulating the gap junctions, or by creating a critical mass of transformed cells. Land *et al.* have shown that both *myc*- and *ras*-transformed rat fibroblasts can be suppressed by surrounding normal cells (*95*). Similar observations were made earlier by Stoker in relation to polyoma-transformed cells (*96*). Growth regulatory polypeptides that can inhibit the replication of certain cells, but may stimulate the growth of others has been demonstrated experimentally in several systems (*97*). They include an increasing number of known cellular products such as TGF-β and members of the interferon family (*97, 98*).

Concluding Remarks

It is widely accepted that tumor development and progression are due to sequential changes at the DNA level [for review see (*1*)]. This is reflected by the "reassortment of unit characteristics" at the phenotypic level (*2*). Several of the currently known oncogenes can block specific steps in the maturation progress. Constitutively activated growth factors may inhibit maturation by urging their target cell to proliferate. Truncated growth factor receptors or faulty signal transducers may achieve a similar effect by emitting a continuous "go" signal in the absence of external stimulation. DNA-binding proteins like *myc* of *myb* may block maturation by interfering with

the condensation of chromatin that is the hallmark of terminal differentiation in many cell lineages.

The category of genes described as antioncogenes, tumor suppressor genes, or emerogenes can antagonize tumorigenic behavior at various levels. Somatic hybridization of normal cells with malignant ones has provided evidence that the normal genome may provide the tumor cell with the ability to respond to appropriate differentiation-inducing stimuli in vivo.

The differentiation block imposed by temperature-sensitive v-*src*, a membrane-associated tyrosine kinase, or v-*erb*B, a truncated growth factor receptor, may be permanently lifted by the temporary down-regulation of the oncoprotein. Reexpression of the oncoprotein cannot halt or reverse the process; the potential "go" signal is apparently inactive when the cell has moved out from the sensitive "maturation window." Signals induced by physiological or chemical differentiation (or both) may down-regulate other highly expressed oncoproteins and thereby lift the maturation block. Such signals may even override the high expression of amplified *myc* genes.

Revertants fall into several categories. Deletion or mutational inactivation of viral oncogenes is relatively trivial. Cellular genes that can act beyond the level of oncoprotein expression are more interesting. They have been best documented in revertants isolated from *ras*-transformed fibroblasts. In the most extensively studied case (*14*), the transformed and tumorigenic phenotype of v-K-*ras*–infected fibroblasts was reverted by a dominantly acting cellular gene or genes, in spite of the continued presence of wild-type transforming virus and full expression of the p21 *ras* protein. The same gene or genes could also cancel the transforming action of *src*, *fes*, and all members of the *ras* family, but not *sis*, *fms*, *raf*, polyoma, and SV40. This approach may provide new leads for a functional oncogene classification, based on suppressor sensitivity.

The oncogene terminology has evolved through a series of historical accidents and is actually a misnomer, but it is here to stay. It embraces a wide variety of genes that can influence the cell cycle at different levels. The consensus to collect a variety of normal genes under a common, cancer-related name is based on the fact that they may contribute to tumor development when they get out of hand. As long as this is so, we might as well refer to the normal genes that can antagonize them in one system or another by the common name of emerogenes, as suggested by one of the originators of the oncogene terminology, George Todaro (*98*) (emero: [Greek] to tame, to domesticate). Some of them may control cell maturation. They, or other genes that act at different levels, may overrule the transforming action of highly expressed oncoproteins. They or the normal alleles of the recessive cancer genes may also prevent tumors by obstructing the development of progression of preneoplastic cells. The study of the emerogenes is experimentally more difficult than the pursuit of the oncogenes, but it may turn out to be even more rewarding.

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Research Articles

Reading Frame Selection and Transfer RNA Anticodon Loop Stacking

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Messenger RNA's are translated in successive three-nucleotide steps (a reading frame), therefore decoding must proceed in only one of three possible frames. A molecular model for correct propagation of the frame is presented based on (i) the measured translational properties of transfer RNA's (tRNA's) that contain an extra nucleotide in the anticodon loop and (ii) a straightforward concept about anticodon loop structure. The model explains the high accuracy of reading frame maintenance by normal tRNA's, as well as activities of all characterized frameshift suppressor tRNA's that have altered anticodon loops.

MAINTENANCE OF THE TRANSLATIONAL READING FRAME is essential for useful gene expression. However, the detailed mechanism by which ribosomes, transfer RNA's (tRNA's), and the message interact to minimize frameshifts has not been clearly defined.

Studies of the activities of tRNA's with eight, rather than the normal seven, nucleotides (nt's) in the anticodon loop have suggested that the length of the translational step is metered by the tRNA. Several such tRNA's have been isolated by selective suppression of single nucleotide insertion (frameshift) mutations in *Salmonella* (*1–3*) and yeast (*4, 5*).

However, previous data do not suggest a unified set of translocational properties. For example, some frameshift suppressor tRNA's act only when four anticodon nucleotide pairs can be formed, while others do not require a fourth nucleotide pair (*1–5*). None of these tRNA's has been tested for translation of 3-nt codons. Missense suppressors exist (*6, 7*) that contain an extra nucleotide in the anticodon loop, but decode 3-nt codons. Finally, there is a wild-type yeast mitochondrial tRNA with an 8-nt anticodon loop that presumably favors 3-nt codons (*8*). The rule that unites these observations was not evident.

We surmised that measurement of both 3-nt and 4-nt translation by individual 8-nt anticodon loop tRNA's would clarify reading frame maintenance. To systematically study the translational activities of such tRNA's, we constructed tRNA genes that contain each nucleotide inserted 5′ to the anticodon of an amber suppressor tRNA. Each of these tRNA's was tested for both 3- and 4-nt decoding efficiencies of an amber codon (UAG) created in *Escherichia coli lacZ*. We varied the nucleotide 3′ to the amber codon in the message in order to detect possible fourth nucleotide pair interactions between each tRNA's anticodon loop and the message.

Our mutant tRNA's translate the same 4-nt message sequence in these messages as a 3- or a 4-nt codon. We suggest that two readily

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