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).
93. The author gratefully acknowledges the help of C. Hickey in preparing this manuscript and the help of P. Hinds, D. Cobrinik, S. Egan, S. Day, and T. Jacks for comments on the text. The author is an American Cancer Society Research Professor and is supported by grants from the National Cancer Institute (5R35-CA39826) and the American Cancer Society (CD 355B).

Growth Factors and Cancer

STUART A. AARONSON

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.

The author is in the Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892.

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

sustained growth factor stimulation over a period of several hours (Fig. 1). If the signal is disrupted for a short period of time, the cell reverts to the G_0 state (*4*). There is also a critical period in G_1 during which simultaneous stimulation by both factors is needed to allow progression through the cell cycle (*5, 6*). After this restriction point, only the presence of a "progression" factor, such as insulin-like growth factor 1 (IGF-1), is needed (*7*). Cytokines such as transforming growth factor β (TGFβ), interferon, or tumor necrosis factor (TNF) can antagonize the proliferative effects of growth factors. In the case of TGFβ, these effects can be observed even when cells are treated with the cytokine relatively late in G_1 (*8*).

In some cell types, the absence of growth factor stimulation causes the rapid onset of programmed cell death or apoptosis (*9*). Certain growth factors can also promote differentiation of a progenitor cell, while at the same time stimulating proliferation; others acting on the same cell induce only proliferation (*10*). Thus, there must be specific biochemical signals responsible for differentiation that only certain factors can trigger (*11, 12*). The actions of a series of growth factors can cause a hematopoietic progenitor to move through stages to a terminally differentiated phenotype (*10*). However, at intermediate stages, in the absence of continued stimulation by the factor, this commitment is reversible (*13*). Although the differentiation program of the cell governs the diversity of phenotypic responses elicited, there are some common, highly conserved biochemical pathways for mitogenic signaling. For example, transfection of cells with DNA encoding foreign receptors often allows coupling of the appropriate ligand to mitogenic signal transduction pathways inherently expressed by the cells (*14*).

The Growth Factor Connection to Cancer

In the early 1980's, approaches aimed at identifying the functions of retroviral oncogenes converged with efforts to investigate normal mitogenic signaling by growth factors. A number of retroviral oncogene products were found to be similar to the protein kinase encoded by v-*src* product (*15*). Unlike many protein kinases that phosphorylate serine or threonine residues, the v-*src* product is a protein kinase that specifically phosphorylates tyrosine residues (*16*). Purification and sequencing of growth factors and their receptors revealed that the platelet derived growth factor (PDGF) B-chain is similar to the predicted v-*sis* oncogene product (*17*) and that the v-*erb*B product, which has sequence similarity to the v-*src* product, is a truncated form of the EGF receptor (*18*). Binding of EGF to its receptor results in autophosphorylation of the receptor on tyrosine (*19*). As will be illustrated, oncogenes activated by a variety of mechanisms (*20*) frequently have been shown to encode growth factors, receptor tyrosine kinases, or other enzymes that participate in mitogenic signaling.

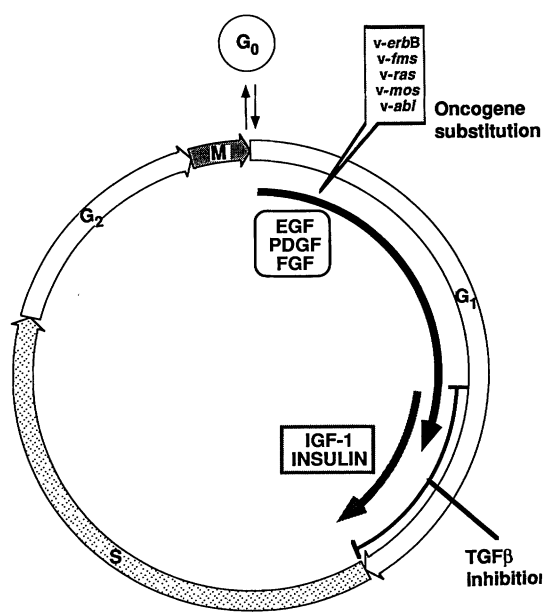

Fig. 1. Growth factor requirements during the cell cycle. A schematic representation of requirements for the coordinated actions of two complementing growth factors to induce cell DNA synthesis. In BALB-MK cells, several oncogenes can specifically substitute for the competence factor requirement. The ability of TGFβ to inhibit the onset of DNA synthesis, even when added in late G_1 is also depicted.

Growth Factor Receptors with Tyrosine Kinase Activity

Cells of most if not all major tissue types are targets of growth factors that mediate their effects by means of receptors with intrinsic tyrosine kinase activity. These receptors have an extracellular ligand-binding domain and an intracellular tyrosine-kinase domain responsible for transducing the mitogenic signal (Fig. 2). Ligand binding induces formation of receptor dimers or oligomers (*21*) and molecular interactions between adjacent cytoplasmic domains lead to activation of kinase function. Most evidence indicates that the transmembrane domain does not directly influence signal transduc-

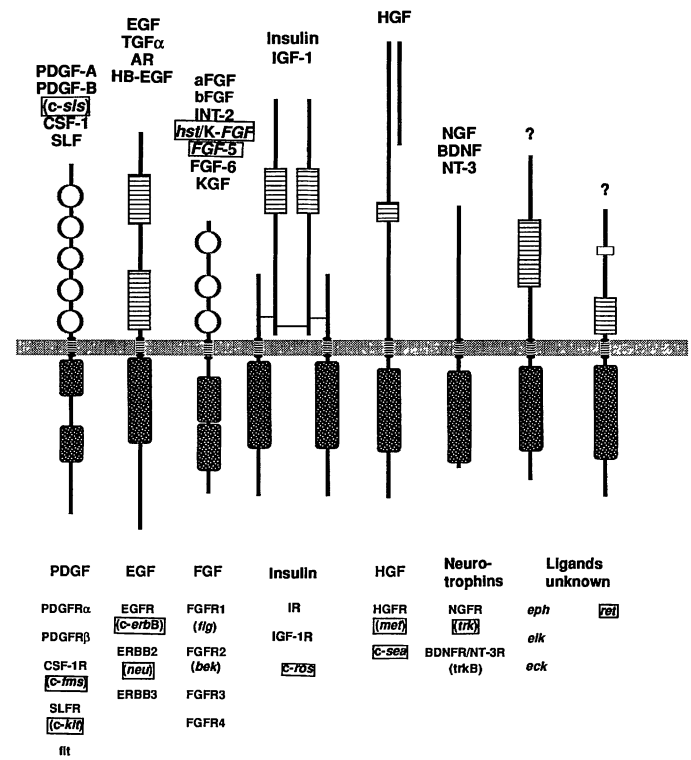

Fig. 2. Transmembrane tyrosine kinases. Structural features of various receptor tyrosine kinase receptors are shown. Each receptor family is designated by a prototype ligand. Growth factors known to bind to receptors of a given family are listed above, and receptors that constitute each family are listed below. Boxes denote those growth factors or receptors whose genes were initially identified as activated oncogenes. The c-*onc* designation is used to specify cellular homologs of retroviral oncogenes. Open circles illustrate immunoglobulin-like repeats. Dashed boxes indicate cysteine-rich domains. Dotted boxes indicate conserved tyrosine kinase domains.

tion and is instead a passive anchor of the receptor to the membrane. However, point mutations in the transmembrane domain of one receptor-like protein, the product of the *neu* or *erb*B-2 gene, enhances its transforming properties (*22*). The tyrosine kinase domain is the most conserved and is absolutely required for receptor signaling. Mutation of a single lysine in the ATP (adenosine triphosphate) binding site, which destroys the ability of the receptor to phosphorylate tyrosine residues, completely inactivates biological function (*23*). The receptor itself is often the major tyrosine phosphorylated species observed after the receptor is activated by ligand binding. Tyrosine phosphorylation may modulate kinase activity, but certainly affects the ability of the kinase to interact with substrates (*24*).

