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Oncoprotein Networks

Review

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The increasing complexity of growth control pathways has been paralleled by the increasingly diverse functions uncovered for oncoproteins and tumor suppressor proteins. Our awareness of the interconnections between growth factor- and growth inhibitor-initiated signaling pathways has furthered our understanding of mechanisms underlying malignant transformation, but at the same time has indicated how complex cell growth control networks will be. In the past few years, there has been enormous progress in elucidating the details of mitogenic signaling. The positioning of multiple oncoproteins and tumor suppressor proteins on the same pathway has underscored the importance of some of these signaling pathways in transformation. For instance, ErbB, Ras, and Raf all lie on the ERK MAP kinase (MAPK) pathway, which through phosphorylation of members of the Ets protein family leads to induction of immediate early genes like c-*fos*.

The functions of oncoproteins and tumor suppressor proteins have been more than adequately reviewed in several places in the past few years. Taking this into account, and given the space constraints, I have chosen to discuss examples of more recently discovered oncoproteins and tumor suppressor proteins that illustrate new principles of cell regulation, emphasizing how such principles allow us to establish a regulatory network. The repertoire of cell surface receptor types involved in oncogenesis has grown, and I begin with an overview of several types of receptor-driven pathways implicated in oncogenesis. I then trace oncoprotein-activated signaling pathways to the nucleus and discuss the intriguing connections between the actin cytoskeleton and transformation. I end by reviewing the role that oncoproteins play in triggering cell cycle progression and preventing apoptosis.

Receptor Pathways to Oncogenesis

Receptor Protein-Tyrosine Kinases

The role of mutant activated receptor protein-tyrosine kinases (PTKs) in oncogenesis is well established. An important principle in the activation of receptor PTKs is ligand-mediated dimerization. Increasing evidence indicates that oncogenic activation of receptor PTKs occurs through mutations that lead to constitutive dimerization and activation of the cytoplasmic catalytic domain. This process has been particularly well established in the case of Tpr-Met, where the N-terminal Tpr domain contains a leucine zipper that is essential for dimerization of the attached Met/HGF receptor domain, which leads to constitutive activation and oncogenic transformation (Rodrigues and Park, 1993). There are many other examples of mutations that cause constitutive receptor PTK dimerization. Point mutations in the Neu/ErbB2 transmembrane domain and in the extracellular domain of the CSF-1 receptor result in dimerization. The Npm-Alk fusion protein, generated by the t(2;5) chromosomal translocation in anaplastic large cell lymphoma, oligomerizes and is activated by virtue of its N-terminal nucleophosmin (Npm) sequences, which are fused to the entire cytoplasmic domain of Alk, a receptor-like PTK (Morris et al., 1994; Fujimoto et al., 1996). The Tel-PDGFβ receptor fusion, generated by the t(5;12) translocation in chronic myelomonocytic leukemia, joins the N-terminal part of Tel, an Ets family transcription factor, with the entire cytoplasmic domain of the *PDGFβR* PTK gene, resulting in dimerization through a helix-loop-helix motif in the Tel domain and constitutive PTK activation (Golub et al., 1994).

Tel proves to be a common fusion partner in leukemia-associated chromosomal translocations. In acute myeloid leukemias carrying the t(12;9) translocation, most of Tel, including the helix-loop-helix motif, is fused to the Abl nonreceptor PTK upstream of the SH3 domain, resulting in a dimeric protein that is constitutively tyrosine phosphorylated, and localizes to the cytoskeleton (Golub et al., 1996). This activation mechanism is similar to that for Bcr-Abl, the product of the t(9;21) chromosome fusion found in chronic myelogenous leukemia, where the N-terminal Bcr domain provides a coiled–coil type oligomerization domain. However, oncogenic activation of other nonreceptor PTKs does not involve fusion with dimerization partners but rather loss of a negative regulatory function, as is the case for v-Src, where the C-terminal regulatory tyrosine of c-Src is deleted.

Another receptor PTK, Ret, had been found to be oncogenically activated in multiple ways. Ret was originally discovered as an oncoprotein in a transfection assay as an artifactual fusion of the cytoplasmic domain with a fragment of the Rfp zinc finger protein resulting in a dimeric constitutively active form of Ret. Ret has subsequently been shown, however, to be mutated in specific human tumors including thyroid papillary carcinomas. A number of Ret-derived oncoproteins have been characterized, and in every case the cytoplasmic domain is fused to an N-terminal domain that affords constitutive dimerization and activation (e.g. the RIα PKA regulatory subunit). Ret is also of interest because mutant forms have been identified as the cause of hereditary cancers known as multiple endocrine neoplasia (MEN) type 2A and 2B and familial medullary thyroid carcinoma (FMTC) (van Heyningen, 1994). In MEN2A, a variety of mutations in the Ret extracellular domain commonly involving Cys have been found (e.g. Cys634Arg). Such mutations lead to constitutive activation, presumably through dimerization mediated by disulfide bonding of unpaired Cys. In MEN2B, the mutation is in the catalytic domain (Met918Thr), and this not only activates Ret but also alters its substrate specificity, making it more similar to that of the Src family PTKs.

A novel but indirect mechanism of receptor PTK activation is emerging from the study of v-Cbl, the oncoprotein encoded by the Cas NS-1 murine leukemia retrovirus, which was derived from c-Cbl through C-terminal

truncation. c-Cbl is a cytoplasmic protein that is tyrosine phosphorylated in response to EGF and other cytokines, and associates with the EGF receptor via its N-terminal domain (Galisteo et al., 1995). c-Cbl is homologous to the C. elegans protein Sli1, which was identified as a negative regulator of signaling from the EGF receptor-related Let23 receptor PTK in vulval development (Yoon et al., 1995). c-Cbl may play a similar role in downregulating receptor PTK signaling in mammalian systems. v-Cbl could then act as a dominant negative inhibitor of c-Cbl function by blocking its interaction with receptor PTKs, thus upregulating mitogenic signaling.

Secreted factors that signal through receptor PTKs are also implicated in oncogenesis. At least four of the large family of FGF genes have been identified as oncogenes in human tumors. Moreover, although germ line mutations that constitutively activate FGFR1, -2, and -3 result in developmental abnormalities of the skeletal system rather than hereditary cancers, *FGFR2* is commonly amplified in poorly differentiated gastric carcinomas (Hattori et al., 1992), implying that ectopic expression of FGFs and inappropriate activation of FGF receptor PTKs can result in tumorigenesis.

Secreted factors also play important roles in tumor metastasis and angiogenesis. For instance, hepatocyte growth factor (HGF)/scatter factor may play a role in metastasis, since it increases the motility and invasiveness of epithelial and endothelial cells. The HGF receptor gene (*MET*) is amplified and overexpressed in a number of tumor types, and in some cases the HGF receptor is constitutively activated (Di Renzo et al., 1995). At least three families of growth factor receptor PTKs are involved in angiogenesis, namely the vascular endothelial cell growth factor (VEGF) and FGF receptor families, and the Tie family. Many solid tumors produce VEGF, especially in response to hypoxia, a condition that commonly exists in solid tumors. Coinoculation of fibroblasts producing a retrovirus expressing a truncated "dominant-negative" form of Flk1, one of the VEGF receptors, with a variety of tumor cell types significantly inhibits tumor growth and angiogenesis in nude mice (Millauer et al., 1994). This supports a critical role for VEGF in tumor angiogenesis. IGF2 also proves to be critical for oncogene-induced tumors in vivo (Christofori et al., 1994).

