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# The Molecular Genetics of Cancer

J. MICHAEL BISHOP

**The search for genetic damage in neoplastic cells now occupies a central place in cancer research. Diverse examples of such damage are in hand, and they in turn hint at biochemical explanations for neoplastic growth. The way may be open to solve the riddles of how normal cells govern their replication and why cancer cells do not.**

CANCER MAY BE A MALADY OF GENES, ARISING FROM genetic damage of diverse sorts—recessive and dominant mutations, large rearrangements of DNA and point mutations, all leading to distortions of either the expression or biochemical function of genes. Is this suspicion correct, and, if so, what is the nature of the ailing genes and how do their ailments sustain neoplastic growth? These are the issues that now prevail in the fundamental research on cancer.

The belief that genetic damage might be responsible for cancer grew from diverse roots: the recognition of hereditary predispositions to cancer (*1, 2*), the detection of damaged chromosomes in cancer cells (*3*), the apparent connection between susceptibility to cancer and impaired ability of cells to repair damaged DNA (*4*), and evidence that relates the mutagenic potential of substances to their carcinogenicity (*5*). Now these roots have been joined by the discovery of cellular genes (proto-oncogenes) that in another form (oncogenes) can cause neoplastic growth.

Here I review the means by which proto-oncogenes have been identified and the evidence that damage to these genes may be involved in the genesis of cancer. I will not argue that the genetic ailments already found in cancer cells offer a full explanation for the malignant phenotype, only that these ailments are not merely adventitious and thus are likely to be part of the engine that drives neoplastic growth. The search for genetic damage and the explication of how that damage affects biochemical function represent seminal lines of inquiry into the mysteries of cancer.

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Dedicated to the memory of Richard C. Parker.

## Retroviruses and Cancer Genes

The life cycle of retroviruses has provided the first clues for identifying cellular genes that might participate in tumorigenesis (*6–8*). The single-stranded RNA of the diploid viral genome is transcribed into DNA by reverse transcriptase. Viral DNA is then integrated into chromosomal DNA, and the host cell uses its own machinery to express viral genes. The scenario presents two possibilities for unveiling cancer genes.

First, integration of viral DNA is potentially mutagenic: it can damage cellular genes directly (*7–9*), and it can influence their expression by bringing them under the control of powerful regulatory elements in the viral genome (*10*). These events (called "insertional mutagenesis") have been implicated in tumorigenesis by a variety of retroviruses. Second, recombination between retroviral and cellular genomes can implant cellular genes into the viral genome, and in this new setting the cellular genes may become oncogenic (*6–8*). The genesis of retroviral oncogenes from cellular proto-oncogenes has been called "transduction" (although strictly used, the term applies only after a gene has been transmitted from one cell to another by viral infection). Not all cellular genes are potential oncogenes, of course, but those that are have often come to the attention of investigators as a result of transduction by retroviruses.

In some instances, transduction follows insertional mutagenesis in close conjunction, and it then becomes difficult to say how each has contributed to tumorigenesis. The conjunction occurs at exceptionally high frequency during the induction of erythroleukemia by avian leukosis virus (*11, 12*) and of T-cell lymphoid tumors by feline leukemia virus (*13*), perhaps because the RNA transcribed from the mutant genes is well adapted for incorporation into retroviral particles.

## Retroviral Oncogenes

Twenty retroviral oncogenes are now known that together offer experimental models for most major forms of neoplasia (*14–17*). Each of these genes encodes a protein whose biochemical action adds to our understanding of the mechanisms of neoplastic growth. Moreover, at least nine retroviral oncogenes (v-*abl*, v-*erb*B, v-*ets*, v-*mos*, v-*myb*, v-*myc*, v-H-*ras*, v-K-*ras*, and v-*sis*) have added signifi-

cance because their cellular counterparts (proto-oncogenes) have also been incriminated in tumorigenesis. We might not have recognized these cellular genes or been alert to their tumorigenic potential without the clues provided by retroviral transduction.