Several distinct families of receptors containing structurally related members are illustrated in Fig. 2. A number of these receptors are encoded by protooncogenes. In some cases their viral counterparts, like v-*sis* and v-*erb*B, were initially identified as retroviral oncogenes (*25*); others have been activated by retroviral integration (*26*). Others were detected as cellular oncogenes by DNA transfection (*27*). Still others reflect genes molecularly cloned on the basis of structural similarity to other tyrosine kinases (*28, 29*) or by identification of protein sequence (*30*). The spacing of cysteine residues in their external domains defines either immunoglobulin-like domains, in the case of PDGF and FGF receptor families, or cysteine-rich clusters. The known ligands for each receptor family also show similarities in cysteine spacing but are otherwise dissimilar (*31*). Recently, the *met* (*32*) and *trk* (*33*) proto-oncogene products were identified as the receptors for hepatocyte growth factor (HGF) and NGF, respectively, and two NGF related ligands, brain-derived neurotrophic factor and neurotrophin-3 were found to interact with the *trk*-B product (*34*).

Receptor Tyrosine Kinase Substrates

The PDGF system has served as the prototype for identification of substrates of the receptor tyrosine kinases. Certain enzymes become physically associated with and are phosphorylated by the activated PDGF receptor kinase. These proteins include phospholipase C (PLC-γ) (*35*), phosphatidylinositol 3′ kinase (PI-3K) (*36*), Ras guanosine triphosphatase (GTPase) activating protein (GAP) (*37*), and Src and Src-like tyrosine kinases (*38*). These molecules contain noncatalytic domains called Src homology (SH) regions 2 and 3. SH2 domains bind preferentially to tyrosine phosphorylated proteins and SH3 domains may promote binding to membranes or the cytoskeleton (*24*). The *raf* proto-oncogene product also becomes physically associated with the receptor and tyrosine phosphorylated (*39*), although it lacks SH2 or SH3 domains (Fig. 3).

PLC-γ is one of several PLC isoforms. It hydrolyses phosphatidylinositol 4,5-bisphosphate and generates two second messengers, inositol trisphosphate and diacylglycerol (*40*). The former causes release of stored intracellular calcium and the latter activates protein kinase C (PKC). These second messengers appear rapidly in cells after stimulation by growth factors such as PDGF and the amount of these compounds synthesized in vivo correlates with the ability of a particular receptor kinase to induce tyrosine phosphorylation of PLC-γ (*35*). Phosphorylation of PLC-γ on tyrosine increases its catalytic activity in vitro (*41*). Together, the results indicate that receptor induced tyrosine phosphorylation activates PLC-γ. The actions of a number of tumor promoters are thought to be mediated by PKC (*40*).

The PI-3K, which phosphorylates the inositol ring of phosphatidylinositol in the 3′ position, becomes physically associated with a number of activated tyrosine kinases (*42*). The 85-kD subunit of the protein contains two SH2 domains and an SH3 domain and is tyrosine phosphorylated, but the subunit lacks PI-3K activity (*43*). The catalytic domain is likely associated with a 110-kD protein that is part of a heterodimeric complex with the 85-kD protein (*42*). The transforming ability of polyoma middle T mutants correlates with the functional activity of PI-3K in complexes with $pp60^{c\text{-}src}$ (*44*). Moreover, v-*src* and v-*abl* mutants that fail to associate with PI-3K are nontransforming (*45*). Thus, PI-3K may function in the process of transformation.

GAP regulates the function of the Ras protein (*46*). It stimulates the GTPase activity of the 21-kD guanine nucleotide binding protein Ras (*47*). Ras is a critical component of intracellular mitogenic signaling pathways. Microinjection of oncogenically activated Ras into NIH 3T3 fibroblasts induces DNA synthesis (*48*). GAP acts as a negative regulator of Ras function (*49*). Mutations that cause oncogenic activation of *ras* lead to accumulation of Ras bound to GTP, the active form of the molecule (*47*). These mutations in *ras* block the ability of GAP to promote conversion of Ras to its inactive, GDP-bound form (*46*). GAP may also function in a complex with Ras as an effector of its downstream signaling functions (*50*). Thus, mutations that impair interaction of Ras with GAP also block the biological function of Ras.

Stimulation of certain receptors results in physical interaction of GAP with the receptor kinase (*37*) as well as its association with the cell membrane, the known site of Ras function (*47*). Tyrosine-phosphorylated GAP is also found to be associated in a complex with at least two other tyrosine phosphorylated proteins (p62 and p190) that may modulate Ras function (*51*). Interaction with p190 decreases the ability of GAP to promote GTPase activity of Ras in vitro (*52*). Stimulation of cells with PDGF leads to an increase in the amount of GTP-bound Ras (*53*), consistent with the possibility that tyrosine phosphorylation of GAP associated proteins transiently interrupts its inhibition of Ras function. However, other proteins that regulate Ras, including a protein that promotes release of bound GDP in exchange for GTP, have been identified in yeast (*54*), and there is evidence for such activities in mammalian cells as well (*55*). Thus, further studies are needed to firmly establish the mechanisms responsible for activation of Ras in growth factor-stimulated cells as well as the effector functions of this important signal transducer.

The *src* gene and structurally related family members including *yes* and *fgr* were initially identified as oncogenes of retroviruses. These and the other members of the *src* family encode nonreceptor tyrosine kinases (*56*). Some are expressed only in certain differentiated cell types, and may have highly specialized functions, while others are expressed in many cell types. Src and other related tyrosine kinases are activated rapidly in cells stimulated with PDGF (*38*).

The *raf* proto-oncogene product is a serine-threonine kinase (*57*)

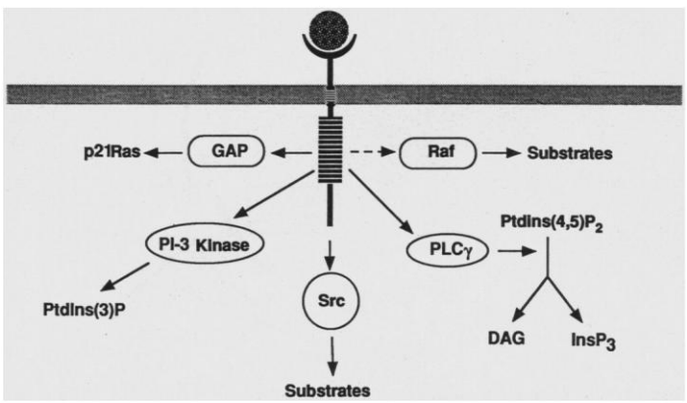

Fig. 3. Substrates of receptor tyrosine kinases. A prototype receptor tyrosine kinase and known intracellular substrates are shown. The substrate specificity of different receptors is described in the text. The dashed line leading to Raf indicates that its activation may be by mechanisms other than direct tyrosine phosphorylation in response to some growth factor receptors.

that is activated by a PKC-independent mechanism in response to a number of growth factors (*58*). Oncogenically activated forms of Raf resulting from deletions or mutations of its NH_2-terminal domain have been identified in tumors by means of gene transfer experiments. The products of the *raf* oncogenes show constitutively increased serine-threonine kinase activity. Thus, the NH_2-terminal domain may normally serve to regulate the catalytic domain. The substrates of Raf remain to be identified.