The Wnt/Wingless Pathway

Another secreted factor that plays a role in tumorigenesis is Wnt1. *wnt1* was one of the first examples of an oncogene activated by provirus insertion, having been discovered as a common site of integration in MMTV-induced mouse mammary carcinomas. Wnt1 was subsequently shown to be a member of a family of secreted proteins that are important for early development; this family includes the Drosophila Wingless protein, which plays multiple roles in Drosophila development by influencing cell fate decisions. Although Wnt1 expression is restricted to the nervous system in vertebrates, being required for the development of the midbrain and cerebellum, other family members are expressed in the mammary gland. Despite a large body of information about Wnt function, mechanistic insight into how Wnt1 transforms has been been slow to emerge. In part this was because the Wnt receptor remained elusive. Very recently, however, proteins in the Frizzled family have been identified as Wnt receptors (Bhanot et al., 1996; Yang-Snyder et al., 1996). Frizzled is a Drosophila polarity gene product required for the development of normal tissue polarity in the epidermis. Frizzled family members have seven transmembrane domains and a cysteine-rich extracellular domain required for Wingless binding. The membrane topology of Frizzled family members is similar to that of G protein-coupled receptors, suggesting that they might be coupled to G proteins, but there is no direct evidence that this is the case.

Although the signaling pathway downstream of Wnt is not fully understood, several components of this pathway are implicated in cancer (Figure 1). Genetic analysis in Drosophila, and the effects of ectopic expression of proteins on Xenopus development have defined a pathway from Wnt leading to Dishevelled, Shaggy/Zeste-white 3 (GSK3), and Armadillo (β-catenin) (Orsulic and Peifer, 1996; Perrimon, 1996). Dishevelled is a cytoplasmic protein with little homology to other proteins, except that it has a PDZ domain; it is phosphorylated in response to Wingless and associates with membranes. Intriguingly, Frizzled family proteins have the SXV C-terminal consensus sequence required for PDZ domain binding, and Dishevelled may interact directly with Frizzled at the membrane.

Shaggy/Zeste-white 3 is a functional homolog of the mammalian glycogen synthase kinase 3 (GSK3) protein kinase. GSK3 normally suppresses the Wnt signaling pathway, and recent biochemical analysis indicates that Wingless decreases GSK3 activity (Cook et al., 1996). Activated Dishevelled itself could be a GSK3 inhibitor. The GSK3 substrates whose dephosphorylation propagates the Wingless signal have not yet been identified, but GSK3 is a cytoplasmic protein, and likely targets are Armadillo or a protein that interacts with Armadillo, such as the adenomatous polyposis coli gene product (APC), which is mutated in most colon cancers (Kinzler and Vogelstein, 1996). Armadillo is a homolog of β-catenin; the catenins are a family of proteins that associate with the cytoplasmic domains of cadherins, which are cell-cell interaction receptors that form part of the adherens junction. APC associates with α- and β-catenins, and by triggering degradation of free β-catenin this interaction appears to be critical for maintaining a low level of free β-catenin. GSK3 phosphorylates APC, increasing β-catenin binding (Rubinfeld et al., 1996). Thus, GSK3 activity could be required for β-catenin degradation. β-catenin itself is also a likely target for GSK3, since wild-type GSK3 phosphorylates β-catenin at a conserved site whose mutation increases its stability (Yost et al., 1996). The net effect of loss of APC or inactivation of GSK3 will be to increase the free pool of β-catenin.

Free β-catenin can signal by virtue of its association with members of TCF/LEF family of HMG box transcription factors, an interaction that allows a LEF-1/β-catenin complex to accumulate in the nucleus and drive transcription (Behrens et al., 1996; Molenaar et al., 1996). Consistent with a signaling function for β-catenin in the Wnt pathway, Wingless causes accumulation of cytoplasmic Armadillo in Drosophila, and Wnt1 expression in CM57G mouse mammary cells leads to upregulation of β- and γ-catenins, due to the stabilization of the soluble pools, which normally turn over extremely rapidly (Papkoff et al., 1996). In this manner, Wnts can induce

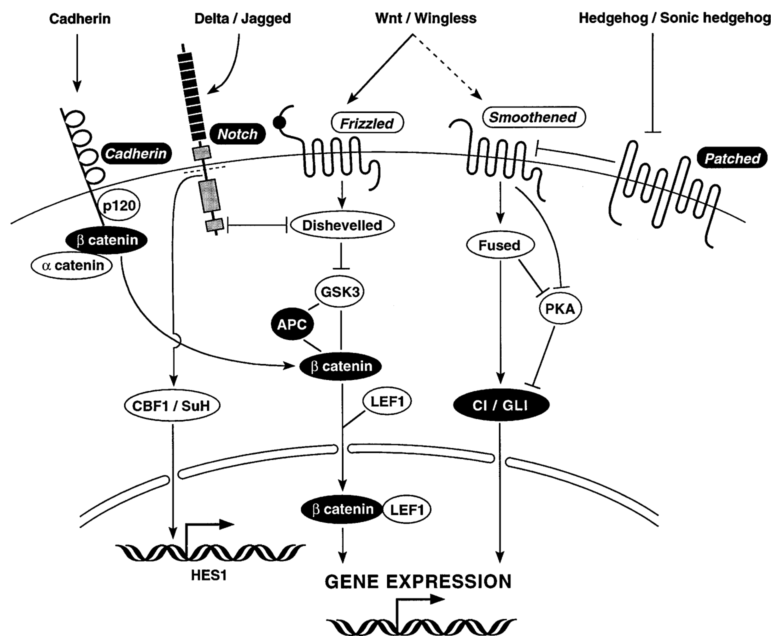

Figure 1. Developmental Signaling Pathways Involved in Cancer

Proteins that have been implicated as oncoproteins or tumor suppressor proteins are in white letters on black fill.

expression of target genes; genes induced by Wingless include those for the transcriptional repressor Engrailed, the TGFβ family member Decapentaplegic, and the cytokine Hedgehog. Autocrine expression of Wnts could then lead to expression of genes that block cellular differentiation, and contribute to the malignant phenotype.

Cadherins and Catenins

Cadherins are surface receptors that mediate specific cell-cell interactions through homotypic association, and also provide a means of cell-cell signaling. There are several potential connections between cadherin adhesion and carcinogenesis. Intracellular signaling via cadherins involves activation of Src family PTKs, which leads to tyrosine phosphorylation of catenins. Cadherin-mediated signaling may act to regulate the availability of free β-catenin (Fagotto et al., 1996), and therefore feed into the same signaling pathway as the Wnt receptor. β-catenin is a v-Src PTK substrate and its phosphorylation perturbs cadherin function in v-Src-transformed cells decreasing cell-cell adhesion (Matsuyoshi et al., 1992). p120 is a catenin-related protein, which binds to cadherins but not APC, and is phosphorylated on tyrosine in response to the activation of the EGF, PDGF, and CSF-1 receptors and in v-Src-transformed cells (Reynolds et al., 1994). A truncated β-catenin, which is detected in certain human cancer cell lines, disrupts the interaction between E-cadherin and α-catenin, and causes loss of intercellular adhesiveness, implying that mutation of β-catenin can play a role in cancer (Oyama et al., 1994). In addition, the expression of E-cadherin is often downregulated in cancer cells, and mutations in E-cadherin have been observed in tumors. It is possible that mutations of β-catenin and E-cadherin not only decrease tumor cell adhesion, but also upregulate the Wnt signaling pathway.

