**Many Oncogenic Mutations Constitutively Activate Signal-Transducing Proteins**

A large number of oncogenes are derived from proto-oncogenes whose encoded proteins are components or regulators of signal transduction pathways — most prominent among them the Ras pathway. As we saw in Chapter 16, Ras is a key component in the transduction of signals from activated receptors to a cascade of protein kinases. In the first part of this pathway, a signal from an activated RTK is carried via two adapter proteins to Ras, converting it to the active GTP-bound form (see Figure 16-10). In the second part of the pathway, activated Ras transmits the signal via two intermediate protein kinases to MAP kinase. The activated MAP kinase then phosphorylates a number of transcription factors that induce synthesis of important growth and proliferation proteins (see Figures 16-13 and 16-14). Virtually every component of this RTK/Ras/MAP kinase signaling cascade has been identified as an oncogene or tumor suppressor gene (Figure 25-14).

Among the best studied oncogenes are the *RASD* genes themselves, which were the first nonviral oncogenes to be recognized. Any one of a number of changes in Ras can lead to its uncontrolled and therefore dominant activity. In particular, if a point mutation substitutes any other amino acid for the glycine at position 12 in the Ras sequence, the normal protein is converted into a constitutively active oncoprotein (see Chapter 16). This single mutation reduces the protein’s GTPase activity, thus maintaining Ras in the active GTP-bound state. Activating Ras mutations short-circuit the first part of the RTK pathway, making upstream activation triggered by ligand binding to the receptor unnecessary. Constitutively active Ras oncoproteins are produced by many types of human tumors, including bladder, colon, mammary, skin, and lung carcinomas, neuroblastomas, and leukemias.

Constitutive Ras activation can also arise from loss-of-function mutations in a GTPase-activating protein (GAP). The normal function of a GAP is to accelerate hydrolysis of GTP and thus the conversion of active GTP-bound Ras to inactive GDP-bound Ras (see Figure 3-35). Loss-of-function mutations in the Ras-GAP protein, the product of the *NF1* gene, leads to sustained activation of downstream signal-transducing proteins. The relationship between *RAS*, an oncogene, and *NF1*, a tumor suppressor gene acting in the same pathway, is a good example of the regulatory circuit shown in Figure 25-12. *NF1* was first discovered as the underlying cause of the familial cancer syndrome neurofibromatosis. Individuals who have inherited a single mutant *NF1* allele then develop neurofibromas, a benign tumor of the sheath cells that surround nerves, caused by loss of both alleles through LOH.

Oncogenes encoding other altered components of the RTK/Ras/MAP kinase pathway have also been identified (see Figure 25-14). For example, constitutively active forms of Raf have been identified in approximately 50 percent of melanomas. As in the case of constitutively active forms of Ras, these mutant Raf forms no longer require regulatory signals coming from the cell surface and signal continuously for cell growth and proliferation.

**Growth Control Pathways Ultimately Regulate Initiation of the Cell Cycle**

Growth stimulatory pathways such as the RTK/Ras/MAP kinase pathway ultimately have two outputs: transcription of a set of genes required for cell growth and activation of the cell cycle to initiate a new round of cell division. During the cell cycle, once a cell progresses past a certain point in G1, called the *restriction point*, it becomes irreversibly committed to entering S phase and replicating its DNA. Cyclin Ds, cyclin-dependent kinases (CDKs), and the Rb protein are all elements of the control system that regulates passage through the restriction point. Many of these proteins that regulate cell cycle initiation are targets for oncogenic mutations.

The pathway that controls entry into the cell cycle is estimated to be misregulated in approximately 80 percent of human cancers. At the heart of this pathway are cyclin D–CDK4/6 complexes and the transcriptional repressor Rb (see Figure 19-16). The expression of cyclin D genes is induced by many extracellular growth factors, or *mitogens*. These cyclins assemble with a partner, CDK4 or CDK6, to generate catalytically active cyclin-CDK complexes whose kinase activity promotes progression through G1. Mitogen withdrawal prior to passage through the restriction point leads to accumulation of two CDK inhibitors. As described in Chapter 19, these two proteins, p15 and p16, bind to cyclin D–CDK4/6 complexes and inhibit their activity, thereby causing G1 arrest.

Most tumors contain an oncogenic mutation that causes the overproduction or loss of one of the components of the pathway that controls entry into S phase, so that the cells are propelled into S phase in the absence of the proper extracellular growth signals. For example, elevated levels of cyclin D1, one of the three cyclin Ds, are found in many human cancers. One mechanism that results in overproduction of cyclin D is translocation. In certain tumors of antibody-producing B lymphocytes, the *cyclin D1* gene is translocated such that its transcription is under the control of an antibody-gene enhancer, causing elevated cyclin D1 production throughout the cell cycle irrespective of extracellular signals. That cyclin D1 can function as an oncoprotein was shown by studies with transgenic mice in which the *cyclin D1* gene was placed under the control of an enhancer specific for mammary duct cells. Initially, the duct cells underwent hyperproliferation, and eventually breast tumors developed in these transgenic mice. A second mechanism that can lead to overproduction of cyclin D is gene amplification. Amplification of the *cyclin D1* gene and concomitant overproduction of the cyclin D1 protein is common in human breast cancers; the extra cyclin D1 helps to drive cells through the cell cycle.