Substrate Specificity of Receptor Kinases

The various receptor kinases tyrosine phosphorylate specific sets of substrates. The substrates phosphorylated by receptors for progression factors such as IGF-1 (Fig. 1), must be distinct, at least in part, from those regulated by receptors for competence factors such as PDGF in order to activate the complementing pathways required for mitogenic signaling. Yet, there are also differences in substrate specificities among receptors for the PDGFs, colony-stimulating factor 1 (CSF-1), EGF, and the fibroblast growth factors (FGFs), whose functions appear to be interchangeable as competence factors. For instance, the PDGF receptor kinase interacts with each of the substrates mentioned above, but the related CSF-1 receptor appears not to phosphorylate PLC-γ or GAP (*59*). The EGF receptor and *erb*B-2 also phosphorylate GAP relatively inefficiently (*60*). The FGF receptors induce tyrosine phosphorylation of a prominent, as yet unidentified substrate, p90 (*61*), which is not phosphorylated in response to a number of other receptors. These findings suggest that different combinations of substrates can activate competence pathways.

Differences in substrate specificity of the receptors may determine phenotypic responses other than proliferation. For example, oligodendrocyte progenitors (OA-2 cells) undergo terminal differentiation in response to PDGF (*62*). Yet, FGF causes the same cells to proliferate rather than differentiate (*12, 63*). Similarly, PC12 neuronal cells proliferate in response to EGF but terminally differentiate as sensory neurons after treatment with NGF (*64*). In hematopoietic progenitor cells, the transfected CSF-1 receptor couples with pathways that induce monocyte differentiation as well as mitogenesis (*13*), whereas certain other growth factors stimulate only proliferation. While such differences might be explained by the specific pattern of substrates of various receptors, our understanding is still incomplete. Thus, further efforts will be needed to identify the full spectrum of second messengers recruited by different tyrosine kinase receptors as well as the specific functions of each with respect to mitogenic signaling and other cellular responses.

The connections between biochemical signals emanating from primary receptor substrates and resulting changes in the nucleus remain largely undefined. However, mitogenic signaling clearly affects the transcriptional activation of specific sets of genes and the inactivation of others. The nuclear effectors of gene activation are transcription factors that bind to DNA as homomeric or heteromeric dimers (*65*) and phosphorylation appears to modulate their functions as well (*66*). Within the complex regulatory network of transcription factors linked to mitogenic signaling pathways, a number including those encoded by *jun, fos, myc, myb, rel*, and *ets*, were identified as viral oncogenes (*67, 68*).

Oncogene Subversion of Specific Signaling Pathways

The evidence summarized above indicates that proto-oncogene products act at critical steps in growth factor signaling pathways. Thus, their constitutive activation as oncogene products would be expected to profoundly influence cell proliferation and possibly the differentiated state of the transformed cell. Tumor cells exhibit reduced requirements for serum in culture. The actions of oncogenes have been investigated with respect to their ability to subvert the actions of the two major growth factor signaling cascades. For instance, mouse keratinocytes can be propagated in chemically defined medium containing only two complementing growth factors, EGF and IGF-1 (*69*). Introduction of various oncogenically activated receptor kinases or *ras* or *raf* oncogenes completely alleviates the requirement for EGF but not IGF-1 (Fig. 1). These findings support the concept that the signaling pathway of competence factors ordinarily limits growth in vivo and that genetic changes activating critical regulatory molecules within this pathway are commonly associated with the generation of the malignant cell.

The state of differentiation of a cell can influence the action of an oncogene and affect the phenotype of the transformed cell. For example, the time of expression of the *ras* oncogene during differentiation of keratinocytes determines whether or not malignancy is induced (*70*). PC12 neural cells undergo terminal differentiation in response to *ras* or *src* oncogenes (*71*), and introduction of *ras* into Epstein-Barr Virus–immortalized B lymphoid cells induces plasma cell differentiation (*72*).

Commonly Activated Growth Signaling Molecules in Human Tumors

There is much evidence for genetic aberrations affecting growth factors or their receptors in human malignancies. Expression of PDGF and its receptors has been documented in a high fraction of sarcomas as well as glially derived neoplasms (*73*). In tissue culture, such tumor cells exhibit chronic PDGF receptor activation, demonstrating a functional autocrine loop induced by ligand stimulation of receptors made by the same cell (*74*). Similarly, transforming factor α (TGFα) is frequently detected in carcinomas expressing large amounts of EGF receptors (*75*). In the case of the FGFs, basic fibroblast growth factor (bFGF) is expressed by human melanoma cell lines but not by normal melanocytes, which require bFGF to proliferate (*76*). Because the ligands for some receptors with tyrosine kinase activity are unknown, the contribution of autocrine loops to malignancies may be greater than is presently appreciated.

Among growth factor receptors, the most frequently implicated in human cancer have been members of the EGF receptor family. The EGF receptor gene is often amplified or overexpressed, or both, in squamous cell carcinomas and glioblastomas (*77*). Similarly, *erb*B-2 is often overexpressed in adenocarcinomas of the breast, stomach and ovary (*78*). Overexpression of either gene under appropriate experimental conditions confers the transformed phenotype (*79*). The *erb*B-3 gene is overexpressed in certain breast carcinomas (*29*). Gene amplification or overexpression of the *met* gene encoding the HGF receptor or of *bek* encoding a member of the FGF family has been observed in human stomach carcinoma cell lines (*80*). Similarly, *ret* is activated by gene arrangements in a large fraction of human thyroid carcinomas (*81*).

Among other rate limiting molecules in mitogenic signaling, members of the *ras* family appear to be the most frequently detected as oncogenes. Oncogenic mutations of *ras* have been documented in as many as 30 to 50% of lung and colon carcinomas. In pancreatic carcinoma, the frequency of *ras* mutations approaches 90 to 95% (*82*). This may reflect the critical function of *ras* in mitogenic signal transduction in many cell types. Among the nuclear effectors, the *myc* family appears to be among the most frequently altered, with

activation by mechanisms ranging from specific chromsomal translocations in many B and T cell lymphomas (*83*) to amplification and overexpression in carcinomas of the lung, neuroblastomas, and a variety of other malignancies (*20*).

Involvement of Other Mitogenic Signaling Systems in Malignancy

Binding of ligands to at least two classes of receptors distinct from membrane spanning tyrosine kinases is known to stimulate cell proliferation. One class includes the receptors for interleukin-2 (IL-2), IL-3, IL-4, IL-6, IL-7, granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, and erythropoietin (Epo). These receptors are membrane glycoproteins with a single hydrophobic transmembrane domain (*84*). Their external domains are similar in size and contain several conserved cysteines in their NH_2-terminal portions (Fig. 4). In contrast, their cytoplasmic domains vary in length, show little if any sequence similarity, and possess no tyrosine kinase domain. Some of these receptors require associated proteins for high affinity ligand binding (*85*).

Little is known of the biochemical pathways by which these receptors stimulate proliferation, although their activation can lead to the appearance of tyrosine phosphorylated proteins (*86*) and increased amounts of GTP (guanosine triphosphate)-bound Ras (*87*). Binding of IL-2 to its receptor activates the tyrosine kinase Lck (*88*). Thus, the Src family of tyrosine kinases (which includes Lck) may participate in signal transduction by this class of receptors. Certain in vitro mutations of the Epo receptor constitutively activate the receptor and cause transformation of appropriate hematopoietic target cells (*89*). Erythroblastic leukemia induced by the spleen focus-forming virus is due to molecular mimicry of Epo by a recombinant *env* gene product of this defective retrovirus (*90*). In human T cell tumors associated with HTLV-1 infection, viral gene products appear to stimulate proliferation of affected cells by increasing expression of both IL-2 and its receptor (*91*).