The Hedgehog/Patched Pathway

The Hedgehog/Patched/Smoothened system, which is intimately connected with the Wingless/Frizzled system in determining cell fate decisions in Drosophila, is also implicated in cancer. Patched is a transmembrane protein that acts in opposition to the secreted Hedgehog protein in controlling cell fates and growth in many tissues (Nusse, 1996). Vertebrate homologs of Sonic hedgehog, Smoothened, and Patched have been identified, and recent evidence indicates that Patched is a receptor for Sonic hedgehog (Marigo et al., 1996; Stone et al., 1996). Smoothened, a serpentine receptor-like protein distantly related to Frizzled, is negatively regulated in a tonic fashion by Patched, possibly through direct interaction (Stone et al., 1996), and this inhibition is relieved upon binding of Hedgehog (Figure 1). Activated Smoothened downregulates cAMP-dependent protein kinase (PKA), presumably through decreasing cAMP levels. In principle, this could occur through negative regulation of adenylyl cyclase by a G_i family protein activated by a G protein-coupled receptor such as Smoothened, but this effect is more likely to be indirect and involve the Fused protein kinase (Therond et al., 1996). The net effect is to activate the Cubitus interruptus zinc finger transcription factor resulting in induction of target genes such as *wingless*. Wingless activation of Frizzled in a neighboring cell leads to increased Hedgehog production, which acts back on the Wingless-producing cell to induce further Wingless expression. Smoothened might also act as an autocrine receptor for Wingless; this would activate the Smoothened signaling pathway, leading to increased Wingless expression. In combination, these Hedgehog/Wingless feedback loops act to reinforce the mutually distinct fates of cells at a compartment boundary.

Mutations of the human homolog of the *Patched* gene are responsible for the hereditary nevoid basal cell carcinoma syndrome, which is an autosomal dominant disorder characterized by multiple basal cell carcinomas and a variety of other tumors and developmental abnormalities, mapping to chromosome 9q22.3. Familial and sporadic nevoid basal cell carcinomas display loss of heterozygosity in this region, consistent with the *Patched*

gene being a tumor suppressor gene (Gailani et al., 1996; Hahn et al., 1996a; Johnson et al., 1996c). Assuming that in vertebrates Patched signaling is required for cell fate decisions and differentiation, as it is in Drosophila, then loss of Patched would lead to constitutive activation of Smoothened, causing inappropriate growth of basal layer cells, ultimately resulting in carcinogenesis. In principle, an activating mutation in Smoothened that blocks its interaction with Patched could also play a role in carcinogenesis. Intriguingly, the *GLI1* gene, which encodes a zinc finger transcription factor related to Cubitus interruptus, is amplified in a subset of human tumors (Kinzler and Vogelstein, 1990); this could have the same consequence as abrogating negative regulation of Smoothened by Patched.

The Notch Pathway

At least three members of the growing Notch/Lin12 family of receptors (currently four in mammals), Tan1 (Notch1), Notch2, and Int3 (Notch4), are associated with cancer. Notch was initially described in Drosophila, where it acts as a suppressor of cellular differentiation when activated by interaction with one of its ligands on an adjacent cell, such as Delta or Serrate. A great deal has been learned about the Notch/Lin12 signaling pathway from genetic analysis in Drosophila and C. elegans. The cytoplasmic juxtamembrane domain of Notch interacts with Suppressor of hairless [Su(H)], which is a transcriptional repressor that acts together with Hairless (Honjo, 1996). When Notch is activated by a ligand, Su(H) is released and migrates to the nucleus, where it induces expression of Hairy and Enhancer of split, which are bHLH transcriptional repressors that block neural differentiation. There is still a debate about how Su(H) is released, with one attractive model proposing that the cytoplasmic domain of Notch is cleaved and then translocates to the nucleus with Su(H). The bound Notch would then mask the Su(H) repression domain and convert it into a transcriptional activator (Hsieh et al., 1996). Genetic analysis shows that Dishevelled is antagonistic to Notch. Dishevelled interacts directly with the C-terminus of Notch, and thereby potentially blocks Notch signaling, thus providing a molecular mechanism for the inhibitory crosstalk between the Wnt and Notch pathways.

Homologs of all these components of the Notch signaling pathway have been identified in vertebrates. There are at least four Notch family receptors in mammals, and several ligands, such as Jagged. The DNA-binding protein RBP-Jκ/CBF1/KBF2 (CBF1) is the Su(H) homolog, and the Hes family of bHLH proteins are Hairy/Enhancer of split homologs. Notch function is also conserved in vertebrates. A truncated form of Xenopus Notch lacking the extracellular domain affects cell fate decisions in embryos, and a soluble form of the mouse Notch intracellular domain blocks MyoD-induced myogenesis. Activated forms of mouse Notch associate with CBF1, triggering transcriptional activation through the CBF1-binding sites in the *HES1* promoter (Jarriault et al., 1995), and Hes1 can block MyoD-induced myogenesis in some systems. A CBF1-independent pathway may also be involved, however, in Notch-mediated antagonism of MyoD-induced myogenesis (Shawber et al., 1996).

Truncation of the Notch extracellular domain activates Notch signaling constitutively in Drosophila and vertebrates. All the putative Notch-derived oncoproteins have deletions in the extracellular domain consistent with their being constitutively activated. Tan1 (Notch1) was initially identified as the product of a chromosomal translocation, t(7;9), in a T lymphoblastic leukemia (Ellisen et al., 1991). Tan1 induces T cell leukemias when bone marrow cells infected with a retrovirus expressing Tan1 are reintroduced into mice, establishing its credentials as an oncoprotein (Pear et al., 1996). Full-length and, less commonly, truncated Notch1 is frequently overexpressed in thymomas arising in MMTV-c-Myc transgenic mice, suggesting that the Notch pathways can collaborate with c-Myc in oncogenesis (Girard et al., 1996). *Int3* (*Notch4*) was identified as a common integration site for the MMTV provirus in MMTV-induced mammary carcinomas, resulting in production of an N-terminally truncated protein. Expression of Int3 from a transgene induces mammary carcinomas (Jhappan et al., 1992). A third Notch family member, Notch2, has been implicated in tumorigenesis through the isolation of recombinant feline leukemia virus genomes from virally induced thymic lymphomas that encode an N-terminally truncated form of Notch2 that is targeted to the nucleus (Rohn et al., 1996). In every case, it seems likely that the truncated Notch protein is signaling constitutively, thus blocking differentiation and causing continuous proliferation.

Another intriguing connection between the Notch signaling pathway and transformation comes from studies on Epstein Barr virus. EBNA2, an EBV-encoded protein needed for transformation of B cells, is an activator of latent viral and cellular genes. However, EBNA2 does not interact directly with EBNA2-responsive sequences in its target genes. Instead, it has been found to interact with CBF1 and drive transactivation of genes to which CBF1 is bound, thus converting a repressor into an activator (Zimber-Strobl et al., 1994), possibly in a manner analogous to Notch bound to CBF1.

Cytokine Receptors as Oncoproteins

Until recently, members of the cytokine receptor family had not been implicated in oncogenesis. However, the murine myeloproliferative leukemia virus oncoprotein, v-Mpl, is a mutant form of the newly discovered receptor for thrombopoietin (TPO), a megakaryocyte growth factor. The TPO receptor (c-Mpl) is a typical member of the cytokine receptor family, which activates JAK2 and STAT3/STAT5, and is coupled to the ERK MAPK pathway through Shc (Gurney et al., 1995; Mu et al., 1995). v-Mpl has part of the viral Env protein fused to the C-terminus of the TPO receptor starting just to the N-terminal side of the transmembrane domain. v-Mpl retains the extracellular domain cytokine receptor WSXWS motif, and is presumably activated through constitutive dimerization, thus generating a continuous growth signal and blocking differentiation. However, the exact downstream signals that are required for v-Mpl-mediated transformation are not known; it is not necessarily the case that the JAK/STAT pathway is essential, since proliferation induced by the erythropoietin (EPO) receptor does not require STAT activation. Activation of the ERK MAPK pathway and other pathways may be

important in transformation. In this connection, it is notable that association with and activation of the EPO receptor at the cell surface by an oncogenic retroviral Env protein, gp55, is responsible for leukemic transformation by Friend leukemia virus (Li et al., 1995b).