Two different possibilities have been proposed to explain why the transduced oncogenes of retroviruses are pathogenic even though they derive from seemingly harmless cellular genes. First, expression of the transduced genes is driven by potent viral signals that the host cell often cannot control; sustained and abundant expression of an otherwise normal gene might cause neoplastic growth. Efforts to test this possibility have given ambiguous results and remain incomplete. When expressed at high levels by experimental means, most of the proto-oncogenes tested to date can transform established lines of cells but not primary explants of normal cells. There may be exceptions in both regards: the proto-oncogene c-*src* has not elicited a completely neoplastic phenotype in established cells (*18*), whereas c-*myc* and c-H-*ras* transform primary as well as established cultures when carried by viral vectors (*19, 20*).

Second, transduced genes generally acquire mutations while en route from proto-oncogene to oncogene (*6, 15*). Comparisons of retroviral oncogenes with their cellular progenitors have uncovered point mutations, deletions, and genetic substitutions in the viral alleles. In three instances, this genetic damage appears to release the biochemical activities of the gene products from allosteric controls: the mutations in v-*src* (*21*) and v-*erb*B (*22*) confer higher constitutive activity on the protein–tyrosine kinases encoded by the genes (they might also alter substrate specificities of the enzymes, but we have no evidence for this as yet), and mutations in the various alleles of v-*ras* appear to diminish the ability of the gene products to govern themselves by the hydrolysis of guanosine 5′-triphosphate (GTP) (*23*). Transformation by these three retroviral oncogenes may therefore result from sustained levels of otherwise normal biochemical activities.

## Insertional Mutagenesis and Proto-Oncogenes

Retroviruses that do not have oncogenes may nevertheless cause cancer. This frequently occurs by means of insertional mutagenesis (*10, 15*) as was first shown in the study of chicken lymphomas. In these tumors, the cellular gene c-*myc* has been activated by insertions of retroviral DNA upstream, within, or (on rare occasion) downstream of the gene (*24*). The activation of transcription from c-*myc* is generally thought to be the first of several steps in tumorigenesis. Ungoverned expression of a previously regulated or silent gene arises because the integrated viral DNA provides either an overpowering promoter from which transcription of the cellular gene now initiates or a transcriptional enhancer that energizes resident promoters for the cellular gene. Insertion of viral DNA may also elicit point mutations within an adjacent cellular gene (*25*), but the functional significance of these is not yet clear.

Five of the proto-oncogenes encountered first as retroviral oncogenes (c-*erb*B, c-*mos*, c-*myb*, c-*myc*, and c-H-*ras*) have also figured in tumorigenesis by insertional mutagenesis (*24, 26, 27*). Two genes that encode hemopoietic growth factors (IL2 and IL3) have been activated by insertional mutagenesis in leukemia cells, and, as a result, the cells do not require the factors from external sources (*28*). A cellular gene whose product (the protein p53 or "nonviral T antigen") was first implicated in neoplastic transformation by findings with papovaviruses has since been implicated by insertional mutagenesis, as well (*29*). In addition, a dozen new proto-oncogenes have been discovered by tracking retroviral DNA to its residence in the cellular genome (*15, 16*).

The details of insertional mutagenesis have helped illuminate the importance of genetic damage found in the transduced oncogenes of retroviruses.

1) The induction of erythroleukemia by avian leukosis virus in chickens apparently begins with the insertional mutagenesis of c-*erb*B (*11*), a gene that encodes the cell surface receptor for epidermal growth factor (*30*). The mutant allele of c-*erb*B resulting from insertion of retroviral DNA is a remarkable facsimile of the transduced oncogene v-*erb*B, duplicating an amino-terminal truncation of the gene product that is apparently responsible for transforming activity (*11*).

2) The transduction that gave rise to the viral oncogene v-*myb* truncated the proto-oncogene c-*myb* at both of its ends (*31*). Insertional mutagenesis by a murine retrovirus in myeloid leukemia cells duplicates these truncations separately: at the 5′ end of c-*myb* in some instances, at the 3′ end in others (*26*). Perhaps the combination of the two lesions in v-*myb* accounts for the rapid and apparently unaided tumorigenesis by the viral oncogene.