Another class of molecules capable of causing mitogenic stimulation of certain cell types are neurotransmitters (Fig. 4). The topography of these receptors includes, in addition to their seven transmembrane domains, an extracellular NH_2-terminal domain and a cytoplasmic COOH-terminal tail or large intracellular loop containing regulatory serine and threonine residues (Fig. 4). The heterodimeric guanine nucleotide binding proteins (G proteins) activated by such receptors can be coupled to various effectors including adenylyl cyclase, phospholipase C, and K^+ channels (*92*). These receptors may also stimulate tyrosine phosphorylation, but the kinases responsible remain to be identified (*93*). Related genes encode the α_1-, α_2-, β_1-, and β_2-adrenergic receptors, the muscarinic acetylcholine receptors (mACHR), the serotonin receptors, the substance K receptor, the dopamine receptors, the bombesin receptor, and the endothelin receptor (*94*). The *mas* oncogene isolated by gene transfer from a human carcinoma encodes a seven membrane spanning receptor (*95*). Overexpression of certain acetylcholine or serotonin receptor subtypes after transfection in NIH 3T3 cells causes ligand-dependent transformation (*96*).

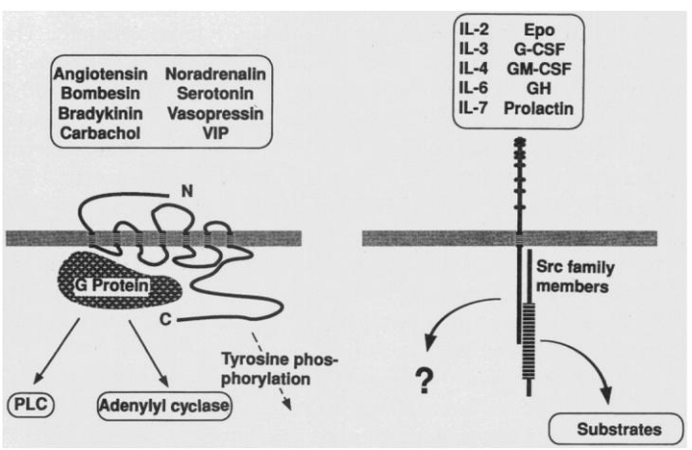

Fig. 4. Mitogenic signaling by other receptor classes. The structures and ligands of two classes of receptors lacking intrinsic tyrosine kinase activity and capable of mitogenic signaling are shown. These receptors possess either a seven transmembrane domain motif (left) or a single transmembrane domain (right). The former couples with G proteins that interact with phospholipase C (PLC) or adenylate cyclase.

Bombesin-like peptides are secreted by neuroectodermally derived small cell lung carcinomas and stimulate growth of these cells (*97*). Moreover, antibodies to bombesin have been reported to inhibit tumor cell proliferation in vitro and in vivo (*98*). These findings raise the possibility that autocrine stimulation by ligands for other G protein–coupled receptors may occur in tumors as well. It would follow that genes that act at rate limiting steps in signal transduction might be subject to oncogenic activation in specialized cell types in which neurotransmitters are normally mitogenic. Indeed, this appears to be the case. For instance, growth hormone–secreting pituitary tumors and endocrine tumors of the adrenal cortex and ovary frequently exhibit point mutations in G proteins that interact with adenylyl cyclase (*99*). The affected residues would lead to constitutive activity and increased intracellular concentrations of cAMP.

The ability to identify human oncogenes has to a large extent been limited by assay techniques. For instance, mutated forms of hematopoietic growth factor receptors efficiently couple with mitogenic signaling pathways and induce transformation in a hematopoietic progenitor cell, but fail to do so when transfected into NIH 3T3 fibroblasts, which are frequently used for detection of transforming genes (*89*). Similarly, overexpression of only G protein–coupled receptors linked to phosphatidylinositol turnover, but not those coupled to adenylyl cyclase, appears to cause ligand-dependent transformation of NIH 3T3 cells (*100*). To detect an oncogene by gene transfer, it must be small enough to be transfected and its promoter must allow a high level of expression in the recipient cell. Some of these problems have recently been overcome by the development of efficient cloning vectors allowing stable expression (*101*). It seems likely that as these approaches are combined with efforts to increase the efficiency of stable transfection of recipient cells other than NIH 3T3, the number of molecules identified as being critically involved in mitogenic signaling and cancer will expand.

Paracrine Influences on Tumor Progression

Growth factors released by one cell type and influencing proliferation of another cell (paracrine stimulation) may also play important roles in tumor progression. For instance, the ability of steroid hormones to stimulate epithelial cell proliferation in sex hormone–responsive tissues such as breast and prostate appears to be mediated at least in part by hormonal effects on stromal cells (*102*). Stromal cells, in turn, influence parenchymal cells by increasing production of growth factors, decreasing production of inhibitory cytokines, or both. Hormonal influences on growth of breast and prostate tumors can be striking (*103*). Chronic wounding and increased cell proliferation associated with some diseases can lead to higher risk of cancer (*104*). Moreover, there are a number of animal studies in which chronic injury and repair in response to agents possessing no known mutagenic actions is associated with increased cancer risk (*105*). Thus, genetic lesions associated with some early cancers may

allow their clonal selection in response to paracrine-acting growth factors. A case in point involves the BCL-2 oncogene, which is activated in low grade B cell lymphomas by a chromosomal translocation of a rearranged immunoglobulin gene (*106*). BCL-2 acts to block apoptosis, but is not by itself capable of inducing cell proliferation (*107*). Presumably, rescue from programmed cell death allows the cell expressing BCL-2 to proliferate preferentially in response to mitogenic signals in vivo. This selective growth advantage can result in the eventual selection of a more malignant variant (*108*).

Recently, paracrine effectors for epithelial cells of major tissues have been identified and cloned. These include keratinocyte growth factor (KGF) (*109*) and HGF (*110, 111*). Scatter factor, a motility factor that causes dispersion of epithelial cells (*112*) is homologous to HGF (*112, 113*). The identification of such factors may help to explain how stromal cells indirectly influence normal epithelial cell growth in response to hormones and how these cells provide an environment conducive to proliferation, invasion, and even metastasis of epithelial tumor cells. Another aspect of malignancy is neo-angiogenesis, the process by which new vasculature is developed to support nourishment of malignant cells (*114*). A number of growth factors including the FGFs and EGF are chemotactic for endothelial cells and induce their proliferation. Such angiogenic factors may be released by stromal cells as part of an aberrant wound-healing response to tumor cell proliferation or by the tumor cell itself.

Stromal cells can also inhibit growth of oncogene-transformed cells in vivo (*115*). These findings indicate that inhibitory cytokines released by stromal cells can inhibit tumor cell proliferation and that loss of responsiveness to such factors could provide a strong selective growth advantage in vivo. Genetic alterations in tumors include loss of function of certain genes that ordinarily act in as yet undefined ways to hold proliferation in check. The latter have been termed tumor suppressor or anti-oncogenes and include as prototypes the pRb, the product of the retinoblastoma gene (Rb) (*116*) and p53 (*117*). The functions of p53 and Rb are required at the critical G_1 to S-phase transition (*117, 118*), the period during which TGFβ is able to block progression through the cell cycle (Fig. 1). Growth inhibition by TGFβ correlates with suppression of phosphorylation of pRb, which may inactivate its function at the G_1 to S-phase transition (*119*). Moreover, a correlation has been found between the lack of TGFβ responsiveness of tumors and inactivation of the Rb gene (*120*). These findings are consistent with the possibility that some tumor suppressor genes may encode proteins involved in the biochemical cascade stimulated by inhibitory cytokines.

Implications for Cancer Intervention

Our present knowledge of the role of growth factor signaling pathways in cancer offers opportunities for improvements in diagnosis and prognosis as well as for therapeutic intervention. Molecular probes specific for genetic rearrangements activating various oncogenes in B and T cell lymphomas and in chronic myelogenous leukemia are being used for diagnosis and to follow the course of treatment (*121*). Amplification of *erb*B-2 in breast or ovarian cancer (*122*) as well as N-*myc* in neuroblastoma (*123*) appear to be prognostic indicators of more aggressive tumors. Other applications of this type will likely derive from the systematic analysis of oncogenes and tumor suppressor genes altered in various tumors.