TGFβ Receptors

The TGFβ receptor and downstream signaling components are also targets for mutation in cancer. For instance, the gene encoding the TGFβ receptor type II is commonly inactivated in colon carcinoma with microsatellite instability (Markowitz et al., 1995), and the *SMAD2* and *SMAD4* (*DPC4*) genes have also been found inactivated or deleted in colon carcinoma (Eppert et al., 1996; Hahn et al., 1996b). Certain members of the Smad family (Smad1, -2, -3, and -5) are directly phosphorylated by ligand-activated TGFβ family receptors, oligomerize with Smad4, and are then translocated into the nucleus. Once in the nucleus, Smad protein oligomers act as transcriptional activator subunits (Liu et al., 1996) and mediate TGFβ-induced gene expression. For instance, Xenopus Smad2 interacts with the sequence-specific DNA binding protein, FAST-1, a member of the winged helix family of proteins, that binds to an activin-response element in the *Mix.2* gene. Smad2 and Smad4 associate in response to TGFβ, and this interaction is required for growth inhibition (Lagna et al., 1996). Mutant Smad2 proteins carrying point mutations found in colon carcinomas are no longer phosphorylated or translocated into the nucleus in response to TGFβ (Eppert et al., 1996). Collectively, these observations provide strong evidence that TGFβ receptor signaling through activation of the Smad pathway plays an important role in tumor suppression.

Ras Family Proteins and Cancer

Activating mutations in H-Ras, K-Ras and N-Ras have all been found in human tumors, and the frequency of Ras mutations is among the highest for any gene in human cancers. Recently, two less well-known Ras-like genes, *TC21* and *R-Ras*, have become potential targets for mutational activation in human tumors. TC21 mutated at residues 22 and and 61 (equivalent to Ras 12 and 61) transforms NIH3T3 cells (Graham et al., 1994), and activating mutations in TC21 have been found in human tumors (Huang et al., 1995); R-Ras mutated at residues 38 or 87 can transform NIH3T3 cells, although mutations in R-Ras have not been detected in human tumors (Cox et al., 1994; Saez et al., 1994). Ras GAPs, which promote Ras GTPase activity and downregulate Ras signaling, have also been implicated in tumorigenesis. Neurofibromin, the product of the *NF1* tumor suppressor gene that is mutated in type 1 neurofibromatosis, is a Ras GAP with a central domain related to p120GAP. The lack of neurofibromin leads to elevated basal levels of Ras GTP in NF1-derived schwannomas (DeClue et al., 1992), which could play a causal role in the etiology of these tumors.

A number of effectors for Ras proteins have been identified, which bind preferentially to Ras in the GTP-bound state. These include Raf1, the p110 PI3 kinase catalytic subunit, PKCζ, RalGDS, Rin1, and MEKK1. Several of these have been implicated in tumorigenesis. For example, v-Raf is a retroviral oncoprotein, and Raf1, which is a MAPKKK, can be oncogenically activated by simple addition of a membrane-targeting signal. Dissection of which of these effectors is required for transformation by Ras has been made possible by the use of activated Ras mutants carrying point mutations at specific sites in the effector domain (White et al., 1995). These results indicate that multiple cellular pathways, including Raf1, are activated by Ha-Ras and contribute to Ha-Ras-induced mammalian cell transformation. Indeed, it has been shown that Ras activation of Raf/MAPK-independent pathways is sufficient to cause tumorigenic transformation (Khosravi-Far et al., 1996). Among the targets for Ras required for transformation is Rac, a member of the Rho family of small G proteins. Rac itself has modest transforming activity when Gly12 is mutated to Val, but it cooperates with activated Raf1 for transformation, and dominant negative Asn17 mutant Rac blocks Ras transformation (Khosravi-Far et al., 1995; Qiu et al., 1995). The Ras-induced pathway leading to Rac activation is not known. Rho is also required for Ras transformation and G1 progression.

Rho Family Proteins and the Cytoskeleton

Hints that the cytoskeleton might play a critical role in transformation have been in evidence for many years. Transformed cells show altered patterns of expression of cytoskeletal proteins; for instance, novel tropomyosin isoforms are expressed. Transformed cells commonly have disorganized actin cytoskeletons, which may be tied to the ability of transformed cells to grow in an anchorage-independent fashion. Normally adherent cells require integrin engagement to proliferate, and the concentration of ligand-occupied integrins into focal adhesions is a key factor in the anchorage and organization of actin microfilament bundles into stress fiber arrays.

The Rho family of small G proteins, which regulate actin-containing structures and plasma membrane topology, has recently assumed increasing importance in oncogenesis. Rho causes actin stress fiber formation, Rac stimulates membrane ruffling and lammellipodia formation, and Cdc42 induces filopodia formation (Nobes and Hall, 1995). A number of potential effector proteins have been found to associate preferentially with the GTP-bound forms of Rho family proteins. Cdc42 and Rac bind many proteins in common through a short recognition sequence (CRIB) with the consensus ISXPX$_4$ FXHXXHVG (Burbelo et al., 1995). Among the direct targets for Cdc42 and Rac are several protein kinases, including Pak1 and Rho kinase. Activated Rac and Cdc42 stimulate the JNK/SAPK and p38 MAPKs (Coso et al., 1995; Minden et al., 1995), possibly through binding of Rac/Cdc42 to Pak1, which leads to its activation (Bagrodia et al., 1995). Although it is not yet clear precisely which targets are essential for different responses induced by each family member, the use of specific point mutations in the effector domains of these proteins has begun to allow a dissection of the different pathways that can be activated (Lamarche et al., 1996). Thus, activation of Pak1 proves not to be required for Rac/Cdc42-induced cytoskeletal changes or G1/S progression. In contrast, a Rac mutant unable to interact with the Rho kinase cannot induce lamellipodia or G1 progression (Lamarche et al., 1996).

The importance of Rho family small G proteins in transformation is emphasized by the large number of mutant activated GDP/GTP exchange factors for this family that have been found to possess fibroblast transforming activity. The first example was Dbl, an oncoprotein derived through artifactual truncation that occurred during transfection. Additional examples include Vav, Lbc, Tiam1, Tim, Ost, Ect2, Lsc, and FGD1. The function of these multiple exchange factors is not fully understood, but they all have a core domain containing a region of ~330 residues known as the Dbl-homology domain, together with an upstream pleckstrin homology domain, which is required for GDP/GTP exchange (Quilliam et al., 1995). The specificities of most of these exchange factors remain to be determined, but Dbl and Vav are pleiotropic exchange factors that can activate all three family members, whereas FGD1 can only activate Cdc42 (Olson et al., 1996). The ability of FGD1 to transform can be explained by the ability of Cdc42 to activate Rac, which in turn can activate Rho, thus leading to activation of all three family members. The essential role in transformation of the Rho family proteins underscores the critical connection between the cytoskeleton and transformation.