There is as yet no direct evidence that activation of proto-oncogenes by insertional mutagenesis is tumorigenic, but the argument nevertheless has considerable logical force. First, several of the genes attacked by integration were already known as progenitors of retroviral oncogenes. Second, integration of retroviral DNA displays little (if any) specificity within the cellular genome, yet integration in the tumors affects specific proto-oncogenes—the consequence of selection for cells that have undergone neoplastic transformation. Third, retroviral vectors have been used to demonstrate that the mutant proto-oncogenes have biological activity. Examples include c-*myc* and c-*erb*B, the mutant versions of which can transform cells in culture and induce tumors in animals (*19, 32, 33*); and the proto-oncogene *int*-1, first identified by insertional mutagenesis (*34*) and also able to elicit phenotypic changes in cultured cells when carried in a viral vector (*35*).

## Damaged Chromosomes in Cancer Cells

Cancer cells have provided clues to oncogenes in the form of microscopically visible damage to chromosomes. Three types of damage have been especially revealing: translocations between (or inversions within) chromosomes, deletions affecting discrete portions of chromosomes, and abnormal amplification of large domains within chromosomes. Translocations and amplification have typically affected proto-oncogenes of the conventional sort, whereas deletions on occasion may signal the existence of a different type of genetic element also involved in tumorigenesis.

## Chromosomal Translocations

Many observers at first demeaned chromosomal translocations by regarding them as a manifestation of adventitious genetic damage. Attitudes changed, however, when molecular dissections revealed that the breakpoints where portions of two chromosomes are joined together by translocations can lie within or adjacent to proto-oncogenes (*36*). Once again, the argument in favor of etiological significance was sustained by the fact that several translocations affect proto-oncogenes already known from the study of retroviruses (*15, 16*). In other instances, the DNA that adjoins breakpoints may harbor new candidates for designation as proto-oncogenes (*37*). One candidate (a locus implicated in human lymphomas and known as *bcl*-2) has now been sequenced, and its gene products have been provisionally identified (*38*).

Translocations can affect either the expression or the biochemical function of proto-oncogenes. Effects on expression are exemplified

by the translocations that join c-*myc* to various immunoglobulin genes in Burkitt lymphoma (*36*) and mouse plasmacytomas (*39*). The consequences of these joinings remain controversial, but several possibilities have come into focus. First, transcription of c-*myc* into RNA may be released from its usual controls, allowing expression of the gene at inappropriate times. Second, regulatory influences provided by the immunoglobulin genes may drive the expression of c-*myc* to levels that are higher than normal. Third, damage inflicted on c-*myc* by translocation may increase the stability of messenger RNA (mRNA) derived from the gene. In consequence, the abundance of the mRNA would increase and become less accessible to rapid modulation. At least some of the structural signals governing the stability of the mRNA for c-*myc* are apparently located in the first (untranslated) exon of the gene, and excision of this exon by translocation (if excision occurs) could therefore be the root of the problem (*40*). Fourth, truncation of the mRNA for c-*myc* by loss of the first exon might enhance translation of the RNA into protein (*41*), although the balance of available evidence now argues against this possibility (*42*). Point mutations have also been observed in translocated forms of c-*myc* (*43*), but the functional significance of these mutations has not been explored.

The Philadelphia chromosome that typifies the cells of chronic myelogenous leukemia embodies the second kind of genetic damage imposed by translocations. A reciprocal exchange between chromosomes 9 and 22 relocates a portion of the proto-oncogene c-*abl* and fuses it with a newly recognized genetic locus known for the moment as *bcr* (for "breakpoint cluster region") (*44, 45*). The genetic fusion in turn creates a chimeric protein that includes the functional domain of the c-*abl* gene product, but whose enzymatic activity is ostensibly more robust than that of the normal gene product (*46*). The translocation has thus produced a mutation that affects the biochemical function rather than the level of expression of a gene product, although the latter may be abnormal as well (*44, 47*). These are gratifying findings because they represent a molecular description of the first chromosomal translocation to be found in human malignancy (*48*).

