28. M. C. Yu, J. H. C. Ho, S. H. Lai, *Cancer Res.* **46**, 956 (1986).
29. J. V. Joossens and J. Geboers, *Nutr. Cancer* **2**, 250 (1981); P. Correa, *Cancer Res.* **48**, 3554 (1988); T. Hiryama, *Gann Monogr.* **11**, 3 (1971); W. Haenszel, M. Kurihara, M. Sequ, *J. Natl. Cancer Inst.* **49**, 969 (1972); L. Bernstein, B. Henderson, *J. Cancer Res. Clin. Oncol.* **110**, 184 (1985); S. Tsugane *et al.*, *Cancer Causes Control* **2**, 165 (1991).
30. W. C. Willett *et al.*, *N. Engl. J. Med.* **323**, 1664 (1990).
31. J. W. Morgan, G. E. Fraser, R. L. Phillips, *Am. J. Epidemiol.* **128**, 918 (1988).
32. W. Willett *et al.*, *N. Engl. J. Med.* **316**, 22 (1987).
33. P. K. Mills *et al.*, *Cancer* **64**, 582 (1989).
34. G. R. Howe, C. M. Friederreich, M. Jain, *J. Natl. Cancer Inst.* **83**, 336 (1991).
35. P. K. Mills *et al.*, *Cancer* **64**, 598 (1989).
36. A. W. Hsing *et al.*, *Cancer Res.* **50**, 6836 (1990).
37. G. N. Stemmerman, A. M. Y. Nomura, L. K. Heilbrun, *ibid.* **44**, 4633 (1984).
38. C. Garland *et al.*, *Lancet* **i**, 307 (1985).
39. T. Hiryama, in *Diet, Nutrition and Cancer*, Y. Hayashi *et al.*, Eds. (Japan Scientific Societies, Tokyo, 1986), p. 41.
40. P. Y. Jones *et al.*, *J. Natl. Cancer Inst.* **79**, 465 (1987).
41. R. K. Severson, A. M. Y. Nomura, J. S. Grove, *Cancer Res.* **49**, 1857 (1989).
42. M. J. Wargovich, V. W. Eng, H. L. Newmark, *Cancer Lett.* **23**, 253 (1984).
43. E. Lanza and P. Greenwald, *Cancer Prev.* ((*Philadelphia*) **89**, 1 (September 1989).
44. M. Shike and S. J. Winawer, *ibid.* **90**, 1 (February 1990).
45. R. L. Prentice and L. Sheppard, *Cancer Causes Control* **1**, 81 (1990).
46. G. Howe, *ibid.*, p. 99; J. E. Hiler and A. J. McMichael, *ibid.*, p. 101; W. C. Willett and M. D. Stampfer, *ibid.*, p. 103; R. L. Prentice and L. Sheppard, *ibid.*, p. 53.
47. W. J. Blot *et al.*, *Cancer Res.* **48**, 3282 (1988); J. M. Elwood, J. C. G. Pearson, D. H. Skippen, *Int. J. Cancer* **34**, 603 (1984).
48. S. Barra *et al.*, *Int. J. Cancer* **46**, 1017 (1990); A. J. Tuyns, G. Péqoiqnot, J. S. Abbatucci, *ibid.* **23**, 443 (1979).
49. A. H. Wu, A. Paganini-Hill, R. K. Ross, *Br. J. Cancer* **55**, 687 (1987); M. P. Longnecker *et al.*, *Cancer Causes Control* **1**, 59 (1990); U. A. Simanowski *et al.*, *Gut* **27**, 278 (1986).
50. M. P. Longnecker *et al.*, *J. Am. Med. Assoc.* **260**, 652 (1988).
51. G. Block, *Am. J. Clin. Nutr.* **53**, 2705 (1991).
52. E. R. Greenberg *et al.*, *N. Engl. J. Med.* **323**, 789 (1990).
53. B. E. Henderson, R. Ross, L. Bernstein, *Cancer Res.* **48**, 246 (1988).
54. T. J. A. Key and M. C. Pike, *Eur. J. Cancer Clin. Oncol.* **24**, 29 (1988).
55. ———, *Br. J. Cancer* **57**, 205 (1988).
56. L. Bernstein and B. Henderson, *Cancer Prev.* (*Philadelphia*) **90**, 1 (January 1990).
57. L. Bergkvist *et al.*, *N. Engl. J. Med.* **321**, 293 (1985).
58. L. Bernstein *et al.*, *Cancer Causes Control* **1**, 51 (1990); H. Shimizu *et al.*, *Br. J. Cancer* **62**, 451 (1990); T. J. A. Key *et al.*, *ibid.*, p. 631; M. C. Pike, in *Accomplishments in Cancer Research 1989*, J. G. Fortner and J. E. Rhodes, Eds. (Lippincott, Philadelphia, 1990), p. 327.
59. J. Casagrande *et al.*, *Lancet* **ii**, 170 (1979).
60. R. K. Ross, A. Paganini-Hill, B. E. Henderson, in *Genitourinary Cancer*, D. G. Skinner and G. Lieskovsky, Eds. (Saunders, Philadelphia, 1988), p. 40.
61. R. L. Noble, *Cancer Res.* **37**, 1929 (1977).
62. M. C. Bosland, *Prostatic Carcinogenesis* (University of Utrecht, Utrecht, 1989).
63. R. K. Ross *et al.*, *J. Natl. Cancer Inst.* **76**, 45 (1986).
64. B. E. Henderson *et al.*, *Br. J. Cancer* **57**, 216 (1988).
65. L. Bernstein *et al.*, *ibid.* **55**, 681 (1987); S. D. Harlow and G. M. Matanoski, *Am. J. Epidemiol.* **133**, 38 (1991).
66. D. Garabrant, J. Peters, T. Mack, *Am. J. Epidemiol.* **119**, 1005 (1984).
67. V. C. Jordan, *Cancer Prev.* (*Philadelphia*) **90**, 1 (August 1990); R. L. Prentice, *J. Natl. Cancer Inst.* **82**, 1310 (1990); D. V. Spicer, M. C. Pike, B. E. Henderson, *ibid.* **83**, 63 (1991).
68. L. Villard and M. Murphy, *Int. J. Epidemiol.* **19**, 255 (1990).
69. M. C. Pike *et al.*, *Br. J. Cancer* **60**, 42 (1989); D. V. Spicer *et al.*, *Contraception* **44**, 289 (1991).
70. B. E. Henderson *et al.*, *Br. J. Cancer* **48**, 437 (1983).
71. A. Herbst *et al.*, *Am. J. Obstet. Gynecol.* **154**, 814 (1986).
72. R. K. Ross *et al.*, *Cancer Res.* **49**, 1045 (1989); S. Patierno *et al.*, *ibid.*, p. 1038; A. Paganini-Hill, A. Chao, R. K. Ross, *Br. Med. J.* **299**, 1247 (1989).
73. S. Preston-Martin, D. C. Thomas, M. C. Yu, *Br. J. Cancer* **59**, 639 (1989).
74. D. C. Thomas and S. Preston-Martin, *Technical Report No. 32* (Department of Preventive Medicine, University of Southern California, Los Angeles, CA).
75. B. E. Henderson, *J. Natl. Cancer Inst.* **81**, 320 (1989).
76. R. P. Beasley, C. C. Lin, L. U. Hwang, *Lancet* **ii**, 1129 (1981); H. C. Whittle *et al.*, *ibid.* **337**, 747 (1991).
77. H. zur Hausen, *Science* **254**, 1167 (1991).
78. W. L. Peterson, *N. Engl. J. Med.* **334**, 1043 (1991).
79. D. Forman *et al.*, *Int. J. Cancer* **46**, 608 (1990); A. Nomura *et al.*, *N. Engl. J. Med.* **325**, 1132 (1991); J. Parsonnet *et al.*, *ibid.*, p. 1127.
80. J. Peto, B. E. Henderson, M. C. Pike, in *Banbury Report 9: Quantification of Occupational Cancer*, R. Peto and M. Scheiderman, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1981), p. 51.

Tumor Suppressor Genes

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For the past decade, cellular oncogenes have attracted the attention of biologists intent on understanding the molecular origins of cancer. As the present decade unfolds, oncogenes are yielding their place at center stage to a second group of actors, the tumor suppressor genes, which promise to teach us equally important lessons about the molecular mechanisms of cancer pathogenesis.

THE PROLIFERATION OF NORMAL CELLS IS THOUGHT TO BE regulated by growth-promoting proto-oncogenes counterbalanced by growth-constraining tumor suppressor genes. Mutations that potentiate the activities of proto-oncogenes create the oncogenes that force the growth of tumor cells. Conversely, genetic lesions that inactivate suppressor genes liberate the cell from the constraints imposed by these genes, yielding the unconstrained growth of the cancer cell. These two end results—deregulated growth resulting from oncogene activation or from suppressor gene inactivation—would seem to be similar if not identical. However, accumulating evidence suggests that they are indeed quite different physiologically and that the progression of many tumors to full malignancy requires both types of changes in the tumor cell genome.