Several strategies have already been developed to exploit the agonist properties of growth factors in the clinical management of malignancies. For instance, administration of hematopoietic growth factors can ameliorate the toxicity of chemotherapy (*124*). It is possible that the synchronization of tumor cell proliferation by application of exogenous growth factors may increase the effectiveness of chemotherapeutic agents that act at a particular stage of the cell cycle. Certain cytokines such as interferon α and IL-2 are being applied to cancer treatment (*125*). These cytokines act to cause tumor cell destruction. In fact, interferon α is the treatment of choice in hairy cell leukemia (*126*). The complex paracrine networks that may influence tumor cell proliferation present another possible target for tumor intervention. For example, somatostatin antagonists have been reported to inhibit the proliferation of some tumors in vivo (*127*). Such antagonists may decrease the amount of circulating IGF-1 (*128*).

It is possible that more specific and effective means of targeting tumor cells may in the future be based upon intervention at those critical points in mitogenic signaling at which oncogenes are commonly activated. Blockade of the actions of autocrine or paracrine acting growth factors could be achieved by specific antagonists. One of the most promising target of more specific therapy may be the growth factor receptor itself. One strategy involves administration of monoclonal antibodies that induce receptor downregulation. In experimental models, such antibodies have been shown to impair tumor cell proliferation both in vitro and in vivo (*129*). Tumors that overexpress receptors might also be targeted with radioisotopes or toxins such as ricin or pseudomonasexotoxin A linked to monoclonal antibodies to the receptor or the specific ligand (*130*). In theory, this strategy would have the advantage of being both tumoricidal and having a high degree of specificity for the tumor cell.

The importance of tyrosine phosphorylation in mitogenic signal transduction provides another possible target for therapy. Erbstatin, isolated from actinomycetes (*131*), is a prototype tyrosine analogue. Tyrphostins are related molecules, which also block phosphorylation of tyrosine residues (*132*). It may be possible to design tyrphostins that selectively inhibit tyrosine kinases, and tyrphostins have been reported to inhibit cell proliferation in culture at concentrations exhibiting little toxicity (*132*).

The oncogenic activation of Ras in many tumors has led to efforts to inhibit its function. One target of such approaches is the posttranslational modification of the Ras molecule required for its membrane localization and essential for its function (*47*). If yeast or animal cells are inoculated with inhibitors that block farnesylation of Ras, cell proliferation is inhibited (*133*). In fact, peptides as small as four amino acids can serve as substrates for the purified farnesyl protein transferase (*134*). Exogenously administered peptides or their analogues may act as competitive inhibitors of the enzyme and thus inhibit the function of Ras. There is, of course, no certainty that any of these approaches will be more successful than available forms of cancer therapy. Nonetheless, it is hoped that the important insights gained over the past several years concerning the underlying mechanisms responsible for the malignant process will provide rational new approaches and eventual improvements in therapy for this disease.

REFERENCES AND NOTES

1. R. Levi-Montalcini, *Science* **237**, 1154 (1987).
2. S. Cohen, *Bioscience Reports* **6**, 1017 (1986).
3. W. J. Pledger, C. D. Stiles, H. N. Antoniades, C. D. Scher, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4481 (1977); *ibid.* **75**, 2839 (1978).
4. B. Westermark and C. H. Heldin, *J. Cell Physiol.* **124**, 43 (1985).
5. E. B. Leof, W. Wharton, J. J. Van Wyk, W. J. Pledger, *Exp. Cell Res.* **141**, 107 (1982); E. B. Leof, J. J. Van Wyk, E. J. O'Keefe, W. J. Pledger, *ibid.* **147**, 202 (1983).
6. D. Wexler, T. P. Fleming P. P. Di Fiore, S. A. Aaronson, unpublished observations.
7. A. B. Pardee, *Science* **246**, 603 (1989).
8. H. L. Moses, E. Y. Yang, J. A. Pietenpol, *Cell* **63**, 245 (1990).
9. A. H. Wyllie *et al.*, *Br. J. Cancer* **56**, 251 (1987); G. T. Williams, *Cell* **65**, 1097 (1991).
10. D. Metcalf, *Nature* **339**, 27 (1989).
11. F. Walker, N. A. Nicola, D. Metcalf, A. N. Burgess, *Cell* **43**, 269 (1985); B. C.