Other Cytoskeletal Connections

Merlin/schwannomin, the product of the NF2 tumor suppressor gene, which is mutated in neurofibromatosis type 2 patients, is a member of the ezrin/radixin/moesin (ERM) family (Belliveau et al., 1995). ERM family proteins bind to F-actin and are localized to microvillar cores in many cell types. The function of these proteins is not fully understood, but they can all interact with the cytoplasmic domain of CD44 (Tsukita et al., 1994), which is a cell surface receptor for hyaluronan. CD44 shows striking variability in the structure of its extracellular domain as a result of alternative splicing, and some isoforms are strongly correlated with the metastatic phenotype. Recent evidence suggests that Rho regulates the CD44/ERM interaction and, conversely, that this interaction may regulate Rho activation (Hirao et al., 1996). The absence of merlin could therefore affect Rho function and play a role in transformation.

Plasma Membrane-to-Nucleus Signaling Pathways

Our knowledge of signaling pathways activated by growth factor receptor PTKs has been advancing rapidly (Figure 2). A number of SH2/SH3 adaptor proteins play critical roles in transducing signals from activated receptor PTKs to downstream signaling pathways. A good example is Grb2, which recruits the Ras GDP/GTP exchange factor Sos to the plasma membrane upon binding to activated receptor PTKs. Adaptors such as Crk, Nck, and Shc have been implicated in oncogenesis, either through their conversion into viral oncoproteins (v-Crk), or else because they transform cells when overexpressed (Nck and Shc). Shc is an intermediary in the activation of Ras and the ERK MAPK pathway. Nck may be connected to the actin cytoskeleton and Rho family proteins, since Nck SH3 domains bind known Rac and/or Cdc42 targets, including Pak1. An Nck/Pak1 complex is translocated to activated growth factor receptor PTKs via the Nck SH2 domain, increasing Pak1 activity (Galisteo et al., 1996). Pak1 in turn can lead to activation of the JNK/SAPK and possibly the ERK MAPK pathways.

MAP Kinase Pathways and Transformation

The MAP kinase module, which consists of three protein kinases in series, a MAPK, a MAPKK, and a MAPKKK, has emerged as one of the most important membrane-to-nucleus signaling pathways in eukaryotes. This module is highly conserved and extremely versatile. In yeast, MAPKs are used for a variety of functions, transmitting the response to extracellular stimuli such as pheromones, osmotic stress, and cell wall integrity. In higher eukaryotes, the MAPK module has been adopted to transmit responses from many different types of surface receptor, including receptor PTKs, G protein-coupled receptors, cytokine receptors, and possibly even the TGFβ receptor family. Activated MAPKs are translocated into the nucleus. In mammals, a number of nuclear MAPK targets have been identified, including the ternary complex factors in the Ets family that stimulate the expression of the c-*fos* gene via the SRE. Not surprisingly, constitutive activation of MAP kinase-mediated mitogenic signaling pathways elicits transformation.

The three well-characterized MAPK pathways in vertebrates are the ERK, JNK/SAPK, and p38 pathways. So far, only the ERK MAPK pathway has been implicated in oncogenesis, even though the c-Jun protooncoprotein is activated by JNK/SAPK phosphorylation and is required for Ras transformation (Johnson et al., 1996b). Moreover, the JNK/SAPK and p38 pathways lead to the activation of many of the same transcription factors, such as Elk1 and Sap1a. Perhaps transformation requires phosphorylation of targets unique to ERK MAPKs, or else the balance of substrate phosphorylation is critical and is not achieved by p38 or JNK/SAPK. Indeed, whereas constitutively activated mutant MEK1s, which activate ERK1/2, elicit transformation of fibroblasts (Cowley et al., 1994; Mansour et al., 1994), an activated mutant MEKK1, which activates both JNK/SAPK and p38, results in apoptosis (Johnson et al., 1996a). A proapoptotic function for JNK/SAPK has also been noted in PC12 cells, where it is counteracted by activation of ERK (Xia et al., 1995). Transformation of fibroblasts by upstream activators of ERK MAPK, such as activated Ras and Raf1, is blocked by a dominant negative mutant of MEK1, establishing that activation of ERK MAPK is required for transformation (Cowley et al., 1994). One critical function for the Ras/ERK MAPK pathway could be mitogen-induced cyclin D expression, which is needed for G1 progression (Lavoie et al., 1996). Ras has other effectors, however, which may play roles in transformation. Indeed, it should be noted that transformation by activated MEK1 is blocked by microinjected Ras antibody showing that direct activation of ERK1 targets is not sufficient for transformation (Cowley et al., 1994); ERK-induced autocrine expression of growth factors that act back on surface receptors that signal through Ras may be important for transformation.

Another oncoprotein connected with the ERK MAPK pathway is Tpl2, originally identified as the product of an oncogene associated with the progression of Moloney murine leukemia virus-induced T cell lymphomas in rats. The same gene was identified as an oncogene in a transfection assay, and called *Cot*. In both cases, the oncoprotein is C-terminally truncated; while this truncation may potentiate transforming activity, however, it is not

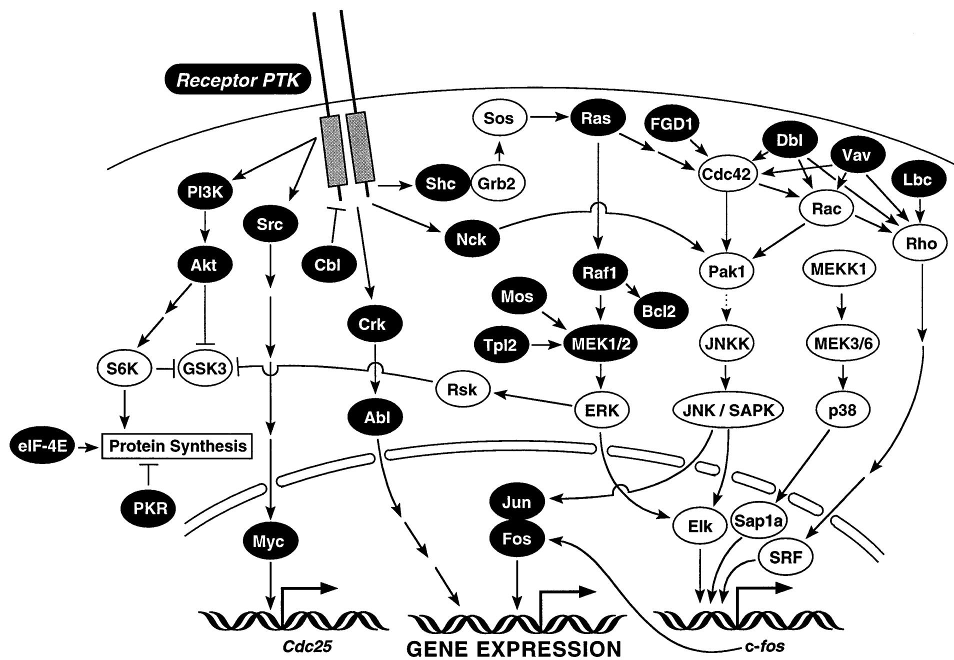

Figure 2. Membrane-to-Nucleus Signaling Pathways Involved in Cancer
Proteins that have been implicated as oncoproteins or tumor suppressor proteins are in white letters on black fill.

needed for transformation. Tpl2/Cot is a protein-serine kinase related to the MAPKKK family, and has been shown to phosphorylate and activate MEK1 (and JNKK) in vitro and to activate ERK1 (and JNK/SAPK) in vivo (Salmeron et al., 1996). ERK MAPK activation induced by the Tpl2 protein is blocked by dominant negative mutants of Ras and Raf-1, and a kinase-deficient Tpl2 mutant downregulates mitogenic signals induced by v-Ha-Ras or v-Raf, suggesting that Tpl2 interacts with the ERK MAPK cascade (Patriotis et al., 1994). The fact that three oncoproteins, Raf, Mos, and Tpl2, are direct activators of MEK1 underlines the importance of the ERK MAPK pathway in transformation.