The existence of tumor suppressor genes becomes most apparent when they are missing from cell genomes. This simple fact underlies the experimental difficulties in studying them and the attendant 10-year lag of this research behind that focused on oncogenes. But these barriers to progress have now been breached, due in large part to recently developed strategies of gene isolation. As a consequence, tumor suppressor genes promise as rich a harvest in the 1990's as oncogenes yielded a decade earlier (*1*). This review attempts to place these genes in a conceptual framework and to discuss in some detail six of these that have been isolated as molecular clones.

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A Brief History of Tumor Suppressors

The evidence for the existence of tumor suppressor genes converged from several distinct lines of work. Early evidence came from somatic cell hybridization, which showed that fusion of tumor cells with normal cells almost invariably results in the outgrowth of nontumorigenic hybrids (*2*). These experiments showed that the normal cells were donating genetic information capable of suppressing the neoplastic phenotype of their tumor cell partners. By extension, the tumor cells lacked this genetic information, having lost it during their evolution from normalcy to malignancy.

These hybrid cells had unstable karyotypes and frequently shed chromosomes originating from one or another parent. When the chromosomes from the normal parent were lost, the hybrid cells would often revert back to a tumorigenic state. Correlation of this reversion to tumorigenicity with the loss of specific normal chromosomes led to the conclusion that these chromosomes carried genes that were missing from the genomes of tumor cells and could act to normalize the growth program of the cancer cells (*3*). In sum, this work provided the first clue that cancer cells often lose critical growth-regulating information during their progression toward to full malignancy.

Human genetics provided a second clue that suggested the existence of tumor suppressor genes. Twenty years ago, Knudson postulated that the rare childhood eye tumor retinoblastoma is triggered by two successive lesions in the cell genome (*4*). In proposing this, he addressed the two forms of this disease—familial and sporadic. In sporadic retinoblastoma, seen in children without a family history of the disease, he argued that both lesions are sustained in the retinal cell lineage as somatic mutations occurring long after conception. For familial retinoblastoma, he proposed that one of the two mutations is acquired from a genetically afflicted parent or originates during gametogenesis; the second required mutation then occurs as a somatic event.

This work had little apparent connection with tumor suppressor genes until the nature of these germline and somatic mutations became apparent; they serve to inactivate a chromosome 13–associated gene termed RB. Such a conclusion was first indicated by karyotypic analyses that occasionally uncovered interstitial deletions that involved the q14 band of chromosome 13 prepared from retinoblastoma tumor cells (*5*). An elegant series of genetic analyses culminating in 1983 led to the proof that Knudson's two elusive genetic targets were the two copies of the 13q14-associated RB gene, and that the two mutational events proposed by him involved the inactivation of both functional copies of this gene (*6*).

The demonstrated loss of RB gene function caused this gene to become allied with the "tumor suppressing" genes uncovered through cell fusion experiments. In both cases, critical genetic information was apparently lost from tumor cell genomes. Moreover, it became clear that single inactive RB alleles act within cells throughout the body in a recessive manner, as children who are effectively hemizygous for RB undergo essentially normal development. Only the rare cell that loses its remaining wild-type allele shows evidence of growth deregulation.

A third route for discovering tumor suppressor genes was suggested by the genetic mechanisms used by evolving tumor cells to eliminate both copies of genes like RB. The first copy of a suppressor gene is inactivated by a somatic (or a germline) mutation. The chromosomal region carrying the surviving wild-type allele may then be replaced by a duplicated copy of the homologous chromosome region that carries the mutant allele. This elimination of the wild-type allele, which may be accomplished by mechanisms like chromosomal nondisjunction, mitotic recombination, or gene conversion, occurs with a frequency as high as 10^{-3} to 10^{-4} per cell generation. This mechanism is therefore greatly favored over independent knockouts of the second copy of the gene, which occur with a frequency of 10^{-6} per cell generation. Most tumors that lack functional copies of a suppressor gene (like RB) display two identically mutated alleles.

These steps that lead to homozygosity of a mutant suppressor allele usually involve the flanking chromosomal regions as well. Accordingly, anonymous DNA markers mapping to nearby chromosomal sites, which may have shown heterozygosity prior to tumor progression, will suffer a parallel reduction to homozygosity [or loss of heterozygosity (LOH)]. Indeed, the repeated observation of LOH of a specific chromosomal marker in cells from a particular tumor type suggests the presence of a closely mapping tumor suppressor gene, the loss of which is involved in tumor pathogenesis (*7*). For example, a chromosome 18q DNA marker that is highly polymorphic (and therefore heterozygous in most genomes) was found in a homozygous state in 70% of advanced colon carcinomas (*8*). This suggested the presence of a suppressor gene locus mapping nearby on the chromosome, although the precise localization and identification of the gene still required extensive molecular cloning.

To generalize, one can cast a broad net for tumor suppressor loci by using a large repertoire of polymorphic DNA markers to survey systematically tumor cell genomes, looking for repeated instances of LOH (*7*). Indeed, this genetic strategy has revolutionized the research field. The fruits of these various search strategies are displayed in Table 1.

Table 1. Suppressor genes in human tumors.

Detected by cell hybridization or chromosome transfer*	
Chromosomal location	Tumor type
1p	Neuroblastoma
3p	Renal ca.
6	Endometrial ca.
9	Endometrial ca.
11	Neuroblastoma (*81*); cervical ca. (*82*); Wilms tumor

Detected through loss of heterozygosity† or direct molecular probing	
Chromosomal location	Tumor type
1p	Melanoma; MEN type 2; neuroblastoma; medullary thyroid ca; pheochromocytoma; ductal cell ca.
1q	Breast ca.
3p	SCLC; adeno ca. of lung (*83*); cervical ca.; von Hippel-Lindau disease, renal cell ca.
5q	Familial adenomatous polyposis; colorectal ca.
9q	Bladder ca. (*84*)
10q	Astrocytoma (*85*); MEN type 2 (*86*)
11p	Wilms tumor; rhabdomyosarcoma; breast ca.; hepatoblastoma; transitional cell bladder ca. (*84*), lung ca. (*87*)
11q	MEN type 1 (*88*)
13q	Retinoblastoma; osteosarcoma; SCLC; ductal breast ca.; stomach ca.; bladder ca.; colon ca. (*17*)
17p	SCLC; colorectal ca.; breast ca., osteosarcoma; astrocytoma (*89*) squamous cell lung ca. (*90*); others in reference (*91*)
17q	NF type 1 (*23*)
18q	Colorectoral ca. (*8*)
22q	NF type 2 (*92*); meningioma; acoustic neuroma; pheochromocytoma

*Data are from Table 1 of (*1*) and references therein, unless otherwise noted. †Data are from Table 2 of (*1*), unless otherwise noted. MEN, multiple endocrine neoplasia; SCLC, small cell lung carcinoma; NF, neurofibromatosis; ca, carcinoma.

Congenital Cancer Predisposition

Cancer-predisposing alleles must operate under narrow constraints. They may not deregulate cell growth to a substantial extent during development, lest the resulting dysmorphogenesis lead to embryonic or fetal lethality. Often their action may become manifest only after two or three decades of life, a delay that is essential if the allele is to be passed on to the succeeding generation to create a truly familial disease. These prerequisites may well explain why the tumor-causing alleles of proto-oncogenes (that is, "activated" oncogenes) have never been found to be transmitted in the germ line. Activated oncogenes almost invariably act dominantly on cell phenotype and their presence would seem to be too disruptive for normal development. In contrast, the recessive action of mutant suppressor gene alleles permits any resulting phenotypic effects to be delayed for long periods of time after conception. These alleles are effectively latent until they are exposed by a reduction to homozygosity in one or another cell.

Two other factors conspire to limit or delay the phenotypic effects of suppressor gene inactivation. First, only a subset of the cell types present during a particular stage of development may respond to the homozygous loss of a suppressor gene by exhibiting some type of growth deregulation (for example, RB inactivation affecting only primitive retinoblasts in the early years of life). Second, most cell types would appear to require a succession of genetic changes that affect multiple distinct genes before they will proliferate into tumor cell masses (*9*).

Mechanisms of Action of Tumor Suppressor Genes

A goal of those studying tumor suppressor genes is to understand the functions of these genes in normal cell physiology and the reasons why their elimination contributes to uncontrolled growth of tumor cells. One productive way of rationalizing how these genes function comes from a focus on the interactions between a normal cell and its neighbors within a tissue. Intercellular communication would appear to be the primary means of ensuring the architectural integrity of normal tissue. Implicit in this is the notion that normal cells make few, if any, decisions autonomously and rely instead on cues received from their surroundings.