The karyotypic instability of cancer cells is commonplace and could represent effect rather than cause. But several diverse observations nevertheless suggest that the translocation of proto-oncogenes can play a role in tumorigenesis. Some translocations occur with great consistency in particular tumors (*3*) and can affect the same proto-oncogene in different species [for example, c-*myc* in B-cell tumors (*15*)]. Three of the proto-oncogenes first recognized during the study of retroviral oncogenes (c-*abl*, c-*ets*, and c-*myb*) have now been implicated in translocations that exemplify various forms of malignancy (*36, 44, 45, 49*). Translocation of a proto-oncogene can damage both the structure and the function of the gene in ways that echo those found in the transduced and overtly oncogenic version of the same gene (*50*). Finally, mice carrying an experimentally introduced facsimile of translocated c-*myc* in their germinal DNA develop lymphoid tumors (*51*).

## Gene Amplification and Proto-Oncogenes

Focal amplification of domains within chromosomes is a scheduled and purposeful event during the life cycles of diverse organisms (*52*). In mammals, however, gene amplification is an unscheduled aberration whose presence is often signaled by two karyotypic abnormalities, double-minute chromosomes and homogeneously staining regions that disrupt the normal banding patterns of chromosomes. Amplified DNA was first encountered in mammalian cells as a means by which leukemia cells acquire resistance to the chemotherapeutic agent methotrexate (*53*), but it is now clear that untreated cancer cells can also contain amplified DNA and that the amplification can include proto-oncogenes.

Amplification of proto-oncogenes has been found in two patterns: as an occasional feature of diverse tumors (*15, 54*) and as a recurrent abnormality of specific proto-oncogenes in particular tumors (*55, 56*). Here c-*myc* has again been a touchstone because two genes recently shown to be similar to it (genes now designated L-*myc* and N-*myc*) have emerged as important components of amplified DNA in several types of human tumors. The cause of gene amplification in mammalian cells and the mechanisms by which it occurs remain enigmatic, but the phenomenon has generally been found only in cells that have taken at least some of the steps toward neoplastic growth (*52*). If amplification does not occur in normal cells [the issue remains unresolved; for example, see (*57*)], then it will play a role not in the initiation but in the later steps of tumorigenesis. It is indeed progression of the neoplastic phenotype in which amplification of proto-oncogenes has generally been implicated (*55, 58*).

Why should we attribute etiological significance to gene amplification in cancer cells? There are three reasons: because amplification frequently affects proto-oncogenes whose ability to alter the proliferation of cells has been demonstrated in other settings (*15, 54, 59*); because the amplification of a proto-oncogene sometimes correlates with a particular feature of cancer cells, as if one were cause and the other effect (*55, 58*); and because amplified DNA persists in mammalian cells only if it provides a selective advantage to the cells (*52, 53*), as it must be doing in the cancer cells where it has survived countless rounds of cell division.

## Finding Oncogenes by Gene Transfer

The DNA of tumor cells often contains oncogenes that can transform cells in culture to neoplastic growth (*60*). Transforming activity has been detected in the DNA of approximately 20% of all the specimens tested, cell lines and original tumors alike, representing a large variety of malignancies. Oncogenes identified by gene transfer are mutant alleles of normal cellular genes (*61–63*). The mutations account for the transforming activity of the oncogenes; they have so far proved to be somatic mutations, restricted to tumor tissue; they presumably arise from either the initial action of a carcinogen or a misstep by the machinery that replicates and sustains the integrity of cellular DNA.

The first of these oncogenes to be identified were alleles of c-*ras* containing point mutations that cause single substitutions of amino acids in the gene products, generally at residue 12 or 61 (*15*). The monotony of the mutations probably represents a consequence of biological selection; therefore, the mutant genes likely were important in the genesis of the tumors from which they were isolated. An alternative explanation would be "hot spots" for mutagenesis within the c-*ras* genes, but transforming alleles obtained by random mutagenesis of c-*ras* in vitro carry mutations in the same locales as the mutations isolated from tumors (*64*).

Gene transfer has now revealed a diverse assortment of oncogenes, many of which are newly identified (*15, 16*). Most of these carry abnormalities that can be traced to the tumor cells from which the DNA originated, but a few were damaged (and thus became active) during experimental manipulations. We know little about the genetic damage responsible for the transforming activity of these genes: at least one (*neu*) owes its activity to point mutations that cause an amino acid substitution at a single residue within the gene product (*62*), whereas two others (*mas* and *met*) are apparently active because of damage to the elements that control their expression (*63*).