PI3 Kinase and Transformation

The PI3 kinase (PI3K) pathway is another receptor-activated pathway implicated in oncogenesis. PI3K activity is stimulated by many types of extracellular signal, especially those involving activation of receptor PTKs. Through the use of receptor PTK mutants that fail to activate PI3K and PI3K inhibitors, such as wortmannin, a role for PI3K has been delineated in a number of cellular responses, including 70K S6 kinase activation, ruffling, and chemotaxis. PI3K first came into view as an activity associated with middle T antigen, the transforming protein of polyoma virus, implicating it in transformation. Subsequent work showed that binding of PI3K to MT antigen, via P.Tyr315, which is phosphorylated by the MT-associated c-Src PTK, is crucial for transformation. The importance of PI3K in transformation has been underscored by the recent identification of a new avian sarcoma virus, ASV16, whose oncogene encodes a Gag-PI3K p110α catalytic subunit fusion oncoprotein, Gag-P3K, which has PI3K activity.

PI3K generates 3′ phosphoinositides, PI3,4,5P_3, PI3,4P_2, and PI3P, and PI3K activation increases the levels of PI3,4,5P_3 and PI3,4P_2 in cells. It is assumed that these act as second messengers, but identification of their effector proteins has proved difficult. Recently, however, c-Akt, which was originally identified as a protooncoprotein, has emerged as a prime candidate. c-Akt is a protein-serine kinase with an N-terminal pleckstrin homology (PH) domain, which, like other PH domains, binds to phosphoinositides. In vivo, c-Akt is activated by stimuli that activate PI3K, as well as by mutationally activated forms of the p110 PI3K catalytic subunit, and is blocked by inhibitors of PI3K activity. Mutation of a conserved residue in the PH domain blocks c-Akt activation (Franke et al., 1995). c-Akt activation in vivo requires phosphorylation of two residues, one in the activation loop and one in the C-terminal tail. In vitro, c-Akt activity has been reported to be stimulated preferentially but weakly by PI3,4P_2 (Franke et al., 1995; Klippel et al., 1997), although in other studies no stimulation was observed (James et al., 1996). The extent of phosphoinositide activation is significantly less than observed in vivo, suggesting that phosphorylation of the activating sites in c-Akt plays an important role in its activation in vivo (Kohn et al., 1996). v-Akt, the oncogene product of the AKT8 murine retrovirus, is a Gag-Akt fusion protein, in which Gag is joined to the PH domain. Unlike c-Akt, v-Akt is myristoylated, and constitutively membrane-associated and activated. Thus, a plausible mechanism of Akt activation in the cell involves inducible binding of Akt to PI3,4P_2 in the plasma membrane, where the translocated protein is activated through trans- or autophosphorylation, which may be facilitated through PH domain-mediated dimerization. Consistent with this, membrane-anchored forms of Akt are activated regardless of whether the PH domain is present (Kohn et al., 1996). However, even if the major role of

$PI3,4P_2$ in c-Akt activation is to recruit the protein to the membrane, it may still act as a true second messenger with other effectors.

The critical targets for Akt phosphorylation are not known, but it has been shown that Akt can phosphorylate and inhibit GSK3 in response to insulin, providing an intriguing connection to the Wnt pathway (Cross et al., 1995). Akt may also be upstream of the 70K S6 kinase, which can phosphorylate and inhibit GSK3 as well. Finally, recent evidence suggests that activated Akt plays a role in protection from apoptosis, which could also be important for its transforming activity.

Other membrane-to-nucleus signaling pathways are involved in transformation. For instance, at least two components of the NF-κB system have been identified as oncoproteins, i.e., Rel and Bcl3. As indicated above, the Notch and Wnt receptor signal pathways also play roles in oncogenesis. However, another well-characterized membrane-to-nucleus signaling system, the JAK/STAT pathway, has to date not been implicated in carcinogenesis; indeed, proliferative signaling via cytokine receptors, such as the EPO receptor, does not require activation of JAK/STAT. Nevertheless, certain activated mutants of Hopscotch, the Drosophila JAK homolog, cause a leukemia-like phenotype owing to overproliferation of blood cells, which requires Drosophila STAT (Yan et al., 1996). It would be interesting to look for similar activating mutants in human leukemias.

Nuclear Proteins and Cancer

The number of oncogenic proteins that act in the nucleus has continued to rise steadily. These include novel transcription factors, chromatin regulatory proteins, and a variety of transcriptional regulators. Many of these have been identified as the products of fusion genes generated by chromosomal translocations in leukemias. Several oncogenes encode transcriptional activators, and they will not be reviewed here. It should be noted, however, that the thorny issue of the critical target genes that need to be induced by such oncoproteins is still unresolved. In this connection, v-Jun appears to have attenuated transcriptional activation function compared to c-Jun (Gao et al., 1996), and this may be true for other oncoproteins that are transcriptional activators.

A building theme is the function of oncoproteins as transcriptional repressors. The first example was v-ErbA, which is a repressor of thyroxine receptor (c-ErbA) induced erythroid differentiation genes. WT1, the product of the Wilms' tumor suppressor gene, has also been shown to be a repressor of certain genes, antagonizing the Egr family of transcriptional activators. Another recent example is v-Qin, the oncogene product of ASV31, which acts as a repressor and is a member of the HNF-3/forkhead family of transcriptional factors (Li et al., 1995a). Evi1, whose expression is activated in murine myeloid leukemias by retroviral insertions and in human acute myelogenous leukemia by chromosomal translocations and inversions, interferes with myeloid differentiation and is also a repressor (Lopingco and Perkins, 1996).

The promyelocytic leukemia Kruppel-like zinc finger (PLZF) protein, which is fused to the retinoic acid receptor α (RARα) protein as a result of a variant t(11;17) chromosomal translocation in a small subset of acute promyelocytic leukemias (Chen et al., 1993), represses expression of a cyclin A promoter reporter gene. One of the two possible PLZF-RARα fusion proteins contains the RARα transactivation domains and the PLZF DNA-binding domain, and this may allow it to transactivate rather than repress cyclin A expression, which is essential for cell cycle progression and growth. CHOP, which was fingered as an oncoprotein through its identification as a chromosomal translocation fusion partner of the Ews RNA-binding protein in a myxoid liposarcoma, is a member of the C/EBP family of basic-leucine zipper transcription factors. CHOP can act as an antagonist of C/EBP-induced transcription, and block C/EBP-induced differentiation of adipocytes (Batchvarova et al., 1995). Transformation of fibroblasts requires a contribution from the Ews fusion partner, but CHOP may act as an oncoprotein by preventing C/EBP-induced expression of differentiation genes.

It is also clear that coactivator proteins, which do not bind directly to DNA, are potential targets for oncoproteins, as evidenced by the fact that adenovirus E1A binds to the CBP and p300 coactivators, resulting in inactivation of certain CBP/p300 transcription stimulatory functions (Arany et al., 1995). Another example of an oncoprotein that does not bind directly to DNA is Bmi1, which is a chromatin structural protein related to the Polycomb family of Drosophila repressors that is upregulated by proviral integration in MuLV-induced thymomas (van Lohuizen et al., 1991). Such proteins appear to regulate and switch off large blocks of chromatin. Bmi1 may act by blocking expression of genes required for lymphoid differentiation.