Individual cells within a tissue must receive two types of growth-regulating signals from their environment. One class of contextual signals are growth-promoting; cells encourage growth of their neighbors through mitogenic signals, conveyed largely by polypeptide growth factors (*10*) (see the article by S. Aaronson, this issue). Many of the proteins encoded by cellular oncogenes constitutively activate intracellular signaling pathways used by the normal cell to process exogenous mitogenic stimuli. In this way, oncoproteins induce a cellular state similar to that experienced when a normal cell is exposed to growth factors. As a consequence, the oncogene-bearing cell acquires growth autonomy, as it is no longer dependent on mitogenic stimuli from its surroundings.

Cells must also actively inhibit the growth of their neighbors. One way of conceptualizing how tumor suppressor genes and their products normally function is as follows: they are components of the intracellular signaling pathways that enable a cell to receive and process growth-inhibitory signals from its surroundings. When a cell loses critical components of this signaling network, it loses responsiveness to certain extracellular growth-inhibitory signals even though these signals may still be present in its environment. In this sense, the products of the various suppressor genes should not be seen as intrinsically cytostatic factors; rather, they serve as transducers of negative growth signals that originate elsewhere inside or outside the cell.

Independent of the details of normal biological function, this model provides an operational definition of a tumor suppressor gene: a genetic element whose loss or inactivation allows a cell to display one or another phenotype of neoplastic growth deregulation. Such a definition excludes genes that are cytostatic or cytotoxic when introduced into a cell and inappropriately overexpressed. Thus, many genes and gene products will antagonize growth when they are forced on a cell by cloning and gene transfer, but this provides no testimony as to whether these genes are normally used by the cell to down-regulate its own proliferation or whether loss of such genes confers growth advantage on evolving, premalignant cell clones.

The exogenous signals that normally persuade a cell to cease proliferating appear to be conveyed by a variety of molecules that are poorly characterized. One type of signal is suggested by the contact inhibition displayed by normal cells growing in monolayer culture (*11*). This phenomenon suggests that cell-to-cell contact mediated by still unknown surface molecules allows a cell to sense the presence of close neighbors and to shut down its growth program in response.

Diffusible growth inhibitors would also seem to play an important role in carrying intercellular anti-mitogenic signals. A substantial literature testifies to the importance of gap junctional communication as an important means of passing growth-inhibitory signals between cells (*12*). This suggests the exchange of low molecular weight signaling molecules (<1000 daltons) able to pass through these channels, but their precise nature is obscure.

Equally important are hormones and macromolecular growth inhibitors that may be passed between cells. The most well known of these is TGF-β (tumor growth factor–β), which, in its three polypeptide variant forms (types 1, 2, and 3), inhibits growth of a variety of cell types (*13*). Other polypeptides and hormones that induce end stage differentiation should be seen in the same light; they, too, act to persuade a cell to stop growing by inducing it to enter a post-mitotic state.

How does a cell respond to these various negative signals? Growth shutdown is achieved via three alternative responses. Most simply, a cell in exponential growth may pause in one or another phase of its growth cycle. Often mentioned in this context is a hold-up at the end of the G1 phase just prior to DNA synthesis (S phase) (*14*). Alternatively, cells may be induced to undergo an end stage, post-mitotic differentiation (*15*). This represents an irreversible commitment and serves once again to limit cell proliferation. Most drastic is a commitment by the cell to undergo senescence (aging) or apoptosis (programmed cell death) (*16*).

Together, these responses define the arena of action of suppressor genes and their encoded proteins. Biochemically they serve as transducers of anti-proliferative signals; biologically, they serve as part of the response machinery that enables a cell to stop progression through the cell cycle, to differentiate, to senesce, or to die.

Our understanding of suppressor gene biology, as sketched in rough outline above, lags far behind the molecular descriptions of these genes and their products. Below are six anecdotes woven around six well-studied suppressor genes. Because this narrative is told from the perspective of the individual cell, I will begin with a gene whose product functions at the cell exterior, and then move inward.

The Deleted in Colon Carcinoma (DCC) Gene

Carcinogenesis in the colon is particularly well studied by virtue of the accessibility of this organ through colonoscopy. The process of tumorigenesis in the gut clearly involves multiple steps. These

include hyperplastic but otherwise normal appearing epithelium, adenomatous polyps at various stages of dysplasia, noninvasive, and, ultimately, invasive carcinomas.

The availability of biopsy material representative of these stages of colonic tumor has made it possible to develop a chronicle of somatic mutations that accompany and apparently underly each of these conversions. Of the large (but not small) adenomas, 60% carry a mutated, activated allele of the oncogene K-*ras*; LOH on chromosome 5 is seen almost as frequently at these early stages. The subsequent evolution of these polyps into frank carcinomas is accompanied in a majority of tumors by the inactivation-mutation of the chromosome 18–associated DCC tumor suppressor gene and, subsequently, the *p53* suppressor gene on chromosome 17 (*17, 18*).

This order of events is not followed precisely during the formation of every colonic tumor, but represents only a favored route. Nonetheless, we learn several important concepts. First, multistep carcinogenesis can be rationalized by a series of definable genetic changes that are successively accumulated in the genome of the evolving cancer cell. Second, carcinogenesis can (and may often) involve both the activation of oncogenes and the inactivation of suppressor genes. In most colon carcinomas, these alterations seem to be effected through somatic mutations.

DCC was uncovered through the use of polymorphic DNA markers that showed a loss of heterozygosity of the long arm of chromosome 18 in the 18q21.3 region (*8*). Such an LOH suggested the elimination of the wild-type copy of a suppressor gene in this chromosomal region and its replacement by a duplicated copy of an already mutated recessive allele. Vogelstein and co-workers, who catalogued the successive genetic changes in colon carcinogenesis (*19*), proceeded to isolate the DCC gene, which encompasses more than one million base pairs (*20*).

The amino acid sequence deduced from the nucleotide sequence of DCC showed that it encodes a 190-kD transmembrane phosphoprotein having the attributes of a cell surface receptor (*19*). The presence of fibronectin type III and C2 immunoglobulin-like regions in its extracellular domain are reminiscent of similarly structured cell adhesion molecules (CAMs) and suggest binding to an extracellular matrix or basement membrane component (*21*). Nonetheless, it is unlikely that the DCC protein is simply a mechanical device that tethers the cell to the extracellular matrix. Rather, it is likely to be a signal-transducing receptor whose loss confers a growth advantage on evolving tumor cells.

While the extracellular ligand for DCC is not known, it is probably widely distributed, as DCC is expressed in a large number of cell types. The chromosome 18q LOH is seen in other tumor types (*22*), and this suggests a function for DCC in human carcinogenesis far broader than that initially suspected.

The Neurofibromatosis Tumor Suppressor Gene

Von Recklinghausen neurofibromatosis (NF-1) affects ~1 in 3500 in the general population. It involves cells originating in the embryonic neural crest and creates a variety of benign growths, including neurofibromas and café-au-lait spots on the skin, neurofibromas of spinal and peripheral nerves, pheochromocytomas, and, occasionally, malignant tumors such as Schwannomas and neurofibrosarcomas. Like retinoblastoma, this disease is triggered by predisposing alleles acquired from an affected parent or, with equal frequency, by alleles created through new germline mutations. Elegant molecular techniques were used to isolate the NF-1 gene, located in the 17q11.2 chromosomal region (*23*).

The protein encoded by the NF-1 complementary DNA (cDNA) shares structural similarity with three proteins that interact with the products of the *ras* proto-oncogene (*24*). The closest structural relatedness found to date is shared with the IRA1 and IRA2 proteins of the yeast *Saccharomyces cerevisiae*, with lesser similarity shared with the GTPase (guanosine triphosphatase)-activating protein (GAP) of mammals (*24, 25*). These structural similarities suggest that NF-1 participates in a much-studied but poorly understood signaling pathway triggered by the $p21^{ras}$ proteins, likely operating at the cytoplasmic face of the plasma membrane.

Like the α subunits of the heterotrimeric GTP (guanosine triphosphate) binding proteins (G proteins), $p21^{ras}$ is probably excited by an activator upstream in its signaling pathway and probably passes these signals on to a downstream target (its effector). $p21^{ras}$ can exist in either a physiologically quiescent GDP-binding state or an GTP-binding signal-emitting state. Oncogenic $p21^{ras}$ proteins are trapped in the excited, signal-emitting state because the mechanism normally employed to delimit their excitation period, hydrolysis of their bound GTP to GDP, is dysfunctional as a result of mutations that alter protein structure. As a consequence, oncogenic $p21^{ras}$ remains in the excited state for extended periods of time rather than for the brief interval typical of its normal counterpart (*25*).

GAP and NF-1 potently stimulate the GTPase activity intrinsic to $p21^{ras}$. Indeed, interaction of $p21^{ras}$ with GAP can increase hydrolysis of $p21^{ras}$-bound GTP by as much as a 1000-fold (*26*). This stimulated GTPase activity and the resulting down-regulation of $p21^{ras}$ function are compatible with two alternative physiologic schemes: (i) GAP down-regulates activated $p21^{ras}$. Thus, GAP may intercept activated (GTP-binding) $p21^{ras}$ before it has a chance to seek out and stimulate its effector; by causing $p21^{ras}$ to hydrolyze its bound GTP, GAP succeeds in aborting the excitation period of $p21^{ras}$, thereby interdicting signal flow to the bona fide effector. (ii) GAP is a downstream effector that becomes excited when it encounters activated $p21^{ras}$. Having received an excitatory pulse from activated, GTP-binding $p21^{ras}$, GAP will release its own, downstream mitogenic signal and then cause the $p21^{ras}$ molecule to hydrolyze its GTP, thereby terminating their brief but productive encounter.

