The Genetic Basis of Cancer

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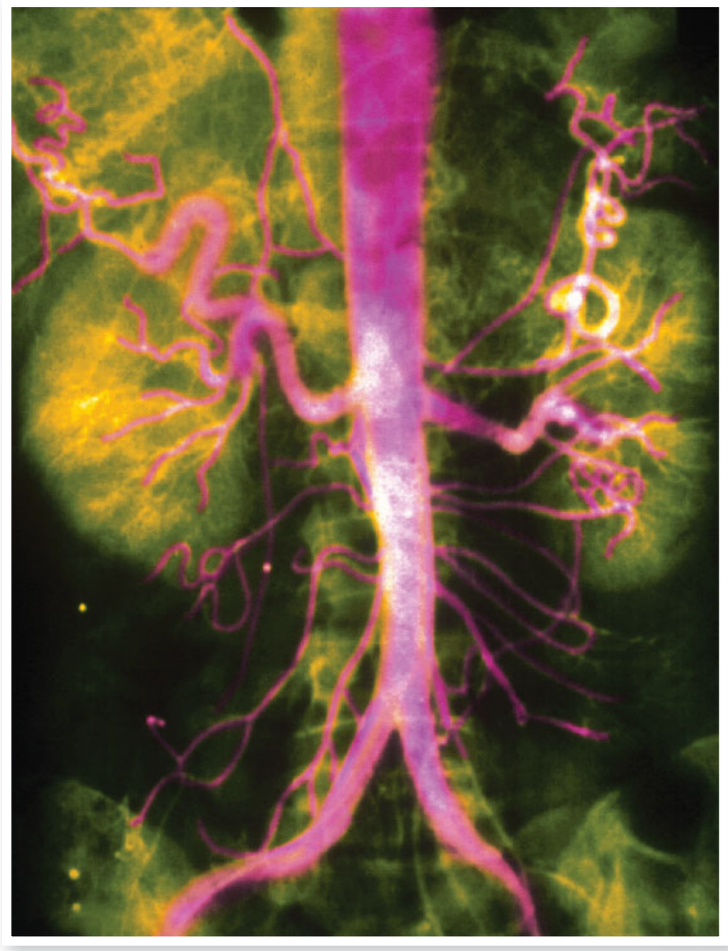
CHAPTER OUTLINE

- Cancer: A Genetic Disease
- Oncogenes
- Tumor Suppressor Genes
- Genetic Pathways to Cancer

A Molecular Family Connection

When Allison Romano started looking at colleges and universities, she wanted to find a school where she could study genetics in depth, maybe even do some hands-on research. Her plans were, in a sense, genetically motivated. At age 12 she was diagnosed with a tumor on one of her adrenal glands. This tumor was removed surgically, and after a lengthy convalescence, Allison returned to seventh grade, healthy and happy, and imbued with an interest in learning about the disease that had afflicted her. In high school, the courses Allison took reinforced this interest. She read a lot and met several students who enjoyed studying biology. Then another adrenal tumor appeared, but this time not in Allison. Rather, the tumor was found in her father. Louis Romano's tumor—the size of a golf ball—was successfully removed, and Louis recovered fully.



Colored X-ray image of a pheochromocytoma showing excessive blood vessel growth into the tumor area.

After this incident, the oncologist suspected that both Louis and Allison had developed adrenal tumors—a rare form of cancer called pheochromocytoma—because they carried a mutation in the *VHL* gene, located in the short arm of chromosome 3. Published research had shown that such mutations are sometimes associated with this type of cancer. The oncologist therefore sent DNA samples from Louis and Allison to a genetics laboratory. DNA tests showed that both Louis and Allison were heterozygous for a mutant *VHL* allele. At nucleotide 490 in the *VHL* gene, a G:C base pair had been changed into an A:T base pair, causing serine to be substituted for glycine at position 93 in the polypeptide encoded by the gene.

When Allison learned of this result, she resolved to study genetics. Her older sister, who showed no sign of pheochromocytoma, asked to be tested for the mutant allele and was found to have it. Her doctor then advised her to have regular screenings for any sign of pheochromocytoma. Louis Romano's two siblings—both asymptomatic—were also informed about the *VHL* mutation, but neither of them opted for testing. Allison subsequently majored in biology at a large university and worked for two semesters in a cancer genetics lab. Her project, on the identification of cancer-related genes in mice, was presented as a poster at the university's annual undergraduate research symposium, where her father and sister could see how she had found purpose in their family's molecular connection.

Cancer: A Genetic Disease

Mutations in genes that control cell growth and division are responsible for cancer.