The point mutations found in c-*ras* genes are analogous to mutations in v-*ras* and thus have the same biochemical effect: the mutant protein apparently does not regulate itself by hydrolysis of GTP (*22*). In contrast, the protein encoded by *neu* is a cell-surface receptor for a presently unknown hormone: it spans the plasma membrane, displays tyrosine kinase activity based in the cytoplasmic domain of the protein, and possesses an extracellular domain similar to that of the receptor for epidermal growth factor (*62, 65*). The mutations in *neu* identified thus far are located within the portion of the gene product that traverses the plasma membrane. By unknown means, changes in this portion of the protein must activate it, perhaps by forcing it into a configuration similar to that achieved by the binding of a physiological ligand. The activation of *neu* may therefore be another example of how the loss of allosteric control can confer pathogenicity on the product of a proto-oncogene.

Oncogenes detected so far by gene transfer in randomly selected specimens of tumor cells have occurred sporadically: no single type of tumor has consistently harbored such an oncogene, nor has point mutation of a given proto-oncogene been consistently implicated in the genesis of a specific tumor. However, there are now examples in which experimental carcinogenesis has repeatedly evoked the same neoplasm, carrying the same oncogene with a mutation that can be traced to the original carcinogen (*66–68*). In some instances, mutation of the proto-oncogene is demonstrably an early event that apparently initiates but does not suffice for tumorigenesis: further events are required such as the action of a tumor promoter or the occurrence of additional genetic damage or both (*68*). The consistency observed in these experimental systems presumably reflects the strict protocols used to induce tumorigenesis. By contrast, the naturally occurring tumors from which mutant genes have been isolated only sporadically are presumably attributable to diverse etiological agents, encountered at various times during the life span of the host.

The use of gene transfer to detect oncogenes has been criticized because the cells used in the assays are themselves abnormal. The criticism seems specious: whatever its idiosyncrasies, gene transfer has proved a sensitive device by which to ferret out genetic lesions in cancer cells, and its utility may grow when the types of cells used in the assay are diversified (*69*). The abundance of genetic lesions detected by gene transfer, their restriction to neoplastic cells (except for the occasional instances when they arise from damage incurred during experimental manipulation), their reproducible occurrence in diverse experimental tumors, their correspondence to the carcinogens used in experimental settings, their phenotypic effects on both established and primary cultures of cells, their frequent presence in proto-oncogenes that have counterparts among the tumorigenic oncogenes of retroviruses, and their resemblance to the damage found in retroviral oncogenes all combine to make a powerful argument for authenticity and etiological importance.

## The Functions of Proto-Oncogenes and Oncogenes

There are almost 40 proto-oncogenes and oncogenes (*15, 16*), yet we can so far name only four biochemical mechanisms by which this rich diversity of proteins may act: protein phosphorylation, with either tyrosine or serine and threonine as the substrate amino acids (*70*); metabolic regulation by proteins that bind GTP in the manner of the familiar G or N proteins (*71*); control of gene expression by influencing the biogenesis of mRNA (*72*); and participation in the replication of DNA (*73*). The details of these mechanisms have been reviewed recently (*14, 17, 74*).

**Table 1.** Recessive genetic lesions in human cancer.

| Tumor | Chromosomal locus | References |
|---|---|---|
| Retinoblastoma and osteosarcoma | 13(q14) | (*84*) |
| Wilms's tumor (nephroblastoma) | 11(p13) | (*85*) |
| Embryonal tumors of the kidney, muscle, liver, and adrenal gland (Beckwith-Wiedemann syndrome) | 11p | (*86*) |
| Bladder carcinoma | 11p | (*87*) |
| Acoustic neuroma or meningioma | 22 | (*88*) |

There are three explanations for how genetic damage might cause the malfunction of a proto-oncogene or its product.