Alterations of GAP are not known to be associated with any pathology. However, deletions of NF-1 from neuroectodermal cells leads to tumors like neurofibrosarcomas. NF-1 may act as a pure down-regulator of $p21^{ras}$ and block *ras*-mediated mitogenic signaling; this is consistent with the apparent function of NF-1 as an anti-proliferative protein. In the absence of NF-1, a neuroectodermal cell might lack the device that it uses to dampens *ras* mitogenic signals. Cancer may then ensue.

An alternative model is that NF-1 acts as a downstream effector and down-regulator of $p21^{ras}$. $p21^{ras}$ can act as a source of either mitogenic or differentiation-inducing signals, depending on cell type (*27*). In one well-studied pheochromocytoma, representing a neuroectodermal cell related to the precursors of neurofibromas, $p21^{ras}$ can induce differentiation rather than the proliferation seen in many other cell types. Thus, $p21^{ras}$ might use several alternative downstream effectors, depending on whether it releases a mitogenic signal (for example, via GAP) or a differentiative signal (for example, via NF-1). By this logic, a neurofibroma cell may proliferate abnormally because it lacks NF-1, which normally transduces signals causing it to differentiate. In such a cell, all of the signaling energy of $p21^{ras}$ may then be deflected toward mitogenesis.

The Retinoblastoma Gene

The RB gene encompasses 180 kb of DNA mapping to chromosome 13q14 (*28*) and encodes a 105-kD nuclear phosphoprotein

(pRB) (*29*). The nuclear localization of pRB and its DNA binding ability suggest a role in transcriptional regulation. Its tissue distribution (*30*) would imply participation in growth regulation in a variety of cell and tumor types. However, RB gene inactivation seems restricted to a narrow subset of tumors. Aside from the retinoblastomas and osteosarcomas that are seen because of germline heterozygosity at the RB locus (*31*), RB inactivation has been observed in retinoblastomas and sarcomas of purely somatic mutational origin, and in several other, more common tumors in which gene inactivation is also presumed to derive exclusively from somatic events. These include most if not all small cell lung carcinomas, as well as a portion of non–small cell lung, bladder, and breast carcinomas (*32*).

Interest in pRB was increased substantially when it was discovered that this protein exists within DNA tumor virus–transformed cells in the form of complexes with various virus-encoded oncoproteins. Human adenovirus, SV40, and human papillomavirus (HPV) each specify an oncoprotein—E1A, large T antigen, and E7, respectively—that forms complexes with the host cell pRB (*33*).

The ability of these viral oncoproteins to complex with pRB suggests that these DNA tumor viruses transform cells through their ability to cripple a vital cellular growth-suppressing mechanism. By targeting pRB for complex formation, these viral proteins may inactivate its function, thereby mimicking the state seen in spontaneous human tumors that have lost pRB function through inactivation of the RB gene. In either case, the cell is liberated from growth constraints imposed by pRB.

The viral oncoproteins share a small region of structural similarity that appears to be involved in mediating complex formation with pRB (*34*). pRB, on its side, has a complementary oncoprotein-binding pocket that is a common target for the three viral proteins (*35*). Most intriguing is the observation that this oncoprotein-binding region is almost invariably affected in mutant pRB proteins isolated from human tumor samples (*36*). This is puzzling because these tumors did not confront DNA tumor virus oncoproteins during their progression to malignancy. What advantage could they gain from altering the oncoprotein-binding pocket of pRB?

One attractive solution to this puzzle is that these viral oncoproteins are structural mimics of a cellular protein that must bind to pRB in order for pRB to exert its growth-suppressing actions. Mutations in this pRB domain would then prevent its association with this cellular oncoprotein "homolog." By the same token, occupancy of this pRB domain by one or another viral oncoprotein may preempt and thus preclude its association with the endogenous cellular homolog. Via either mechanism, this association fails to occur, and pRB's agenda of growth-suppression, which depends on binding to this cellular partner protein, is frustrated. Recently, recombinant forms of the pRB oncoprotein-binding domain have been used to demonstrate specific associations with a number of host cell proteins (*37*). This provides strong indication of the existence of cellular proteins with oncoprotein-like domains.

pRB switches between a hyperphosphorylated and relatively unphosphorylated state in a cell cycle–specific manner. It is underphosphorylated in G1, becomes heavily phosphorylated just prior to the G1 to S transition, remains phosphorylated in S, G2, and most of M, and reverts to an underphosphorylated state at or before the M-G1 transition (*38*). This suggests but does not prove that pRB is involved in regulating the cell's progression through its growth cycle.

Importantly, all three viral oncoproteins bind to the underphosphorylated form of pRB. These viral proteins apparently deregulate cell growth by specifically targeting the underphosphorylated form of pRB and ignoring the phosphorylated form, which may therefore be inactive and thus not worthy of their attentions (*39*). This leads to the surmise that pRB is active in growth suppression only in G0 and G1, and is phosphorylated and rendered inactive for the remainder of the cell cycle. In line with this thinking is the finding that unphosphorylated pRB binds tightly to a still unidentified nuclear partner protein via its oncoprotein-binding domain, while phosphorylated pRB is incapable of doing so (*40*). Interaction of pRB with this nuclear partner is crippled in the mutant pRB forms isolated from human tumors. This suggests that such binding is indeed critical to growth suppression by pRB and that the ability to suppress growth may be limited to the G0 and G1 phases of the cell's growth cycle. Further, it is possible that the afferent signals regulating pRB activity come from cell cycle–regulated kinases such as G1-specific cyclin: cdc2 kinase complexes.

None of this provides clear insight into the nature or the efferent downstream signals that pRB releases in order to shut down growth. One provocative result comes from work on keratinocytes, which, when treated with TGF-β, turn off transcription of the *myc* gene and stop proliferating (apparently at the end of G1). In contrast, keratinocytes that carry a pRB-binding oncoprotein continue to express *myc* in the presence of TGF-β (*41*), suggesting that pRB acts as an intermediary in the signaling pathway between the TGF-β receptor and down-regulators of *myc* transcription. When pRB is removed from this pathway through oncoprotein sequestration, *myc* is freed from down-regulation by TGF-β. By extension, cells that carry mutated RB alleles might show deregulated *myc* transcription, which may in turn drive their growth. Cells transfected with the RB cDNA show reduced transcription of *fos* (*42*). It is possible that the loss of pRB function uncouples proto-oncogene expression from negative upstream regulators, thereby mimicking the constitutive expression seen when these same genes are converted into oncogenes by cis-acting mutations.

A distinct mechanism is suggested by another line of work that shows direct physical interaction between the RB protein and two proteins that are known or suspected to be transcription factors. The first and better characterized of these is between pRB and the cellular E2F transcription factor, encountered originally through its ability to up-regulate the adenovirus E2 promoter (*43*). This E2F factor is suspected to bind to a number of host cell promoters, the activity of which it presumably regulates.

E2F activity is increased in the cell in response to mitogens (*43*). On this basis, it appears likely that E2F, like the Myc protein, acts as a transcription factor responsible for orchestrating a part of the cell's mitogen-induced growth program. Complex formation with pRB may well alter E2F activity. However, when the E1A viral oncoprotein complexes with pRB, then the latter releases any bound E2F (*43*). This might suggest that the viral E1A protein can act through its ability to liberate growth-promoting transcription factors from the clutches of pRB. Only the underphosphorylated form of pRB, which is suspected to be active in growth suppression, is able to bind E2F. A similar set of interactions with pRB and E1A has been ascribed to the transcription factor DRTF1, which may in fact be identical to E2F (*44*).

An analogous, though less well-characterized interaction has been uncovered between pRB and the cellular Myc protein (*45*). As before, pRB may act by binding and possibly sequestering a growth-promoting transcription factor, in this case, Myc. This would suggest that E2F and Myc are analogously acting regulators, each assigned to choreograph part of the cell's response to mitogens. Their activities as well as those of yet other analogous factors may be modulated or inhibited through sequestration by pRB.

The *p53* Gene

p53 is a rule-breaker that follows few of the principles used to define other tumor suppressor genes. In spite of this perversity, its true nature has emerged: it, too, acts as a negative regulator of cell growth. p53 was found initially through its association with SV40 large T oncoprotein in virus-transformed cells and soon after as an over-expressed antigen in chemically transformed sarcoma cells (*46*). Because of its nuclear localization, it was thought initially to function in cell transformation like the oncogene-encoded Myc protein. Indeed, in SV40-transformed cells, p53 protein concentration is dramatically increased, reminiscent of the deregulation of *myc* expression seen in a number of tumor models (*46, 47*). Most provocative was the early finding that *p53* cDNAs, like plasmids carrying oncogenic alleles of *myc*, could collaborate with cotransfected *ras* oncogenes in the transformation of embryo fibroblasts (*48*).