We have already seen that inactivating mutations in both *RB* alleles lead to childhood retinoblastoma, a relatively rare type of cancer. However, loss-of-function mutations in the *RB* gene are also found in the more common cancers that arise later in life (see Figure 25-11). These tissues, unlike retinal tissue, probably produce other proteins (e.g., p107 and p130, both structurally related to *RB*) whose function is redundant with that of *RB*, and thus *RB* is not so critical for preventing cancer in these tissues. In the retina, however, regulation of cell cycle entry appears to rely exclusively on the Rb protein, which is why patients heterozygous for the *RB* gene first develop tumors in this tissue. Rb function can be eliminated not only by inactivating mutations, but also by the binding of an inhibitory protein, designated *E7*, that is encoded by human papillomavirus (HPV), another nasty viral trick to create virus-producing tissue. At present, this binding is known to occur only in cervical and oropharyngeal cancers.

The proteins that function as cyclin-CDK inhibitors are also targets for oncogenic mutations. In particular, loss-of-function mutations in p16 (*CDKN2A*) that prevent it from inhibiting cyclin D–CDK4/6 kinase activity are among the most common oncogenic drivers in several cancers (see Figure 25-11 and Table 25-2). Loss of p16 mimics overproduction of cyclin Ds and thus p16 normally acts as a tumor suppressor. Although the *p16* tumor suppressor gene is deleted in some human cancers, the *p16* sequence is normal in others. In some of these latter cancers (e.g., lung cancer), the *p16* gene, or genes encoding other functionally related proteins, is inactivated by hypermethylation of its promoter region, which prevents its transcription. What promotes this change in the methylation of *p16* is not known, but it prevents production of this important cell cycle control protein.

The locus encoding p16 is highly unusual in that it encodes no less than three tumor suppressor genes, which makes it the most vulnerable locus in the human genome to oncogenic changes. In addition to harboring the p16-encoding gene, *CDKN2A*, it has the *CDKN2B* locus immediately upstream, which encodes p15, another cyclin D–CDK4/6 inhibitor (Figure 25-15). The locus also encodes a key activator of the tumor suppressor p53. This protein, p14ARF (p19ARF in the mouse), is encoded by an exon upstream of the first *CDKN2A* exon and shares its exon 2 and exon 3 with *CDKN2A*. As we will see in Section 25.4, this protein controls the stability of p53. Thus mutations in this locus can simultaneously affect the two major tumor-suppressor pathways in the cell, the Rb and p53 pathways.

**Inappropriate Production of Nuclear Transcription Factors Can Induce Transformation**

Mutations that create oncogenes or inactivate tumor suppressor genes eventually cause broad changes in gene expression. These changes can be measured by comparing the amounts of different mRNAs produced in normal cells and in tumor cells. Since the most direct effect on gene expression is exerted by transcription factors, it is not surprising that many oncogenes encode transcription factors. Two such transcription factors with a clear role in tumorigenesis are the FOS and MYC proteins, which stimulate transcription of genes encoding proteins that promote progression through the G1 phase of the cell cycle and the G1-to-S transition. We discuss the deregulation of these proteins below.

JUN and FOS were initially identified in transforming retroviruses and later found to be overexpressed in some human tumors. The *JUN* and *FOS* proto-oncogenes encode proteins that sometimes associate to form a heterodimeric transcription factor, called *AP1,* that binds to a sequence found in promoters and enhancers of many genes (see Figure 8-26a and Chapter 16). These proteins function as oncoproteins by activating the transcription of key genes that encode growth-promoting proteins and by inhibiting the transcription of growth-repressing genes.

Many nuclear proto-oncogene proteins are produced when normal cells are stimulated to grow, indicating their direct role in growth control. For example, platelet-derived growth factor (PDGF) treatment of quiescent mouse 3T3 cells induces an approximately 50-fold increase in the production of the transcription factors FOS as well as MYC, the products of the *FOS* and *MYC* proto-oncogenes. Initially, there is a transient rise of FOS and later a more prolonged rise of MYC (Figure 25-16). The levels of both proteins decline within a few hours, a regulatory effect that may, in normal cells, help to avoid cancer.

The oncogenic forms of *FOS* and *MYC* are due to gain-of-function mutations. In normal cells, the mRNAs for these genes and the proteins they encode are intrinsically unstable and degrade rapidly after the genes are transcribed. Some of the genetic changes that turn *FOS* from a normal gene into an oncogene involve deletions of the sequences that normally make the *FOS* mRNA and protein short-lived. Conversion of the *MYC* proto-oncogene into an oncogene can occur by different mechanisms. In cells of the human tumor known as **Burkitt’s lymphoma**, the *MYC* gene is translocated to a site near the heavy-chain antibody genes, which are normally active in antibody-producing white blood cells (Figure 25-17). The *MYC* translocation is a rare aberration of the normal DNA rearrangements that occur during maturation of antibody-producing cells. The translocated *MYC* gene, now regulated by the antibody-gene enhancer, is continually highly expressed, causing the cell to become cancerous. Localized amplification of a segment of DNA containing the *MYC* gene, which occurs in several human tumors, also causes inappropriately high production of the otherwise normal MYC protein. This mechanism of oncogenic activation is similar to the formation of BCR-ABL oncogene by a translocation that produces the Philadelphia chromosome.