1) The damage might cause constitutive activity: the oncogene or its product cannot be regulated, but the level of expression is no greater than the usual maximum. For example, translocation of c-*myc* may on occasion strip the gene of the elements that normally modulate its transcription; mutations in v-*src*, v-*erb*B, and *neu* apparently confer constitutive activity on the gene products; and the mutant alleles of c-*ras* encode proteins that have suffered a reduction in the biochemical activity (guanosine triphosphatase) by which they normally limit the duration of their own action.

2) The abnormality may be a surfeit of an otherwise normal gene product, the consequence, for example, of gene amplification, translocation into the vicinity of a strong transcriptional enhancer, insertion of retroviral DNA, or transduction into a retroviral genome. It is useful to keep this category distinct from the preceding one because there is experimental evidence that abundance is an important determinant of how the product of a proto-oncogene or oncogene affects cellular phenotype (*75, 76*).

3) Mutations might change the manner in which a protein acts. Examples include alterations in the substrate specificity of a protein kinase or in the specificity of a transcription factor. This possibility has great intuitive appeal, but there is as yet no decisive evidence that it ever applies.

How can we fit these themes into the context of cellular replication? The proliferation of cells is governed by an elaborate circuitry that reaches from the surface of the cell to the nucleus. The products of proto-oncogenes may represent some of the junction boxes in that circuitry (Fig. 1): polypeptide hormones that act on the surface of the cell, receptors for these hormones, proteins that carry signals from the receptors, and nuclear functions that may orchestrate the genetic response to afferent commands. What we now know of oncogenes allows us to view their actions as "short circuits" at the corresponding junction boxes. This imagery is at best only a first approximation. For example, some proto-oncogenes may have roles in regulating differentiation or in the maintenance of fully differentiated cells rather than in cellular proliferation (*6*), a possibility that is not addressed by the circuitry envisioned here.

## Recessive Mutations and Cancer

The possibility that recessive mutations might underlie the neoplastic phenotype has been a persistent theme in cancer research (*77*). The theme has two sources.

1) Experimental fusion of normal and cancer cells often suppresses the neoplastic phenotype (*77–79*). The suppressive activity has been attributed to particular chromosomes contributed by the normal partner in the fusion, and the cancer cells therefore appear to be defective in functions that are required for the regulation of cellular phenotype. The nature of these functions is unknown, but in at least some settings the defect seems to deprive cells of the ability

to differentiate and thus destines them to incessant proliferation (*78, 80*).

2) By inference from karyotypes and by the use of both enzymatic and physical markers for chromosomal domains, evidence has been obtained for the presence of recessive genetic lesions in a variety of human tumors (Table 1). The tumors are either hemi- or homozygous for the lesions and thus may be completely deficient in one or more gene functions (although it is also possible that the damaged genes produce proteins that manifest abnormal activities only in a recessive manner). The chromosomal locus affected by recessive genetic damage in human retinoblastomas has been identified and isolated by molecular cloning (*81, 82*); in this instance, there may be only one gene at fault, and the genetic lesion appears to cause loss of function.

How are these findings related to the evidence that many tumors contain oncogenes whose products act directly on cellular phenotype? Some observers have argued that oncogenes may not act in a dominant fashion (*77, 79*). Correct or not, however, the argument does not belie the fact that the products of oncogenes must be active in the cell for their pathogenic influence to be felt, in possible contrast to the recessive genetic lesions described above. Two kinds of genetic elements may therefore figure in the genesis of cancer. One is pathogenic only if it produces an active protein, the other may play an etiological role when it is inactive or absent. Might these two kinds of elements interact with one another, and, if so, how? The usual answer to this question is that recessive genetic damage might remove regulatory functions and thus unleash potential oncogenes. This scheme has prompted some observers to dub the regulatory elements themselves "anti-oncogenes" (*83*).

Whatever their nature might be, recessive mutations represent an additional dimension in the molecular genetics of cancer, because they may explain the hereditary diatheses to several forms of human cancer (*83–89*). By contrast, there is as yet no evidence to implicate any of the conventional proto-oncogenes or oncogenes in the inheritance of cancer, although the creation of transgenic mice carrying activated versions of c-*myc* has illustrated how inherited abnormalities of proto-oncogenes might contribute to tumorigenesis (*51, 90*).