Some of these results placed p53 in the oncogene camp, but this was a mirage, as the initially used *p53* cDNA clones were mutants. Wild-type *p53* cDNAs were later found to be strongly growth-suppressive and inhibitory of transformation (*49*). Moreover, a small number of tumors were found in which the *p53* alleles were absent or clearly inactivated. This meant that wild-type *p53* acts to suppress growth and transformation, while mutant alleles favor cell growth. Many mutant *p53* alleles favor growth and transformation in cells that continue to carry intact, wild-type *p53* gene copies; accordingly, such mutant *p53* alleles act in a dominant fashion vis à vis the wild-type *p53* allele.

These observations can be rationalized through a simple model that argues that *p53* acts biologically as a tumor suppressor gene whose genetics are dictated by the unusual biochemistry of its encoded protein. Unlike the other suppressor proteins, the normal form of p53 within the cell appears to assemble into homotetramers and higher order homo-oligomeric structures (*50*). Defective subunits of such an oligomerizing protein (for example, mutant p53 molecules) may participate in forming a multi-subunit complex together with wild-type monomers and, in so doing, poison the function of the complex as a whole (*51*).

One further insight comes from the discovery that the cellular heat shock protein Hsc70 is often bound up into these mixed complexes of mutant and wild-type p53 molecules (*52*). Hsc70 may act as a chaperone that brings p53 subunits together and helps them to oligomerize. The presence of Hsc70 in these complexes suggests that the usual brief dalliance of Hsc70 with p53 has turned instead into a tight embrace from which the partners cannot extricate themselves; for unknown reasons, the mutant p53 subunits cause a normally fleeting interaction to hang up at some inauspicious sticking point. As a consequence, large numbers of mutant and wild-type p53 proteins and Hsc70 become trapped in long-lived, unproductive complexes (often in the cytoplasm), and the cell's nucleus is deprived of active p53 complexes, which it requires for negative growth regulation. Wild-type, active p53 normally has a lifetime of only 20 to 30 min (*53*), and cells that carry mutant p53 alleles may accumulate steady-state concentrations of p53 that vastly exceed those seen in the normal cell.

Viral oncoproteins like SV40 and adenovirus E1B seem to mimic this state by sequestering p53 in inactive complexes that increase steady-state p53 concentrations, but prohibit p53 from reaching its normal site of action in the nucleus (*47, 54*). The human papilloma virus E6 oncoprotein seems to achieve a similar end result through another trick: by associating with recently synthesized p53 molecules, it tags them for rapid destruction, apparently at the hands of the cell's ubiquitin-dependent proteolytic machinery (*55*).

p53 and pRB show several parallels: they are both growth-suppressing proteins found in the nucleus and both are targeted for sequestration (or destruction) by the oncoproteins of SV40, adenovirus, and human papillomavirus. It appears that both proteins must be eliminated from the cell's growth-suppressing circuitry in order for full viral transformation to occur. This suggests that p53 and pRB serve distinct, even complementary functions in growth regulation, a point made as well by the observation that both *p53* and RB genes are affected by mutations in many human osteosarcomas (*56*).

The similarities between *p53* and RB are further extended by the recent finding that mutant *p53* alleles can be passed through the germline where they may serve as congenital determinants of cancer predisposition (*57*). Members of families afflicted with the Li-Fraumeni syndrome show tumors appearing in a variety of organ systems including rhabdomyosarcomas, adrenocortical carcinomas, brain tumors, leukemias, melanomas, and carcinomas of the breast, lung, larnyx, and colon.

Somatic mutations of *p53* have been implicated as causal events in the formation of a large and ever increasing number of common tumors, including those involving the hematopoietic organs, bladder, liver, brain, breast, lung, and colon, and other less common tumors including rhabdomyosarcomas (*56, 58*). In fact, *p53* is already documented as the most frequently mutated gene in human cancer.

Why is *p53* such a popular actor? To begin, it may be a centrally important growth regulator in many cell types. But its genetic and biochemical traits are important as well. Point mutations create carcinogenic *p53*, and such simple genetic changes occur readily. Moreover, unlike the *ras* genes, where point mutations productive for cancer are limited to two or three codons, the cancer-favoring missense mutations of *p53* can occur in at least 30 distinct codons in its reading frame (*59*). In addition, these point mutations often create dominant alleles that produce shifts in cell phenotype even without a reduction to homozygosity.

One inconsistency is inherent in this description of *p53* genetics. If point mutations of *p53* create dominant negative alleles that disrupt normal cell regulation and thus embryological development, how can such alleles be tolerated and passed in the germline of Li-Fraumeni families? The answer here may stem from yet another subtlety of *p53* genetics. The various mutant alleles seen in tumors and encoding a variety of amino acid substitutions would seem to range from minimally dysfunctional to strongly penetrant, dominant negative alleles. Li-Fraumeni *p53* alleles may well be weak alleles that lack dominant negative activity and are thus tolerable during ontogeny.

None of this tells us the nature of the signals transduced by p53. Like pRB, it may receive afferent signals in the form of phosphorylation by cdc2 protein kinase (*60*). Like pRB, it may be involved in transcriptional regulation; p53 has a transcriptional activation domain and binds preferentially to certain DNA sequences (*20, 61*). The biological consequence of p53 mutation may be manifested as immortalization of cultured cells (*62*), but a precise understanding of the effects of p53 mutations on evolving tumor cell clones is still elusive.

Wilms Tumor

The pattern of familial and sporadic cases of Wilms tumor (WT) of the kidney is strikingly parallel to that seen for retinoblastoma. This led early workers to describe its underlying genetic mechanisms using a model identical to that of retinoblastoma (*63*). In the case of WT, the locus involved was mapped to human chromosome 11p13 through the sighting of chromosomal deletions carried by children affected with the disorder (*63*). As with retinoblastoma, this tumor

occurs in infants and young children and arises from embryonal precursors, in this case in the kidney.

However, the WT syndrome diverges from retinoblastoma in several important respects. The WT genetic deficit is not associated with a variety of tumors in other tissues; the tumors are histopathologically heterogeneous; and the locus most intensively studied to date (11p13) may only represent one of two or three loci that are involved in WT pathogenesis (*64*).

Two groups have used elegant gene cloning strategies to isolate the gene at 11p13 (*65*). Like the RB and p53 products, the WT-1 protein has the hallmarks of a transcription factor. The four zinc finger domains of this 345–amino acid protein indicate a sequence-specific DNA binding protein (*66*) that shares sequence similarity with a mammalian immediate-early protein variously termed EGR-1, NGFI-A, TIS-8, Krox-24, or Zif 268 (*67*). In fact, the zinc fingers of WT-1 and EGR-1 are more than 60% identical in amino acid sequence.

Zinc fingers have been implicated in direct nucleotide sequence–specific recognition by DNA binding proteins (*66*), and this has encouraged some to inquire whether the two proteins may indeed interact with a common DNA recognition site. Indeed, both EGR-1 and WT-1 bind strongly to the common sequence CGCCCCCGC (*68*). Most recently, they have been found to be functional antagonists as well. Thus, EGR-1 acts as a strong transcriptional activator of promoters that contain the above sequence. Binding of WT-1 to this same sequence element acts to suppress transcriptional activation by EGR-1. Such inhibition may be more complex than that achieved through a simple preemptive occupation of the DNA target site by WT-1, as this suppressor protein may contain a domain that actively antagonizes transcription (*69*).

How can all this be rationalized physiologically? EGR-1 is expressed ubiquitously and is among the most prominent of the cellular genes turned on rapidly in response to serum stimulation (*67*). Its antagonist, WT-1 is expressed in a narrow range of tissues including the kidney, urogenital precursors, and a subset of hematopoietic cells largely in the spleen (*65*). As suggested by its response to mitogens, EGR-1 (like Myc and E2F) appears to be important in programming cell proliferation (*67*). The expression of WT-1 in certain embryonal kidney precursors may interrupt the growth program in these cells by antagonizing EGR-1 protein function, thereby allowing these cells to undertake, as an alternative, a commitment to end stage differentiation. The apparent parallels to pRB function is striking: in each case, the suppressor protein may directly interact with and antagonize mitogen-induced transcription factors.