The *MYC* gene encodes a basic helix-loop-helix protein that acts as part of a set of interacting proteins that can dimerize in various combinations, bind to DNA, and coordinately regulate the transcription of target genes. Other members of this protein set include MAD, MAX, and MNT. MAX can heterodimerize with MYC, MAD, and MNT. MYC-MAX dimers regulate genes that control proliferation, such as cyclins. MAD proteins inhibit MYC proteins, which has led to an interest in using MAD proteins, or drugs that stimulate MAD proteins, to rein in excessive MYC activity that contributes to tumor formation. MYC protein complexes affect transcription by recruiting chromatin-modifying complexes containing histone acetyl transferases (which usually stimulate transcription; see Chapter 8) to MYC target genes. MAD and MNT work with the SIN3 co-repressor protein to bring in histone deacetylases that help to block transcription. Together, all these proteins form a regulatory network that employs protein-protein association, variations in DNA binding, and transcriptional regulation to control cell proliferation. Overproduction of MYC protein tips the scales in favor of cell growth and division.

**Aberrations in Signaling Pathways That Control Development Are Associated with Many Cancers**

During normal development, secreted signals such as Hedgehog (Hh), Wnt, and TGF-β are used to direct cells to particular developmental fates, which may include the property of rapid cell cycling. The effects of such signals must be regulated so that growth is limited to the right time and place. Among the mechanisms available for reining in the effects of these powerful developmental signals are inducible intracellular antagonists, receptor blockers, and competing signals. Mutations that prevent such restraining mechanisms from operating are likely to be oncogenic, causing inappropriate or cancerous growth.

The Hedgehog signaling pathway, which is used repeatedly during development to control differentiation, is a good example of a signaling pathway implicated in cancer induction. In the skin and cerebellum, one of the human Hh proteins, Sonic Hedgehog, stimulates cell division by binding to and inactivating a membrane protein called *Patched1 (PTC1)* (see Figure 16-29). Loss-of-function mutations in *PTC1* permit cell proliferation in the absence of an Hh signal; thus *PTC1* is a tumor suppressor gene. People who inherit a defective copy of *PTC1* have a propensity to develop skin and brain cancer; either can occur when the remaining *PTC1* allele is lost through the mechanism of LOH that we saw for RB and NF1 cancer syndromes. Spontaneous mutations in both copies of this gene have also been observed in sporadic cases of these cancers. Mutations in other genes in the Hh signaling pathway are also associated with cancer. Some such mutations create oncogenes that turn on Hh target genes inappropriately; others are recessive mutations that affect negative regulators such as PTC1.

Many of the signaling pathways described in Chapters 16 and 20 also play roles in controlling embryonic development and cell proliferation in adult tissues. In recent years, mutations affecting components of most of these signaling pathways have been linked to cancer. Indeed, once one gene in a developmental pathway has been linked to a type of human cancer, knowledge of that pathway gleaned from model organisms such as worms, flies, or mice allows focused investigations of the possible involvement of additional pathway genes in other cases of the cancer. For example, *APC*, a gene that is mutated early on in colon cancer, is now known to be part of the Wnt signaling pathway (see Chapter 16). That knowledge, in turn, led to the discovery of β*-*catenin mutations in colon cancer.

Mutations in tumor-suppressor developmental genes promote tumor formation in tissues where the affected gene normally acts to restrain growth. For example, transforming growth factor β (TGF-β), despite its name, primarily acts to inhibit proliferation of many cell types, including most epithelial and immune-system cells. Binding of TGF-β to its receptor activates cytosolic Smad transcription factors (see Figure 16-24). After translocating to the nucleus, Smads can promote expression of the gene encoding p15, an inhibitor of cyclin-dependent kinase 4 (CDK4), which causes cells to arrest in G1. TGF-β signaling also promotes expression of genes encoding extracellular matrix proteins and plasminogen activator inhibitor 1 (PAI-1), which reduces the plasmin-catalyzed degradation of the matrix. Loss-of-function mutations in either TGF-β receptors, as noted above, or in Smads thus promote cell proliferation and probably contribute to the invasiveness and metastasis of tumor cells (Figure 25-18). Such mutations have in fact been found in a variety of human cancers. For example, deletion of the *Smad4* gene occurs in many human pancreatic cancers; retinoblastoma and colon cancer cells lack functional TGF-β receptors and therefore are unresponsive to TGF-β growth inhibition. Originally Smad4 was called *DPC (deleted in pancreatic carcinoma)*.