## Tumor Progression

Malignant tumors arise from a protracted sequence of events whose components have never been decisively counted or named. The common view is that each step in the sequence creates an additional phenotypic aberration. For example, an emerging cancer cell might independently acquire the capabilities for extended proliferation, invasion of adjacent tissue, and metastasis. These steps are broadly defined, however, and each may be the manifestation of more than one new abnormality within the cancer cell.

How much of the progression during tumorigenesis arises from genetic damage is unknown, but there are hints that oncogenes may eventually fit into the scheme. Insertional mutagenesis by retroviruses (*10*) and point mutations induced in proto-oncogenes by chemical carcinogens (*67, 68*) may on occasion exemplify initial steps in tumorigenesis. There has been some provisional success in the pursuit of genes that can confer the ability to metastasize on cells already capable of abnormal proliferation (*91*). In some settings, mutation of c-*ras* may account for the appearance of a new and more aggressive variant of tumor cell (*92*). Amplification of several proto-oncogenes has been implicated as an advanced step in the emergence of highly malignant tumors (*55, 58*). There are examples of tumor cells that display damage to two or more proto-oncogenes, and these genes may embody independent steps in tumorigenesis (*93, 94*). It

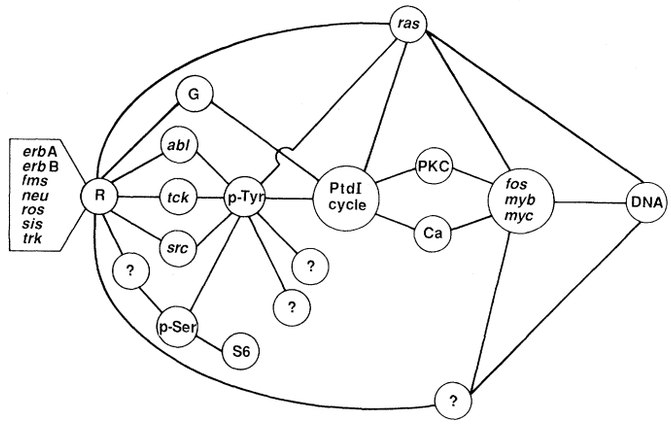


**Fig. 1.** Circuitry for the regulation of cellular proliferation. The diagram illustrates how some of the functions encoded by proto-oncogenes might fit into the circuitry that regulates the proliferation of vertebrate cells. The scheme is in part hypothetical and is not intended to be comprehensive. The supporting literature is too extensive for citation here. Functions encoded by proto-oncogenes have been designated by conventional terminology for the genes themselves. Other abbreviations include: G, G or N regulatory proteins; R, receptor; p-Ser, phosphoserine; p-Tyr, phosphotyrosine; S6, a ribosomal protein that is phosphorylated in response to diverse mitogenic signals; PtdI, phosphatidylinositol; PKC, protein kinase C.

has become increasingly common to find tumor cells in which the same proto-oncogene has been damaged in more than one way (*25, 43, 58, 93, 95*)—a surprising variation on the theme that multiple events are required to achieve the malignant phenotype. Both DNA-mediated gene transfer (*96*) and retroviruses (*97*) have been used to demonstrate experimentally how two different oncogenes can cooperate to generate a neoplastic cell. Finally, even tumorigenesis, initiated by a combination of c-*myc* and c-*ras* may require events beyond the action of the oncogenes, in one instance, the loss of a specific chromosome (*98*).

Although a combination of genetic lesions may contribute to tumorigenesis and embody at least some of the steps in tumor progression, there is no cause at present to assign inevitable roles in tumorigenesis to individual genes. For example, different settings find c-*myc* and c-*ras* implicated in either initiation (*10, 15, 67, 68*) or advanced progression (*55, 58, 92*) of the neoplastic phenotype.

Do all of the separate steps in tumorigenesis always represent damage to different genes? Perhaps not.

1) When recessive mutations figure in tumorigenesis, both copies of a gene have been damaged or deleted (*83–86*). Inherited cases of retinoblastoma and Wilms's tumor dramatize the fact that damage to each copy of a gene is an independent event and thus appears as a separate step in tumorigenesis (*83, 89*). Some observers have even proposed that a complete deficiency for a single genetic function can suffice to account for tumorigenesis, that no other genetic damage need be involved (*77, 83*).