The *erb*A Gene

*erb*A has been considered an oncogene, but detailed examination of its biology persuades one that it is indeed a tumor suppressor which, like mutant alleles of *p53*, acts in a dominant-negative manner to disrupt wild-type function. As a result of the work of Graf and Beug, the biology of transformation leading to avian erythroblastosis, involving the activities of the *erb*A and *erb*B, is better understood than any other tumor model. The v-*erb*B oncogene acts to expand a pool of highly mitotic, undifferentiated erythroid precursor cells, but these are poorly tumorigenic, because they differentiate at high rates into post-mitotic, end stage red cells. v-*erb*A on its own acts to block differentiation of these erythroid precursors, but creates no tumors because it is unable to provide the mitogenic impetus needed to expand the pool of stem cells. The two genes, carried into erythroid precursors by avian erythroblastosis virus, act in concert to create an aggressive erythroleukemia. v-*erb*B drives expansion of the pool of undifferentiated precursor cells, while v-*erb*A blocks their egress into the differentiation pathway (*70*).

The v-*erb*A allele that participates in formation of chicken erythroleukemias is a mutant version of a transcriptional regulatory protein, the chicken thyroid hormone (triiodothyronine) receptor (*71*). Function of the wild-type receptor protein is blocked in the presence of the v-*erb*A (*72*), because the latter occupies critical DNA binding sites in a way that precludes association by the wild-type receptor protein and inhibits transcription. There is evidence that the mutant *erb*A protein acts to inhibit function of the structurally related retinoid receptors as well. Indeed, these receptors, when activated by bound ligand, may be even more important in the normal triggering of avian erythroid stem cell differentiation (*73*). Consequently, when functioning of these various receptors is blocked by the mutant *erb*A protein, entrance into the differentiation program may be unattainable even though the thyroid hormone and retinoids are present in abundance. In chicken red cell precursors, activation of certain erythrocyte-specific transcriptional promoters, such as those controlling the band 3 and carbonic anhydrase genes, is blocked by the mutant v-*erb*A (*74*).

Seen from this perspective, the normal thyroid hormone receptor gene (c-*erb*A) is a suppressor gene that limits proliferation through its ability to promote end stage differentiation. When its activities and those of related retinoic acid receptors are blocked by a dominant negative mutant, large numbers of undifferentiated stem cells accumulate and can serve as precursors of fully malignant cells. A prediction here is that genes for differentiation-promoting receptors like the thyroid and related retinoid receptors should be altered in certain types of human tumors. One type of retinoid receptor gene has been found to be altered in 90% of acute promyelocytic leukemias as a consequence of 15;17 chromosomal translocation, which fuses it to a second unrelated gene termed *myl* (*75*). The biological function of the resulting chimeric protein remains to be elucidated.

Prospectives

This review has been written largely from the viewpoint of those interested in protein function and cell physiology. Such an analysis ignores other important parts of this rapidly expanding research field. Underrepresented here are human genetics and the powerful molecular techniques that have led to the discovery and isolation of at least six suppressor genes.

These various suppressor genes all act in one or another way to constrain cell proliferation, but the resemblance ends there. Accordingly, it is difficult to predict where other, soon-to-be-discovered suppressor proteins will act within the cell or how they will affect cell phenotype.

Our understanding of inborn cancer susceptibility genes (*76*) lags far behind the well-developed descriptions of somatically activated genes like oncogenes. This will be remedied by the rapidly developing phenomenology regarding tumor suppressors. Of the tumor suppressor genes described here, the RB, p53, NF-1, and WT-1 are already clearly implicated in familial cancer, but this is only the beginning. Familial colon cancer seems close to being understood in terms of the recently isolated APC (adenomatous polyposis coli) gene located on chromosome 5 (*77*). And if historical precedent is a useful guide, one may presume that other suppressors known through their somatic inactivation (Table 1) will also be passed in mutant form through the germline. All this will bring genetic diagnosis of many types of cancer predisposition within reach. Indeed, it is already being practiced for retinoblastoma families (*78*).

We may also see new types of cancer therapy arise through

manipulation of suppressor genes. By inserting wild-type suppressor genes into tumor cells that lack them, one may be able to reinstate a semblance of normal growth control, and with this, cause reversion of tumor cells to a more normal growth pattern. Early successes have been reported on this front, including the use of retrovirus transducing vectors to insert wild-type RB gene copies into osteosarcoma and prostate carcinoma cells that lack them (*79*). These genetically reconstituted cells grow more slowly in culture, but lose tumorigenicity when implanted into immunodeficient mice. This echoes earlier work showing that insertion of an entire chromosome 11 causes Wilms tumor cells to lose tumorigenicity (*80*). Still, prospects of novel cancer treatment through somatic gene therapy are not imminent, as such a route is strewn with many technical obstacles. But this work plants the seed of an idea that may be realized in unforeseeable ways. That is, after all, the allure of this field—every month brings something new and unexpected!

Surely, the study of tumor suppressors offers much to those interested in cell and developmental biology. The diagrams describing the regulatory circuitry that governs cell proliferation and differentiation are still incomplete. We now understand in some detail how cell growth is incited, but the other, equally important half of the picture explaining growth shutdown is still shrouded in haze. Suppressors will show us the way!