Pml, which was uncovered as a translocation partner of RARα in promyelocytic leukemia (PML), is a component of a novel nuclear structure, called PODS, whose function is still somewhat enigmatic (Dyck et al., 1994; Koken et al., 1994). In cultured cells, expression of the Pml-RARα protein disrupts PODS in a retinoic acid reversible fashion; since PML is reverted by retinoic treatment of patients, the oncogenic activity of Pml-RARα action is likely to involve disorganization of PODS. Conversely, Pml, through its PODS organizing activity, acts as a growth suppressor. PODS are also disrupted by adenovirus E4-ORF3, which is required for viral replication (Doucas et al., 1996), implying that PODS play a role in DNA replication.

The *VHL* gene is mutated in the hereditary cancer syndrome, von Hippel Lindau disease, which predisposes to renal carcinoma and pheochromocytoma. VHL proves to be an alternative A type subunit of the RNA polymerase II cofactor Elongin (SIII), which stimulates transcription elongation by RNA polymerase II in vitro, and is assumed to ensure complete gene transcription (Duan et al., 1995; Kibel et al., 1995). Elongin is a heterotrimer consisting of a transcriptionally active A subunit together with B and C regulatory subunits. Replacement of the normal Elongin A subunit with VHL inhibits it elongation activity (Duan et al., 1995). Presumably, this regulates the levels of particular transcripts that are subject to transcriptional pausing. The absence of VHL activity could allow the overexpression of specific proteins, including positive growth regulators. Intriguingly, the *ELL*

gene, which is frequently fused to the trithorax-like *MLL* gene on chromosome 11 as a result of t(11;19) translocations in acute myeloid leukemias, encodes a distinct RNA polymerase II elongation factor, providing another connection between the regulation of transcription elongation and cell growth (Shilatifard et al., 1996).

Brca1 and Brca2 are huge proteins whose disruption is the underlying cause of a large fraction of hereditary breast and ovarian cancer. The subcellular localization of Brca1 has been hotly debated, but current opinion favors a nuclear localization. Brca1 is essential for early mouse development, which gives little clue to its function, although it may govern expression of cell cycle regulators. Brca1 has not been shown to have DNA-binding activity, but the C-terminal 335 residues of Brca1 have transactivation function when fused to a DNA-binding domain (Chapman and Verma, 1996). This transactivation activity is abolished by point mutations in this region, which occur naturally in affected individuals, supporting the idea that Brca1 transactivating activity is physiologically relevant.

Finally, the Dek-Can and Set-Can fusion proteins arising from leukemia-associated translocations found in leukemia contain the C-terminal domains of Can, which contains repeats found in nucleoporins. The Can protein is localized to the nuclear and cytoplasmic faces of the nuclear envelope, whereas the Can fusion proteins are found exclusively in the nucleus (Fornerod et al., 1995). Potentially, this altered distribution perturbs nuclear import or export of proteins or RNAs.

Protein Synthesis and Oncogenesis

An increase in the rate of protein synthesis initiation can also lead to transformation. Two different initiation factors have been implicated. Overexpression of eIF-4E, the mRNA cap-binding subunit of the eIF-4F initiation factor, elicits fibroblast transformation (Lazaris-Karatzas et al., 1990; Smith et al., 1990). The second initiation factor, eIF-2, is a heterotrimer that forms a ternary complex with GTP and Met-tRNA$_i$, and is needed to deliver Met-tRNA$_i$ to the initiation complex. eIF-2 is negatively regulated through phosphorylation of Ser-51 in the α subunit; this blocks the action of the eIF-2 GDP-GTP exchange factor, which is needed to reactivate eIF-2 after its cargo is delivered. Phosphorylation of eIF-2α plays a major role in negative regulation of initiation of protein synthesis; two protein kinases are known that phosphorylate eIF-2α at Ser-51, namely the double-stranded RNA dependent protein kinase (PKR) and a heme-regulated protein kinase. Expression of a kinase-inactive mutant form of PKR causes fibroblast transformation (Koromilas et al., 1992; Meurs et al., 1993) as a result of a decrease in eIF-2α phosphorylation. Consistent with a role for eIF-2α phosphorylation in transformation mediated by kinase-inactive PKR, overexpression of an Ala-51 mutant eIF-2α also causes transformation. Presumably, the general increase in protein synthesis initiation capacity results in increased translation of poorly translated mRNAs encoding specific proteins that are important for growth and cell cycle progression. Indeed, enhanced expression of several proteins, including cyclin D1 and ornithine decarboxylase (ODC), has been found in eIF-4E-overexpressing NTH 3T3 cells, although the mechanisms of upregulation appear to differ. Both ODC and cyclin D1 are implicated in G1 progression, and it is interesting to note that eIF-4E requires Ras function and, like Ras, cooperates with v-Myc in transformation. Finally, overexpression of elongation factor EF-1α increases sensitivity to transformation, and an N-terminally truncated form of EF-1α is implicated as an oncoprotein in prostatic cancer.

The Cell Cycle and Cancer

Direct connections between the cell cycle and cancer have been evident for some time (Sherr, 1996). After some initial uncertainty, it is now clear that the p16 Cdk inhibitor gene is a major target for mutational inactivation in human cancers. In addition, specific mutations in p16 are the underlying cause of one type of hereditary melanoblastoma. Surprisingly, there is no evidence that the three other members of the p16 Cdk inhibitor family, p15, p18, and p19, are mutated in human cancer. An emerging paradigm, however, is that one component of the p16-cyclin D/Cdk4-Rb axis is mutationally inactivated in most cancer cells (Sherr, 1996). Thus, a tumor may have mutations that lead to loss of p16 or Rb, or upregulation of cyclin D expression, or a mutation that renders Cdk4 insensitive to p16. The net result is that Rb and Rb family members are constitutively inactivated in such cells, providing a trigger for progression from G1 into S phase. The best evidence is that cyclin D is not required for cell cycle progression in cells bearing a mutation in one component of this axis, and that p16 fails to block the growth of such cells.

The other key component in the G1-to-S transition is cyclin E, which acts in conjunction with Cdk2. Cyclin E is overexpressed in some cancer cells, although the mechanism has not been established (Keyomarsi and Pardee, 1993). Mutations or deletions that eliminate a C-terminal Cdk phosphorylation site enhance cyclin E function by blocking ubiquitin-mediated degradation, thus leading to its accumulation. It is possible that mutations of this type will be found in cancers. So far, none of the members of the p21 family of Cdk inhibitors, which have a broader inhibitory specificity than the p16 family, has been implicated in tumorigenesis, despite an extensive search for mutations in the genes for p21, p27, and p57. It has recently been shown, however, that the adenovirus E1A and human papilloma virus E7 transforming proteins have the ability to counteract the Cdk inhibitory activity of p27 (Mal et al., 1996). This property of E1A may be important in its ability to override TGFβ-induced growth inhibition and trigger the growth of quiescent cells, which have high levels of p27.