Gliniak and L. R. Rohrschneider *ibid.* **63**, 1073 (1990).
12. R. D. McKinnon, T. Matsui, M. Dubois-Dalcq, S. A. Aaronson, *Neuron* **5**, 603 (1990).
13. J. H. Pierce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5613 (1990); L. Rohrschneider and D. Metcalf, *Mol. Cell. Biol.* **9**, 5081 (1989).
14. M. F. Roussel *et al.*, *Nature* **325**, 549 (1987); J. H. Pierce *et al.*, *Science* **239**, 628 (1988); Van Rudden and I. Wagner, *EMBO J.* 7, 2749 (1988).
15. M. Collett and R. Erickson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2021 (1978).
16. T. Hunter and B. Sefton, *ibid.* 77, 1311 (1980).
17. R. Doolittle *et al.*, *Science* **221**, 275 (1983); M. Waterfield, *Nature* **304**, 35 (1983).
18. J. Downward *et al.*, *Nature* **307**, 521 (1984).
19. G. Carpenter and S. Cohen, *J. Biol. Chem.* **165**, 7709 (1990).
20. J. M. Bishop, *Cell* **64**, 235 (1991).
21. A. Ullrich and J. Schlessinger, *ibid.* **61**, 203 (1990).
22. C. I. Bargmann, M. C. Hung, R. A. Weinberg, *ibid.* **45**, 649 (1986); O. Segatto, C. R. King, J. H. Pierce, P. P. Di Fiore, S. A. Aaronson, *Mol. Cell. Biol.* **8**, 5570 (1988).
23. Y. Yarden and A. Ullrich, *Annu. Rev. Biochem.* **57**, 443 (1988).
24. C. A. Koch, D. Anderson, M. F. Moran, C. Ellis, T. Pawson, *Science* **252**, 668 (1991).
25. C. J. Sherr *et al.*, *Cell* **41**, 665 (1985); P. Besmer *et al.*, *Nature* **320**, 415 (1986); D. R. Smith, P. K. Vogt, M. J. Hayman, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5291 (1989); H. Matsushime, L. H. Wang, M. Shibuya, *Mol. Cell. Biol.* **6**, 3000 (1986).
26. C. Dickson, R. Deed, M. Dixon, G. Peters, *Prog. Growth Fact. Res.* **1**, 123 (1989).
27. M. Taira *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2980 (1987); P. Delli Bovi *et al.*, *Cell* **50**, 729 (1987); X. Zhan, B. Bates, X. G. Hu, M. Goldfarb, *Mol. Cell. Biol.* **8**, 3487 (1988); A. L. Schechter *et al.*, *Nature* **312**, 513 (1984); M. Dean *et al.*, *ibid.* **318**, 385 (1985); D. Martin-Zanca, S. H. Hughes, M. Barbacid, *ibid.* **319**, 743 (1986); M. Takahashi and G. M. Cooper, *Mol. Cell. Biol.* **7**, 1378 (1987).
28. M. Shibuya *et al.*, *Oncogene* **5**, 519 (1990); T. Matsui *et al.*, *Science* **243**, 800 (1989); C. R. King, M. H. Kraus, S. A. Aaronson, *ibid.* **229**, 974 (1985); K. Semba, N. Kamata, K. Toyoshima, T. Yamamoto, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6497 (1985); P. L. Lee, D. E. Johnson, L. S. Cousens, V. A. Fried, L. T. Williams, *Science* **245**, 57 (1989); E. B. Pasquale and S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5449 (1989); M. Ruta *et al.*, *Oncogene* **3**, 9 (1988); Y. Hattori *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5983 (1990); S. Kornbluth, K. E. Paulson, H. Hanafusa, *Mol. Cell. Biol.* **8**, 5541 (1988); E. B. Pasquale, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5812 (1990); T. Miki *et al.*, *Science* **251**, 72 (1991); J. Partanen *et al.*, *EMBO J.* **10**, 1347 (1991); K. Keegan, D. E. Johnson, L. T. Williams, M. J. Hayman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1095 (1991); R. Klein, L. F. Parada, F. Coulier, M. Barbacid, *EMBO J.* **8**, 3701 (1989); H. Hirai, Y. Maru, K. Hagiwara, J. Nishida, F. Takaku, *Science* **238**, 1717 (1987); V. Lhoták, P. Greer, K. Letwin, T. Pawson, *Mol. Cell. Biol.* **11**, 2496 (1991); R. A. Lindberg and T. Hunter, *ibid.* **10**, 6316 (1990).
29. M. H. Kraus, W. Issing, T. Miki, N. C. Popescu, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9193 (1989).
30. A. Ullrich *et al.*, *Nature* **313**, 756 (1985); Y. Yarden *et al.*, *ibid.* **323**, 226 (1986); A. Ullrich *et al.*, *EMBO J.* **5**, 2503 (1986).
31. S. A. Aaronson and S. R. Tronick, in *Cancer Medicine*, J. F. Holland *et al.*, Eds. (Lea & Febiger, Philadelphia, ed. 3, 1991).
32. D. P. Bottaro *et al.*, *Science* **251**, 802 (1991); L. Naldini *et al.*, *Oncogene* **6**, 501 (1991).
33. B. L. Hempstead, D. Marin-Zanca, D. R. Kaplan, L. F. Parada, M. V. Chao, *Nature* **350**, 678 (1991); A. R. Nebreda, D. Martin-Zanca, D. R. Kaplan, L. F. Parada, E. Santos, *Science* **252**, 558 (1991); R. Klein, S. Q. Jing, V. Nanduri, E. O'Rourke, M. Barbacid, *Cell* **65**, 189 (1991).
34. D. Soppet *et al.*, *Cell* **65**, 895 (1991).
35. J. Meisenhelder, P.-G. Suh, S. G. Rhee, T. Hunter, *ibid.* **57**, 1109 (1989); M. I. Wahl *et al.*, *Mol. Cell. Biol.* 9, 2934 (1989).
36. D. R. Kaplan *et al.*, *Cell* **50**, 1021 (1987).
37. C. J. Molloy *et al.*, *Nature* **342**, 711 (1989); D. R. Kaplan, D. K. Morrison, G. Wong, F. McCormick, L. T. Williams, *Cell* **61**, 125 (1990); A. Kazlauskas, C. Ellis, T. Pawson, J. A. Cooper, *Science* **247**, 1578 (1990).
38. R. Ralston and J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7845 (1985); R. M. Kypta, Y. Goldberg, E. T. Ulug, S. A. Courtneidge *Cell* **62**, 481 (1990).
39. D. K. Morrison, D. R. Kaplan, U. Rapp, T. M. Roberts, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8855 (1988); D. K. Morrison *et al.*, *Cell* **58**, 649 (1989).
40. A. Berridge and R. F. Irvine, *Nature* **341**, 197 (1989); U. Kikkawa, A. Kishimoto, Y. Nishizuka, *Annu. Rev. Biochem.* **58**, 31 (1989).
41. S. Nishibe *et al.*, *Science* **250**, 1253 (1990); P. J. Goldschmidt-Clermont *et al.*, *ibid.* **251**, 1231 (1991).
42. For review, see L. C. Cantley *et al.*, *Cell* **64**, 281 (1991).
43. J. A. Escobedo *et al.*, *ibid.* **65**, 75 (1991); E. Y. Scolnik *et al.*, *ibid.*, p. 83; M. Otsu *et al.*, *ibid.*, p. 91.
44. M. Whitman, D. R. Kaplan, B. Schaffransen, L. Cantley, T. M. Roberts, *Nature* **315**, 239 (1985); D. R. Kaplan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3624 (1986); S. A. Courtneidge and A. Heber, *Cell* **50**, 1031 (1987).
45. Y. Fukui and H. Hanafusa, *Mol. Cell. Biol.* **9**, 1651 (1989); L. Varticovski, Q. Daley, P. Jackson, D. Baltimore, L. C. Cantley, *ibid.* **11**, 1107 (1991).
46. F. McCormick, *Cell* **56**, 5 (1989).
47. M. Barbacid, *Annu. Rev. Biochem.* **56**, 779 (1987).
48. D. W. Stacey and H. F. Kung, *Nature* **310**, 508 (1984).
49. B. K. Zheng *et al.*, *ibid.* **346**, 754 (1990); K. Tanaka *et al.*, *Mol. Cell. Biol.* 9, 757 (1989); R. Ballester *et al.*, *Cell* **59**, 681 (1989).
50. A. Yatani *et al.*, *Cell* **61**, 769 (1990).
51. A. H. Bouton *et al.*, *Mol. Cell. Biol.* **11**, 945 (1991).
52. M. F. Moran *et al.*, *ibid.* **11**, 1807 (1991).
53. T. Satoh, M. Endo, M. Nakafuku, S. Nakamura, Y. Kasiro, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5993 (1991); J. B. Gibbs, M. S. Marshall, E. M. Scolnick, R. A. Dixon, U. S. Vogel, *J. Biol. Chem.* **265**, 20437 (1990).
54. S. Powers, E. Gonzales, T. Christensen, J. Cubert, D. Broek, *Cell* **65**, 12125 (1991); J. Chant, K. Corrado, J. Pringle, I. Herskowitz *ibid.*, p. 1231; S. Jones, M. L. Vignais, J. R. Broach, *Mol. Cell. Biol.* **11**, 2641 (1991).
55. Y. K. Huang, H. F. Kung, T. Kamata, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8008 (1990); A. Wolfman and I. G. Macara, *Science* **248**, 67 (1990); J. Downward, R. Riehl, L. Wu, R. A. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5998 (1990).
56. J. A. Cooper, in *Peptides and Protein Phosphorylation*, B. Kenpard and P. F. Alewood, Eds. (CRC Press, Inc., Boca Raton, FL, 1990), pp. 4242–4250.
57. U. R. Rapp *et al.*, *Cold Spring Harbor Symposium Quant. Biol.* **53**, 173 (1988).
58. P. Li, K. Wood, H. Mamon, W. Haser, T. Roberts, *Cell* **64**, 479 (1991).
59. J. R. Downing *et al.*, *EMBO J.* **8**, 3345 (1989); M. Reedijk, X. Liu, T. Pawson, *Mol. Cell. Biol.* **10**, 5601 (1990).
60. F. Fazioli *et al.*, *Mol. Cell. Biol.* **11**, 2040 (1991).
61. S. R. Coughlin, P. J. Barr, L. S. Cousens, L. J. Fretto, L. T. Williams, *J. Biol. Chem.* **263**, 988 (1988); D. P. Bottaro *et al.*, *ibid.* **265**, 12767 (1990).
62. M. C. Raff, L. E. Lillien, W. D. Richardson, J. F. Burne, M. D. Noble, *Nature* **333**, 562 (1988).
63. F. Besnard *et al.*, *Int. J. Dev. Neurosci.* 7, 401 (1989).
64. K. Huff, D. End, G. Guroff, *J. Cell. Biol.* **88**, 189 (1981); D. Schubert, S. Heinemann, Y. Kodokoro, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2579 (1977); M. A. Dichter, A. S. Tischler, L. A. Greene, *Nature* **268**, 501 (1977).
65. P. J. Mitchell and R. Tjian, *Science* **245**, 371 (1989); N. Jones, *Cell* **61**, 9 (1990); E. M. Blackwood and R. N. Eisenman, *Science* **251**, 1211 (1991).
66. N. J. Boyle *et al.*, *Cell* **64**, 573 (1991); C. A. Gonzalez and M. R. Montminy, *ibid.* **59**, 675 (1989); R. Ofir, V. J. Dnarki, J. Rashid, I. M. Verma, *Nature* **348**, 78 (1990); J. Ayiverx and P. Sassone-Corsi, *Cell* **64**, 983 (1991).
67. R. N. Eisenman, in *Oncogenes and the Molecular Origin of Cancer* R. E. Weinberg, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989), pp. 175–221.
68. B. Lewin, *Cell* **64**, 303 (1991).
69. J. P. Falco, W. G. Taylor, P. P. Di Fiore, B. E. Weissman, S. A. Aaronson, *Oncogene* **2**, 573 (1988).
70. A. Balmain and I. B. Pragnell, *Nature* **303**, 72 (1983); B. Bailleul *et al.*, *Cell* **62**, 697 (1990).
71. S. Alema, P. Cassalbore, E. Agostini, F. Tabo, *Nature* **316**, 557 (1985); M. Noda *et al.*, *ibid.* **318**, 73 (1985); I. Guerrero, H. Wong, A. Pellicer, D. E. Burstein, *J. Cell Physiol.* **129**, 71 (1986).
72. S. Seremetis *et al.*, *Science* **243**, 660 (1989).
73. C. H. Heldin and B. Westermark, *Br. Med. Bull.* **45**, 453 (1989); M. Maxwell *et al.*, *J. Clin. Invest.* **86**, 131 (1990); M. Nister *et al.*, *Cancer Res.* **48**, 3910 (1988).
74. T. P. Fleming, T. Matsui, C. J. Molloy, K. C. Robbins, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8063 (1989).
75. E. Di Marco *et al.*, *Oncogene* **4**, 831 (1989); R. Derynck *et al.*, *Cancer Res.* **47**, 107 (1987).
76. R. Halaban, B. S. Kwon, S. Ghosh, P. Delli Bovi, A. Baird, *Oncogene Res.* **3**, 177 (1988); R. Halaban *et al.*, *J. Cell Biol.* **107**, 1611 (1988).
77. T. A. Libermann *et al.*, *Nature* **313**, 144 (1985); T. Yamamoto *et al.*, *Cancer Res.* **46**, 414 (1986).
78. D. J. Slamon *et al.*, *Science* **235**, 177 (1985); D. J. Slamon *et al.*, *ibid.* **244**, 707 (1989); J. Yokota *et al.*, *Lancet* **i**, 765 (1986); M. H. Kraus, N. Popescu, S. Amsbaugh, C. R. King, *EMBO J.* **6**, 605 (1987).
79. P. P. Di Fiore *et al.*, *Science* **237**, 178 (1987); R. M. Hudziak, J. Schlessinger, A. Ullrich, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7159 (1987); P. P. Di Fiore *et al.*, *Cell* **51**, 1063 (1987); T. J. Velu *et al.*, *Science* **238**, 1408 (1987); H. Riedel, S. Massoglia, J. Schlessinger, A. Ullrich, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1477 (1988).
80. N. R. Soman, P. Correa, B. A. Ruiz, G. N. Wogan, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4892 (1991); C. Ponzetto *et al.*, *Oncogene* **6**, 553 (1991); S. Giordano, M. F. Di Renzo, R. Ferracini, L. Chiadó-Piot, P. M. Comoglio, *Mol. Cell. Biol.* **8**, 3510 (1988); M. Hattori *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5983 (1990).
81. M. Grieco *et al.*, *Cell* **60**, 557 (1990); A. Fusco *et al.*, *Nature* **328**, 170 (1987).
82. J. L. Bos, *Cancer Res.* **49**, 4682 (1989).
83. J. Kagan and C. M. Croce, *Ann. Oncol.* **2** (Suppl.), 9 (1991); C. L. Sawyers, C. T. Denny, O. N. Witte, *Cell* **64**, 337 (1991).
84. D. Cosman *et al.*, *Trends Biochem. Sci.* **15**, 265 (1990).
85. M. Tsudo, R. W. Kozak, C. K. Goldman, J. A. Waldmann, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9694 (1986); M. Hibi *et al.*, *Cell* **63**, 1149 (1990).
86. A. Morla, J. Schreur, A. Miyajama, J. Wang, *Mol. Cell. Biol.* **8**, 2214 (1988); Y. Konakura *et al.*, *Blood* **76**, 706 (1990); R. Isfort, R. Hunn, R. Frackelton, J. Ihle, *J. Biol. Chem.* **263**, 19203 (1988).
87. T. Satoh, M. Nakafuku, A. Miyajima, Y. Kaziro, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3314 (1991).
88. M. Hatakeyama *et al.*, *Science* **252**, 1523 (1991).
89. A. Yoshinura, G. Longmore, H. Lodish, *Nature* **348**, 647 (1990).
90. S. K. Ruscetti, N. Janesch, A. Chakroborti, S. T. Sawyer, W. D. Hankins, *J. Virol.* **63**, 1057 (1990); J. P. Li, A. D'Andrea, H. Lodish, D. Baltimore, *Nature* **343**, 762 (1990).
91. F. Wong-Staal and R. Gallo, *Nature* **317**, 395 (1985).
92. H. R. Bourne and A. L. DeFranco, in *Oncogenes and the Molecular Origins of Cancer*, R. E. Weinberg, Ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, (1989), pp. 97–124.
93. K. R. Stratton, P. F. Worley, R. L. Huganir, J. M. Baraban, *Proc. Natl. Acad. Sci.*