REFERENCES AND NOTES

1. G. Klein, *Science* **238**, 1539 (1987); R. Sager, *ibid.* **246**, 1406 (1989); G. Klein, *Tumor Suppressor Genes* (Dekker, New York, 1990), p. 273.
2. R. Sager, *Adv. Cancer Res.* **44**, 43 (1985); H. P. Klinger, *Cytogenet. Cell Genet.*; H. Harris, *Cancer Res.* **48**, 3302 (1988).
3. E. J. Stanbridge, *Annu. Rev. Genet.* **24**, 615 (1990); _____, R. R. Flandermeyer, D. W. Daniels, W. Nelson-Rees, *Somatic Cell Genet.* 7, 699 (1981); M. Kaelbling and H. P. Klinger, *Cytogenet. Cell Genet.* **41**, 65 (1986); W. J. Benedict *et al.*, *Cancer Res.* **44**, 3471 (1984); H. Harris *et al.*, *Nature* **223**, 363 (1969); F. Wiener, G. Klein, H. Harris, *J. Cell Sci.* **15**, 177 (1974); S. A. Bader *et al.*, *Cell Growth Differen.* **2**, 245 (1991).
4. A. G. Knudson, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 820 (1971).
5. J. J. Yunis and N. Ramsay, *Am. J. Dis. Child.* **132**, 161 (1978).
6. W. F. Benedict *et al.*, *Science* **219**, 973 (1983); R. Godbout *et al.*, *Nature* **304**, 451 (1983); W. K. Cavenee *et al.*, *ibid.*, p. 179; R. S. Sparkes *et al.*, *Science* **219**, 971 (1983).
7. M. F. Hansen and W. K. Cavenee, *Cancer Res.* **47**, 5518 (1987).
8. B. Vogelstein *et al.*, *N. Engl. J. Med.* **319**, 525 (1988).
9. H. Land, L. F. Parada, R. A. Weinberg, *Science* **222**, 771 (1983); R. A. Weinberg, *ibid.* **230**, 770 (1985).
10. M. B. Sporn and A. B. Roberts, *Nature* **313**, 745 (1985); A. S. Goustin *et al.*, *Cancer Res.* **46**, 1015 (1986); C.-H. Heldin and B. Westermark, *Cell* **37**, 9 (1984); J. Folkman and M. Klagsbrun, *Science* **235**, 442 (1987); D. Metcalf, *ibid.* **229**, 16 (1985).
11. M. G. P. Stoker, *Curr. Top. Dev. Biol.* **2**, 107 (1967).
12. _____, *J. Cell Sci.* **2**, 293 (1967); P. Metha, J. S. Bertram, W. R. Loewenstein, *Cell* **44**, 187 (1986); J. Paul and O. H. Iversen, *Proceedings of the 1986 International Conference on Cancer Research*, New York, NY (Hemisphere, Washington, DC, 1987), p. 145; J. E. Trosko, C. C. Chang, T. J. Slaga, Eds., *Mechanisms of Tumor Promotion* (CRC Press, Boca Raton, 1984), pp. 120–145; M. Bignami *et al.*, *Mol. Carcinogenesis* **1**, 67 (1988); H. Yamasaki, in *Theories of Carcinogenesis*, O. H. Iversen, Ed. (Hemisphere, Washington, DC, 1988), p. 143.
13. J. Massagué, *Cell* **49**, 437 (1982); M. B. Sporn *et al.*, *J. Cell Biol.* **105**, 10039 (1987); M. B. Sporn *et al.*, *Science* **233**, 532 (1986); *Annu. Rev. Cell Biol.* **6**, 597 (1990).
14. L. Diller *et al.*, *Mol. Cell. Biol.* **10**, 772 (1990).
15. E. J. Stanbridge, *Adv. Virol. Oncol.* **6**, 83 (1987); P. A. Marks and R. A. Rifkind, *Tumor Suppressor Genes*, G. Klein, Ed. (Dekker, New York, 1990), p. 201.
16. A. H. Wyllie, *Nature* **284**, 555 (1980); S. Goldstein, *Science* **249**, 1129 (1990); O. Sugawara *et al.*, *ibid.* **247**, 707 (1990).
17. B. Vogelstein *et al.*, *Science* **244**, 207 (1989); J. Marx, *ibid.*, p. 147.
18. J. Bos *et al.*, *Nature* **327**, 293 (1987); K. Forrester *et al.*, *ibid.*, p. 298.
19. E. R. Fearon *et al.*, *Science* **247**, 49 (1990).
20. K. R. Cho, L. Hedrick, K. W. Kinzler, B. Vogelstein, personal communication; B. Vogelstein, personal communication.
21. G. M. Edelman, *Biochemistry* **27**, 3533 (1988).
22. P. Devilee *et al.*, *Oncogene* **6**, 311 (1991).
23. M. R. Wallace *et al.*, *Science* **249**, 181 (1990); D. Viskochil *et al.*, *Cell* **62**, 187 (1990); R. M. Cawthon *et al.*, *ibid.*, p. 193.
24. G. Xu *et al.*, *Cell* **62**, 599 (1990); R. Ballester *et al.*, *ibid.*, p. 851; A. Buchberg *et al.*, *Nature* **347**, 291 (1990).
25. M. Trahey and F. McCormick, *Science* **238**, 542 (1987); K. Tanaka *et al.*, *Mol. Cell. Biol.* **9**, 757 (1989); K. Tanaka *et al.*, *Cell* **60**, 803 (1990); *Mol. Cell. Biol.* **10**, 4303 (1990); M. Barbacid, *Annu. Rev. Biochem.* **56**, 779 (1987).
26. U. Vogel *et al.*, *Nature* **335**, 90 (1988); M. Trahey *et al.*, *Science* **242**, 1697 (1988).
27. D. Bar-Sagi and J. R. Feramisco, *Cell* **42**, 84 (1985); N. Hagag, S. Halegoua, M. Viola, *Nature* **319**, 680 (1986); M. Noda *et al.*, *ibid.* **318**, 73 (1985).
28. S. H. Friend *et al.*, *Nature* **312**, 643 (1986); W.-H. Lee *et al.*, *Science* **235**, 1394 (1987); T. L. McGee, D. W. Yandell, T. P. Dryja, *Gene* **80**, 119 (1989); Y.-K. T. Fung *et al.*, *Science* **236**, 1657 (1987); F. D. Hong *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5502 (1989).
29. W. H. Lee *et al.*, *Nature* **329**, 642 (1987); S. H. Friend *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 9059 (1987).
30. R. Bernards *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6474 (1989).
31. R. D. Jensen and R. W. Miller, *N. Engl. J. Med.* **285**, 307 (1971); M. F. Hansen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6216 (1985).
32. K. Rygaard *et al.*, *Cancer Res.* **50**, 5312 (1990); J. Cheng *et al.*, *Blood* **75**, 730 (1990); J. W. Harbour *et al.*, *Science* **241**, 353 (1988); J. M. Horowitz *et al.*, *ibid.* **243**, 937 (1989); *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2775 (1990); E. Y.-H. P. Lee *et al.*, *Science* **241**, 218 (1988); A. T.'Ang *et al.*, *ibid.*, p. 263; H.-J. Xu *et al.*, *Oncogene* **4**, 807 (1989); J. Yokota *et al.*, *ibid.*, p. 471.
33. P. Whyte *et al.*, *Nature* **334**, 124 (1988); C. Egan, S. T. Bayley, P. E. Branton, *Oncogene* **4**, 383 (1989); A. DeCaprio *et al.*, *Cell* **54**, 275 (1988); N. Dyson *et al.*, *J. Virol.* **64**, 1353 (1990); K. Münger *et al.*, *EMBO J.* **8**, 4099 (1989).
34. J. Figge *et al.*, *J. Virol.* **62**, 1814 (1988).
35. S. Huang *et al.*, *EMBO J.* **9**, 1815 (1990).
36. Q. J. Hu, N. Dyson, E. Harlow, *ibid.*, p. 1147.
37. W. G. Kaelin, Jr. *et al.*, *Cell* **64**, 521 (1991); S. Huang, W.-H. Lee, E. Y.-P. Lee, *Nature* **350**, 160 (1991).
38. J. A. DeCaprio *et al.*, *Cell* **58**, 1085 (1989); K. Mihara *et al.*, *Science* **246**, 1300 (1989); P.-L. Chen *et al.*, *Cell* **58**, 1193 (1989); K. Buchkovich, L. A. Duffy, E. Harlow, *ibid.*, p. 1097; G. H. Stein, M. Beeson, L. Gordon, *Science* **249**, 666 (1990); Y. Furukawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2770 (1990).
39. J. W. Ludlow *et al.*, *Cell* **56**, 57 (1989); P. Hinds, D. Templeton, R. A. Weinberg, unpublished data; K. Münger and P. Howley, personal communication.
40. S. Mittnacht, D. J. Templeton, R. A. Weinberg, in *Accomplishments in Cancer Research* (Lippincott, Philadelphia, 1991), p. 197; D. J. Templeton, S.-H. Park, L. Lanier, R. A. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **8**, 3033 (1991); S. Mittnacht and R. A. Weinberg, *Cell* **65**, 381 (1991).
41. J. A. Pietenpol *et al.*, *Cell* **61**, 777 (1990); *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3758 (1990).
42. P. D. Robbins *et al.*, *Nature* **346**, 668 (1990); *ibid.* **351**, 419 (1991).
43. S. Bagchi, P. Raychaudhuri, J. R. Nevins, *Cell* **62**, 659 (1990); S. P. Chellapan *et al.*, *ibid.* **65**, 1053 (1991); M. Mudryj *et al.*, *ibid.*, p. 1243; T. Chittenden, D. M. Livingston, W. G. Kaelin, Jr., *ibid.*, p. 1073 (1991); S. Bagchi, R. Weinmann, P. Raychaudhuri, *ibid.*, p. 1063; P. Raychaudhuri *et al.*, *Genes Dev.* **5**, 1200 (1991); P. Raychaudhuri, S. Bagchi, S. Neill, J. R. Nevins, *J. Virol.* **64**, 2702 (1990); M. C. Blake and J. C. Azizkhan, *Mol. Cell. Biol.* **9**, 4994 (1989).
44. L. R. Bandara and N. B. LaThangue, *Nature* **351**, 494 (1991); N. B. LaThangue and P. W. J. Rigby, *Cell* **49**, 507 (1987); M. K. Shivji and N. B. LaThangue, *Mol. Cell. Biol.* **11**, 1686 (1991); L. R. Bandara *et al.*, *Nature* **352**, 249 (1991).
45. A. K. Rustgi, N. Dyson, R. Bernards, *Nature* **352**, 541 (1991).
46. A. B. DeLeo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 24201 (1979); D. Lane and L. V. Crawford, *Nature* **278**, 261 (1979); F. McCormick and E. Harlow, *J. Virol.* **34**, 213 (1980); D. H. Linzer and A. J. Levine, *Cell* **17**, 43 (1979).
47. F. McCormick, R. Clark, E. Harlow, R. Tjian, *Nature* **292**, 63 (1981).
48. D. Eliyahu *et al.*, *ibid.* **312**, 646 (1984); L. F. Parada *et al.*, *ibid.*, p. 649.
49. C. A. Finlay *et al.*, *Cell* **57**, 1083 (1989); D. Eliyahu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8763 (1989); S. J. Baker *et al.*, *Science* **249**, 912 (1990).
50. J. E. Stenger *et al.*, *Mol. Carcinogen.*, in press.
51. I. Herskowitz, *Nature* **329**, 219 (1987); S. A. Kraiss *et al.*, *J. Virol.* **62**, 4737 (1988); J. Milner, E. A. Metcalf, A. C. Cook, *Mol. Cell. Biol.* **11**, 12 (1991); M. Montenarh and A. Quaiser, *Oncogene* **4**, 379 (1989); F. I. Schmieg and D. T. Simmons, *J. Virol.* **164**, 132 (1988).
52. C. A. Finlay *et al.*, *Mol. Cell. Biol.* **8**, 531 (1988); O. Pinhasi-Kimhi *et al.*, *Nature* **320**, 182 (1986); P. W. Hinds *et al.*, *Mol. Cell. Biol.* **7**, 2863 (1987); H.-W. Stürzbecher *et al.*, *Oncogene* **1**, 201 (1987); C. F. Clarke *et al.*, *Mol. Cell. Biol.* **8**, 1206 (1988).
53. M. Oren, W. Maltzman, A. J. Levine, *Mol. Cell. Biol.* **1**, 101 (1981).
54. E. Harlow, D. C. Pim, L. V. Crawford, *J. Virol.* **37**, 564 (1981); P. Sarnow *et al.*, *Cell* **28**, 387 (1982).
55. B. A. Werness, A. J. Levine, P. M. Howley, *Science* **248**, 76 (1990); M. Scheffner *et al.*, *Cell* **63**, 1129 (1990).
56. R. R. Weichselbaum, M. Beckette, A. Diamond, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2106 (1988); J. Y. Shew, *Oncogene Res.* **4**, 205 (1989); L. M. Mulligan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5863 (1990).
57. D. Malkin *et al.*, *Science* **250**, 1233 (1991); S. Srivastava *et al.*, *Nature* **348**, 747 (1990).
58. L. V. Crawford, D. C. Pim, R. D. Bulbrook, *Inst. J. Cancer* **30**, 185 (1982); J. Cheng and M. Haas, *Mol. Cell. Biol.* **10**, 5502 (1990); T. Takahashi *et al.*, *Science* **246**, 491 (1989); I. C. Hsu *et al.*, *Nature* **350**, 427 (1991); B. Bressac, M. Kew, J. Wands, M. Ozturk, *ibid.*, p. 429; J. Bartek *et al.*, *Inst. J. Cancer* **46**, 839 (1990); R. Iggo *et al.*, *Lancet* **335**, 675 (1990); S. J. Baker *et al.*, *Cancer Res.* **50**, 7717 (1990); N. R. Rodrigues *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7555 (1990); S. J. Baker *et al.*, *Science* **244**, 217 (1989); J. M. Nigro *et al.*, *Nature* **342**, 705 (1989); D. Sidransky *et al.*, *Science* **252**, 706 (1991); M. Hollstein *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9958 (1990); B. Bressac *et al.*, *ibid.*, p. 1973; A. G. Menon *et al.*, *ibid.*, p. 5435; J. Cheng and M. Haas, *Mol. Cell. Biol.* **10**, 5502 (1990); H. Ahuja *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6783 (1989).
59. A. L. Harris, *Nature* **350**, 377 (1991).