2) The experimental induction of skin carcinomas in mice provides a graphic example of how a single proto-oncogene can suffer two types of genetic damage in sequence (*99*). The initial carcinogen induces benign papillomas that are heterozygous for a point mutation in one allele of a c-*ras* gene. As the tumor progresses to malignancy, however, the mutant allele may become homozygous or amplified, as if additional changes at the c-*ras* locus might contribute to progression.

3) The transformation of cultured cells can be performed in ways that require the combined actions of two different oncogenes (*96*). These findings have been taken to represent the requirement for multiple events in tumorigenesis, and they have been used to suggest that the cooperating oncogenes have different physiological assign-

ments: one drives the proliferation of cells, the second elicits other aspects of the neoplastic phenotype. It is now clear, however, that suitable experimental manipulations can expand the potency of single oncogenes: those that were previously thought to elicit only indefinite growth can in reality bestow other aspects of the neoplastic phenotype on cells (*19, 32, 33*), and those that were previously thought to require another oncogene to drive indefinite growth can in reality do so themselves (*76, 100*).

The notion that tumorigenesis generally arises from a combination of genetic ailments affecting multiple genes remains attractive. I have raised caveats not to challenge that view, but to dramatize how much we have to learn.

## Conclusion

Cancer has myriad causes, but many of these may act in a common way—by damaging DNA. By one means or another, on the basis of circumstantial evidence of considerable variety, damage to diverse proto-oncogenes has been implicated in the genesis of human tumors (Table 2). The same genetic lesions have been found repeatedly in the DNA of human tumors, in original specimens as well as explanted cell lines. With frequencies that seem beyond coincidence, these lesions have involved proto-oncogenes already identified by retroviral transduction, and the damage carried by the genes is of a sort we know to be pathogenic. Provocative correlations can be made between at least some of the genetic lesions and distinctive features of the tumors in which they are found. Several proto-oncogenes are affected by diverse forms of genetic damage: c-*myc* provides the most visible example, because it has figured in mutagenesis by the integration of retroviral DNA, in chromosomal translocations, and in gene amplification.

How can the role of oncogenes in the genesis of human tumors be tested directly? One obvious strategy is to seek changes in the phenotype of cancer cells when the actions of oncogenes are reversed by experimental means. For example, antibodies directed against the products of *ras* and *neu* have been used to suppress the neoplastic phenotype of cells carrying transforming alleles of these genes (*101, 102*). Alternatively, replacement of genes inactivated by recessive mutations might restore cells to normal behavior. A test of this possibility appears to be in the offing for human retinoblastoma (*82*). Given the complexities of the cancer cell, however, neither strategy may prove sufficient. Hints of such difficulties come from the finding that antibodies directed against the *neu* protein cannot suppress the growth of cells expressing transforming alleles of both *neu* and *ras* (*102*).

Genetic damage remains undetected in the great majority of human tumors. We may have to invent new ways to search for this damage, and we must remain open to the possibility that we will not always find it because it is not always there. But as the genome of the cancer cell is better understood, we hope to acquire new devices for the prevention, diagnosis, and therapy of cancer; and we may eventually achieve an even grander goal, to grasp the designs that order the lives of our cells.

**Table 2.** Proto-oncogenes in human tumors. The table lists genetic lesions that are found at some reasonable frequency (varying from 20 to 100%) in the listed tumors. Most of the data on which this summary is based are cited in (*15*).

| Proto-oncogene | Neoplasm | Lesion |
|---|---|---|
| c-*abl* | Chronic myelogenous leukemia | Translocation |
| c-*erb*B | Squamous cell carcinoma | Amplification |
| | Glioblastoma | |
| c-*myc* | Burkitt's lymphoma | Translocation |
| | Small cell carcinoma of lung | Amplification |
| | Carcinoma of the breast | |
| L-*myc* | Small cell carcinoma of lung | Amplification |
| N-*myc* | Neuroblastoma | Amplification |
| | Small cell carcinoma of lung | |
| c-*ras* | Diverse | Point mutation |


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