New players in the regulation of the cell cycle are continually being uncovered, but none with a clear cut involvement in oncogenesis has been discovered recently. However, an intriguing connection has emerged between an old friend, Cdc25, and transformation. Cdc25A and Cdc25B, but not Cdc25C, can cooperate with activated Ras to transform primary rodent fibroblasts (Galaktionov et al., 1995b). Cdc25s are dual-specificity phosphatases that dephosphorylate the negative regulatory phosphates at Thr-14 and Tyr-15 in Cdks,

leading to their activation. Cdc25C activates cyclin B/Cdc2 at the G2/M transition, but the cyclin/Cdk target(s) for Cdc25A and Cdc25B has not been unequivocally established. There is reasonable evidence, however, that Cdc25A acts on Cdk2 complexes, and that Cdc25A activity is required for the G1/S transition. It is possible that cyclin D-Cdk4 complexes may also be Cdc25 targets. The exact connection between Cdc25A and Ras is not clear, but it has been suggested that Ras-activated Raf might directly regulate Cdc25A activity through phosphorylation (Galaktionov et al., 1995a).

Another intriguing connection between Cdc25A and -B and the cell cycle is that the expression of both *Cdc25* genes is increased at the transcriptional level by c-Myc leading to accumulation of Cdc25 protein (Galaktionov et al., 1996). Both *Cdc25* genes contain c-Myc/Max-binding sites in the first intron, which respond transcriptionally to increased c-Myc expression. Moreover, expression of c-Myc leads to activation of cyclin E-Cdk2 and cyclin D-Cdk4 complexes in quiescent cells (Steiner et al., 1995). Further evidence that Cdc25A is an important target for c-Myc is that depletion of Cdc25A by antisense RNA expression or antisense-oligonucleotide treatment blocks c-Myc-induced apoptosis. Consistent with a role for Cdc25A/B in carcinogenesis, Cdc25A/B RNA and protein are found to be overexpressed in a variety of tumors, including breast carcinomas (Galaktionov et al., 1995b). Moreover, adenovirus E1A increases Cdc25A activity and expression in quiescent cells, and Cdc25A is required for adenovirus to induce DNA synthesis (Spitkovsky et al., 1996). Cdc25A and Cdc25B are also targets for TGFβ, being downregulated at the RNA and protein level following TGFβ treatment.

Apoptosis

An emerging theme in carcinogenesis is the acquired ability of tumor cells to avoid undergoing apoptosis in response to DNA damage or other conditions that would induce a normal cell to commit suicide. Our understanding of the mechanisms underlying programmed cell death, or apoptosis, has grown explosively in the past few years. Signal transduction pathways leading from receptors, such as Fas and the TNF receptor, to the activation of effectors of cell death, such as the Caspase protease family, have been elucidated (Nagata, 1997 [this issue of *Cell*]). Bcl2 family proteins are key regulators of this pathway. These proteins hetero- and homodimerize, and have been categorized as either pro-apoptotic (e.g. Bax or Bad) or anti-apoptotic (e.g. Bcl2 or Bcl-X_L). Thus, the balance of pro- versus anti-apoptotic Bcl2 family members in cells dictates cellular response. *bcl2* was identified at the breakpoint of a t(14;18) transcription in acute lymphocytic B cell leukemia. Expression of a *bcl2* transgene results in the expansion of the B cell population due to a failure of these cells to undergo normal programmed cell death and elimination.

Both Bad and Bcl2 have recently been found to be regulated through phosphorylation. Bad is phosphorylated at Ser-112/136 in response to IL3 treatment of cells that require IL3 for survival, and this results in 14–3–3 binding (Zha et al., 1996). Mutation of Ser-112/136 increases the potency of Bad, implying that phosphorylation attenuates its killing activity. Bcl2 associates with Raf1 and localizes Raf1 to mitochondrial membranes (Wang et al., 1996). Moreover, mitochondrially localized Raf1 protects IL3-dependent cells from apoptosis induced by IL3 withdrawal, whereas a kinase-inactive form of Raf1 potentiates killing. Bcl2 has also been found to associate with R-Ras (Fernandez-Sarabia and Bischoff, 1993), which could play a role in Raf1 activation. One potential target for Bcl2-associated Raf1 is Bad itself. A recent clue to the function of Bcl2 family proteins comes from the finding that their structure is related to the diphtheria toxin channel (Muchmore et al., 1996), and that they can act as ion channels in vitro. Channels with distinct properties may be formed by different combinations of Bcl2 family members. This system is also a target for viral intervention; for instance, adenovirus E1B 19K binds to Bax, thus inactivating it (Han et al., 1996).

Another important apoptotic regulator is p53, which is activated by DNA damage; activated p53 induces or represses the expression of a number of target genes (Levine, 1997 [this issue of *Cell*]). Among these is the p21 Cdk inhibitor p21, which acts on cyclin-Cdk4/6 and cyclin-Cdk2 complexes, playing a role in the arrest of cells in G1. Activation of p53 is also required for the induction of apoptosis in response to some but not all signals. The finding that c-Myc induces apoptosis in the absence of growth factors has been another key finding. c-Myc is a double-edged sword, being capable of driving a cell through the cell cycle under appropriate conditions or provoking cell death under suboptimal conditions. Signals from the IGF1 receptor PTK counteract the c-Myc-induced apoptotic pathway at many levels, and this allows c-Myc to express its proliferative potential and explains the requirement for IGF1 for growth of most cultured cells (Harrington et al., 1994).

Adherent endothelial and epithelial cells require integrin engagement for survival, and in the absence of attachment to a substratum undergo apoptosis (Meredith et al., 1993; Boudreau et al., 1995). One of the consequences of the accumulated mutations in a carcinoma may be to provide a signal that mimics integrin occupancy. This will have the net effect of providing a signal that permits cells to grow in the absence of anchorage, and at the same time interdicts the normal apoptotic response to lack of integrin engagement. Interestingly, activated R-Ras increases integrin ligand affinity, whereas dominant negative mutant R-Ras reduces cell adhesiveness (Zhang et al., 1996).

Conclusions

One striking conclusion is that oncogenesis is intimately connected with a failure of incipient tumor cells to differentiate. This can be the consequence of mutations in highly conserved genes that regulate cell fate decisions and differentiation, such as *Notch* and *Patched*. In addition, a number of oncogenic transcription factors act as repressors to block transcription of genes required for differentiation. The importance of constitutively activated mitogenic signaling pathways in oncogenesis has become increasingly obvious. Activated forms of several components of the ERK MAP kinase pathway are oncoproteins, but new mitogenic signaling pathways, such as

the PI3 kinase/Akt pathway, have also been implicated in tumorigenesis. Until recently, Ras family members were the only small G proteins with well-established roles in transformation, but the Rho family has come into prominence in the past few years with the discovery that several oncoproteins act by stimulating members of the Rho family. The actin cytoskeleton clearly plays a dynamic role in cell growth and transformation. This is underscored by the fact that the merlin/schwannomin tumor suppressor is a member of the ERM protein family, which regulate cortical actin structures. Cadherins and catenins, which were once thought of simply as components of the adherens junction, are emerging as players in a new transcytoplasmic signaling system.

Deregulation of cell cycle control is becoming increasingly important, and most cancer cells lack the Rb G1 checkpoint through mutation of one of the elements in the cyclin D/Cdk4/p16/Rb axis. The role of transcriptional deregulation in cancer is a continuing theme, but one that involves not only bona fide transcription factors, but also other types of nuclear regulatory protein. Surprisingly, transformation can be elicited by global changes in the rates of transcription and translation, reinforcing the notion that the transformed phenotype can result from subtle imbalances in the levels of normal growth regulators. Finally, viral oncoproteins are continuing to point the way to important intracellular pathways involved in growth regulation.

The interconnections that are emerging between previously unassociated signaling pathways involved in cancer are truly remarkable. Growth stimulatory and growth inhibitory systems are proving to be inextricably interlinked. We can anticipate that the intricacy of these growth regulatory networks will continue to grow, and that perturbation of such networks through mutation will be a fundamental cause of cancer.

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