60. J. R. Bischoff *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4766 (1990).
61. S. E. Kern *et al.*, *Oncogene* **6**, 131 (1991); S. Fields and S. K. Jang, *Science* **249**, 1046 (1990); R. W. O'Rourke *et al.*, *Oncogene* **5**, 1829 (1990); L. Raycroft, H. Wu, G. Lozano, *Science* **249**, 1049 (1990); S. E. Kern *et al.*, *ibid.* **252**, 1708 (1991).
62. J. R. Jenkins, K. Rudge, G. A. Currie, *Nature* **312**, 651 (1984).
63. A. G. Knudson and L. C. Strong, *J. Natl. Cancer Inst.* **48**, 313 (1972); U. Francke *et al.*, *Cytogenet. Cell Genet.* **24**, 185 (1979).
64. P. Grundy *et al.*, *Nature* **336**, 374 (1988); I. Henry *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3247 (1989); V. Huff *et al.*, *Nature* **336**, 377 (1988); A. E. Reeve *et al.*, *Mol. Cell. Biol.* **9**, 1799 (1989); A. Koufos *et al.*, *Am. J. Hum. Genet.* **44**, 711 (1989).
65. K. M. Call *et al.*, *Cell* **60**, 509 (1990); M. Gessler *et al.*, *Nature* **343**, 774 (1990); K. Pritchard-Jones *et al.*, *ibid.* **346**, 194 (1990); J. Pelletier *et al.*, *Genes Dev.* **5**, 1345 (1991).
66. N. P. Pavletich and C. O. Pabo, *Science* **252**, 809 (1991).
67. V. P. Sukhatme, *J. Am. Soc. Nephrol.* **1**, 859 (1990); V. Sukhatme *et al.*, *Cell* **53**, 37 (1988); B. A. Christy, L. F. Lau, D. Nathans, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7857 (1988); P. Lemaire *et al.*, *ibid.*, p. 4691; J. Milbrandt, *Science* **238**, 797 (1987); R. W. Lim, B. C. Varnum, H. R. Herschman, *Oncogene* **1**, 263 (1987).
68. F. J. Rauscher III *et al.*, *Science* **250**, 1259 (1990).
69. S. L. Madden *et al.*, *ibid.* **253**, 1550 (1991).
70. T. Graf and H. Beug, *Cell* **34**, 7 (1983).
71. J. Sap *et al.*, *Nature* **324**, 635 (1986); C. Weinberger *et al.*, *ibid.*, p. 641.
72. J. Sap *et al.*, *ibid.* **340**, 242 (1989).
73. K. Damm, C. C. Thompson, R. M. Evans, *ibid.* **339**, 593 (1989); K. Damm *et al.*, *EMBO J.* **6**, 375 (1987).
74. M. Zenke *et al.*, *Cell* **52**, 107 (1988); B. Pain *et al.*, *New Biologist* **2**, 284 (1990).
75. H. de Thé *et al.*, *Nature* **347**, 558 (1990); A. Kakizuka *et al.*, *Cell* **66**, 663 (1991).
76. B. Ponder, *Trends Genet.* **6**, 213 (1990).
77. K. W. Kinzler *et al.*, *Science* **253**, 661 (1991); I. Nishisho *et al.*, *ibid.*, p. 665; G. Joslyn *et al.*, *Cell* **66**, 601 (1991); J. Groden *et al.*, *ibid.*, p. 589.
78. D. W. Yandell *et al.*, *N. Engl. J. Med.* **321**, 1689 (1989).
79. J. Sumegi, E. Uzvolgyi, G. Klein, *Cell Growth Differen.* **2**, 247 (1990); H.-J. S. Huang *et al.*, *Science* **242**, 1563 (1988); R. Bookstein *et al.*, *ibid.* **247**, 712 (1990).
80. B. E. Weissman *et al.*, *Science* **236**, 175 (1987).
81. S. A. Bader *et al.*, *Cell Growth Diffen.* **2**, 245 (1991).
82. M. Koi *et al.*, *Mol. Carcinogen.* **2**, 12 (1989); P. J. Saxon, E. S. Srivatsan, E. J. Stanbridge, *EMBO J.* **15**, 3461 (1986).
83. J. Yokota *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 9252 (1987).
84. E. A. Fearon *et al.*, *Nature* **318**, 377 (1985); Y. C. Tsai *et al.*, *Cancer Res.* **50**, 40 (1990).
85. W. Cavenee, H. J. Scrable, C. D. James in *Recessive Oncogenes and Tumor Suppressor Genes*, W. Cavenee, N. Hastie, E. Stanbridge, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989), p. 67; not localized to specific region on chromosome 10.
86. C. G. P. Mathew *et al.*, *Nature* **328**, 524 (1987); N. E. Simpson *et al.*, *ibid.*, p. 528.
87. M. Shiraishi *et al.*, *Jpn. J. Cancer Res.* **78**, 1302 (1987).
88. K. Larsson *et al.*, *Nature* **332**, 85 (1988).
89. C. D. James *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2858 (1989).
90. A. Weston *et al.*, *ibid.*, p. 5099.
91. M. Hollstein *et al.*, *Science* **253**, 49 (1991).
92. B. R. Seizinger *et al.*, *Nature* **322**, 644 (1986); B. R. Seizinger *et al.*, *Science* **236**, 317 (1987); G. A. Rouleau *et al.*, *Nature* **329**, 5419 (1987).
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Growth Factors and Cancer

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Signaling pathways that mediate the normal functions of growth factors are commonly subverted in cancer. Oncogenes identified by a variety of approaches have been shown to funcion at critical steps in mitogenic signaling. Progression through the cell cycle requires the coordinated actions of members of two complementary classes of growth factors, and oncogenes appear to replace the actions of one set of these growth factors. Growth factors can also influence normal cell differentiation, and constitutive activation of growth-promoting pathways in cancer cells can modulate the cell phenotype as well. Paracrine actions of growth factors and cytokines may also influence the stepwise series of genetic events that lead to malignancy. New approaches for cancer therapy are being developed that intervene at various steps in growth factor signaling pathways.

MULTICELLULAR ORGANISMS HAVE HIGHLY COORDINATED mechanisms to control cellular interactions. These complex signaling networks mediate normal embryonic development and are responsible for systemic responses to wounding and infection. The discovery of nerve growth factor (NGF) (*1*) and epidermal growth factor (EGF) (*2*) has led to the identification of a wide array of factors that affect the growth of virtually all cell types. Such factors can act as positive or negative modulators of cell proliferation and influence differentiation. The interaction of growth factors, cytokines and hormones with specific membrane receptors triggers a cascade of intracellular biochemical signals, resulting in the activation and repression of various subsets of genes.

Genetic aberrations in growth factor signaling pathways are inextricably linked to developmental abnormalities and to a variety of chronic diseases, including cancer. Malignant cells arise as a result of a stepwise progression of genetic events that include the unregulated expression of growth factors or components of their signaling pathways. This review focuses on normal aspects of growth factor signal transduction, as well as genetic aberrations in growth factor signaling pathways commonly implicated in human malignancy.

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Stringent Regulation of Mitogenic Responsiveness to Growth Factors

Growth factors cause cells in the resting or G_0 phase to enter and proceed through the cell cycle. The mitogenic response occurs in two parts; the quiescent cell must first be advanced into the G_1 phase of the cell cycle by "competence" factors, traverse the G_1 phase, and then become committed to DNA synthesis under the influence of "progression" factors (*3*). Transition through the G_1 phase requires