U.S.A. 86, 2498 (1989); J. Zachary, J. Gil, W. Lehman, J. Sinnett-Smith, *ibid.* 88, 4577 (1991); T. Force, J. M. Kyriakis, J. Avruch, J. V. Bonventre, *J. Biol. Chem.* 266, 6650 (1991); L. M. T. Leeb-Lundberg and X.-H. Song *ibid.* 266, 7746 (1991); S. J. Shattil and J. S. Brugge, *Curr. Opin. Cell Biol.*, in press.
94. B. F. O'Dowd, R. J. Lefkowitz, M. G. Caron, *Annu. Rev. Neurosci.* 12, 67 (1989); J. Ramachandran, E. G. Peralta, A. Ashkenazi, J. W. Winslow, D. J. Capon, *Bioessays* 10, 54 (1989); H. Y. Lin, E. H. Kaji, G. K. Winkel, H. E. Ives, H. F. Lodish, *Proc. Natl. Acad. Sci. U.S.A.* 88, 3185 (1991).
95. T. R. Jackson, L. A. Blair, J. Marshall, M. Goedert, M. R. Hanley, *Nature* 335, 440 (1988).
96. D. Julius, T. J. Livelli, T. M. Jessell, R. Axel, *Science* 244, 1057 (1989).
97. F. Cuttitta *et al.*, *Nature* 316, 823 (1985).
98. J. L. Mulshine *et al.*, *Ann. N.Y. Acad. Sci.* 547, 360 (1988).
99. C. A. Landis *et al.*, *Nature* 340, 692 (1989); J. Lyons *et al.*, *Science* 249, 655 (1990).
100. J. S. Gutkind, E. A. Novotny, M. R. Brann, K. C. Robbins, *Proc. Natl. Acad. Sci. U.S.A.* 88, 4703 (1991).
101. T. Miki *et al.*, *ibid.* 88, 5167 (1991).
102. A. A. Donjacour and G. R. Cunha, *Cancer Treat. Res.* 53, 335 (1991); M. E. Lippman, and R. B. Dickson, *J. Steroid Biochem.* 34, 107 (1989).
103. S. Sukumar, *Cancer Cells* 2, 199 (1990); C. A. Perez *et al.*, in *Principles and Practice of Oncology*, V. T. De Vita, S. R. Hallman, S. A. Rosenberg, Eds. (Lippincott, New York, 1989), pp. 1023–1058.
104. R. J. Coburn, *Cancer of the Skin* (Saunders, Philadelphia, 1976), pp. 939–949; S. Preston-Martin, M. C. Pike, R. K. Ross, P. A. Jones, B. E. Henderson, *Cancer Res.* 50, 7415 (1990); R. H. Collins, Jr., M. Feldman, J. S. Fordtran, *N. Engl. J. Med.* 316, 1654 (1987).
105. B. N. Ames and L. S. Gold, *Science* 249, 970 (1990); N. R. Drinkwater, *Cancer Cells* 2, 8 (1990).
106. Y. Tsujimoto, L. R. Finger, J. Yunis, P. C. Nowell, C. M. Croce, *Science* 226, 1097 (1984); Y. Tsujimoto *et al. ibid.* 228, 1440 (1985).
107. D. L. Vaux, S. Cory, J. M. Adams, *Nature* 335, 440 (1988); D. Hockenbery, G. Nunez, C. Millman, R. D. Schreiber, S. J. Korsmeyer, *ibid.* 348, 334 (1990).
108. J. C. Reed *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3660 (1990).
109. P. W. Finch, J. S. Rubin, T. Miki, D. Ron, S. A. Aaronson, *Science* 245, 752 (1989).
110. K. Miyazawa *et al.*, *Biochem. Biophys. Res. Commun.* 163, 967 (1989); T. Nakamura *et al.*, *Nature* 342, 440 (1989); J. S. Rubin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 415 (1991).
111. T. Nakamura, K. Nawa, A. Ichihara, *Proc. Natl. Acad. Sci. U.S.A.* 83, 6489 (1986); E. Gohda *et al.*, *J. Clin. Invest.* 81, 414 (1988); R. Zarnegar and G. Michalopoulous, *Cancer Res.* 49, 3314 (1989).
112. E. Gherardi and M. Stoker, *Nature* 346, 228 (1990).
113. K. M. Weidner *et al. J. Cell Biol.* 111, 2097 (1990).
114. L. Liotta, P. S. Steeg, W. G. Stetler-Stevenson, *Cell* 64, 327 (1991).
115. G. P. Dotto *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85, 6389 (1988); M. Bignami, S. Rosa, S. A. La Rocca, G. Falcone, F. Tato, *Oncogene* 2, 509 (1988); A. W. Stoker, C. Hatier, M. J. Bissell, *J. Cell Biol.* 111, 217 (1990).
116. S. H. Friend *et al.*, *Nature* 323, 643 (1986); W. H. Lee *et al.*, *Science* 235, 1394 (1987); Y. K. Fung, *ibid.* 236, 1547 (1987).
117. A. J. Levine, *Nature* 351, 453 (1991).
118. J. A. DeCaprio *et al.*, *Cell* 58, 1085 (1989).
119. M. Laiho, J. A. DeCaprio, J. W. Ludlow, D. M. Livingston, J. Massague, *ibid.* 62, 175 (1990).
120. R. A. Weinberg, *Trends Biochem. Sci.* 15, 199 (1990); W. F. Benedict, H. J. Xu, R. Takahashi, *Cancer Invest.* 8, 535 (1990); H. L. Moses, E. Y. Yang, J. A. Pietenpol, *Ciba Found. Symp.* 157, 66 (1991).
121. S. Sell, *Clin. Lab. Med.* 10, 1–37 (1990).
122. D. J. Slamon *et al.*, *Science* 244, 707 (1989).
123. G. M. Brodeur, R. C. Seeger, M. Schwab, H. E. Varmus, J. M. Bishop, *ibid.* 224, 1121 (1984).
124. J. Crawford *et al.*, *N. Eng. J. Med.* 325, 164 (1991).
125. J. J. Mule and S. A. Rosenberg, *Important Adv. Oncol.* (1989), p. 99.
126. M. C. Lill and D. W. Golde, *Blood* 4, 238 (1990).
127. Editorial, *Euro. J. Clin. Invest.* 17, 281 (1987); A. V. Schally, *Cancer Res.* 48, 6977 (1988); A. V. Schally *et al.*, *Proc. Soc. Exp. Biol. Med.* 175, 259 (1984); J. J. Shepherd and G. B. Senator, *Lancet* ii, 574 (1986).
128. K. Y. Ho, A. J. Weissberger, P. Marbach, L. Lazarus, *Ann. Int. Med.* 112, 173 (1990); S. W. J. Lamberts, *Acta. Endocrinol. (Copenhagen)* 286, 54 (1987).
129. R. Kumar, H. M. Shepard, J. Mendelsohn, *Mol. Cell. Biol.* 11, 979 (1991); J. A. Drebin, V. C. Link, D. F. Stern, R. A. Weinberg, M. I. Greene, *Cell* 41, 697 (1985); R. M. Hudziak *et al.*, *Mol. Cell. Biol.* 9, 1165 (1989).
130. I. Pastan (see accompanying article).
131. A. M. Umezawa *et al.*, *J. Antibiotics* 39, 170 (1986).
132. P. Yaish, A. Gazit, C. Gilan, A. Levitzki, *Science* 242, 933 (1988).
133. W. R. Schafer *et al.*, *ibid.* 245, 379 (1989); W. A. Maltese and K. M. Sheridan, *J. Cell Physiol.* 133, 471 (1987).
134. Y. Reiss, J. L. Goldstein, M. C. Seabra, P. J. Casey, M. S. Brown, *Cell* 62, 81 (1990).
135. I gratefully acknowledge my colleagues S. R. Tronick, J. H. Pierce, P. P. Di Fiore and M. Kraus, who provided helpful suggestions and critical comments. The scope of this review and space limitations precluded comprehensive referencing.

Chromosome Aberrations and Cancer

ELLEN SOLOMON, JULIAN BORROW, AUDREY D. GODDARD

Cancer may be defined as a progressive series of genetic events that occur in a single clone of cells because of alterations in a limited number of specific genes: the oncogenes and tumor suppressor genes. The association of consistent chromosome aberrations with particular types of cancer has led to the identification of some of these genes and the elucidation of their mechanisms of action. Consistent chromosome aberrations are observed not only in rare tumor types but also in the relatively common lung, colon, and breast cancers. Identification of additional mutated genes through other chromosomal abnormalities will lead to a more complete molecular description of oncogenesis.

CANCER IS THE RESULT OF THE ACCUMULATION OF MULtiple genetic changes (*1*). Each alteration, whether an initiating or a progression-associated event, may be mediated through a gross chromosomal change and therefore has the potential to be cytogenetically visible. A corollary of this idea is that the molecular characterization of chromosomal rearrangements will lead to the identification of genes involved in cancer. This review will focus on those chromosomal aberrations for which the affected genes have been cloned and characterized. Readers interested in the burgeoning cancer cytogenetics literature are referred to other reviews (*2*).

The authors are at the Somatic Cell Genetics Laboratory, Imperial Cancer Research Fund, London WC2A 3PX, United Kingdom.

Tumor Cytogenetics

The common tumor chromosome aberrations are generally classified as structural or numerical. Structural alterations include translocations,