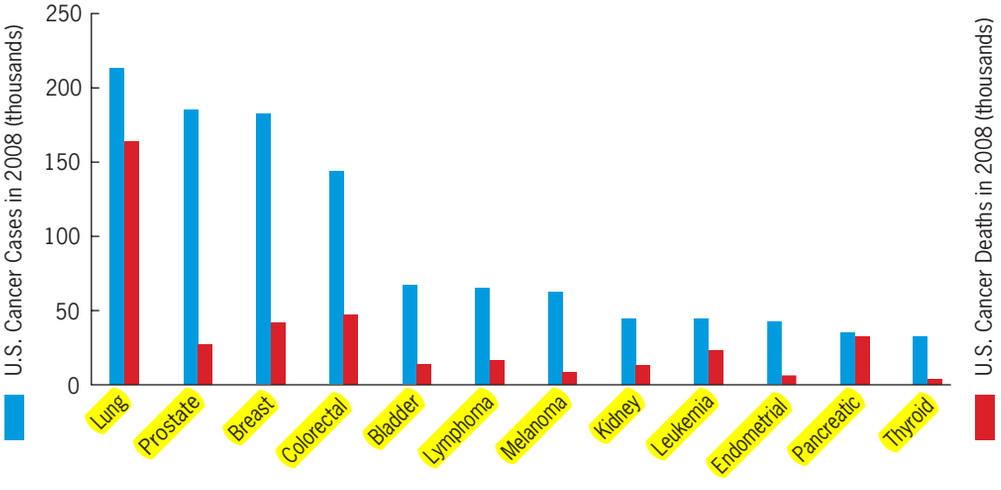
Cancerous tumors kill several hundred thousand Americans every year. What causes tumors to form, and what causes some of them to spread? Why do some types of tumors tend to be found in families? Is the tendency to develop cancer inherited? Do environmental factors contribute to the development of cancer? These and other questions have stimulated an enormous amount of research on the basic biology of cancer. Although many details are still unclear, the fundamental finding is that cancers result from genetic malfunctions. In some instances, these malfunctions may be triggered or exacerbated by environmental factors such as diet, excessive exposure to sunlight, or chemical pollutants. Cancers arise when critical genes are mutated. These mutations can cause biochemical processes to go awry and lead to the unregulated proliferation of cells. Without regulation, cancer cells divide ceaselessly, piling up on top of each other to form tumors. When cells detach from a tumor and invade the surrounding tissues, the tumor is *malignant*. When the cells do not invade the surrounding tissues, the tumor is *benign*. Malignant tumors may spread to other locations in the body, forming secondary tumors. This process is called **metastasis,** from Greek words meaning to "change state." In both benign and malignant tumors, something has gone wrong with the systems that control cell division. Researchers have now firmly established that this loss of control is due to underlying genetic changes.

THE MANY FORMS OF CANCER

Cancer is not a single disease, but rather a group of diseases. Cancers can originate in many different tissues of the body. Some grow aggressively, others more slowly. Some types of cancer can be stopped by appropriate medical treatment; others cannot. ■ **Figure 21.1** shows the frequencies of new cases of different types of cancer in the United States, as well as the number of fatalities attributed to each type. Lung cancer is the most prevalent type, in large measure due to the effects of cigarette smoking. Breast cancer and prostate cancer are also fairly common.

The most prevalent types of cancer are derived from cell populations that divide actively, for example, from epithelial cells in the intestines, lungs, or prostate gland. Rarer forms of cancer develop from cell populations that typically do not divide, for example, from differentiated muscle or nerve cells.

Although the death rate from cancer is still high, enormous progress has been made in detecting and treating different types of cancer. The techniques of molecular genetics have enabled scientists to characterize cancers in ways that were not previously possible, and they have allowed them to devise new strategies for cancer therapy. There is little doubt that the large investment in basic cancer research is paying off.



■ **FIGURE 21.1** Estimated number of new cases and deaths from specific types of cancer in the United States in 2008.

Cancer cells can be obtained for experimental study by removing tissue from a tumor and dissociating it into its constituent cells. With appropriate nutrients, these dissociated tumor cells can be cultured *in vitro*, sometimes indefinitely. Cancer cells can also be derived from cultures of normal cells by treating the cells with agents that induce the cancerous state. Radiation, mutagenic chemicals, and certain types of viruses can irreversibly transform normal cells into cancerous cells. The agents that cause this type of transformation are called **carcinogens.**

The abiding characteristic of all cancer cells is that their growth is unregulated. When normal cells are cultured *in vitro*, they form a single cell layer—a monolayer—on the surface of the culture medium. Cancer cells, by contrast, overgrow each other, piling up on the surface of the culture medium to form masses. This unregulated pileup occurs because cancer cells do not respond to the chemical signals that inhibit cell division and because they cannot form stable associations with their neighbors.

The external abnormalities that are apparent in a culture of cancer cells are correlated with profound intracellular abnormalities. Cancer cells often have a disorganized cytoskeleton, they may synthesize unusual proteins and display them on their surfaces, and they frequently have abnormal chromosome numbers—that is, they are aneuploid.

CANCER AND THE CELL CYCLE

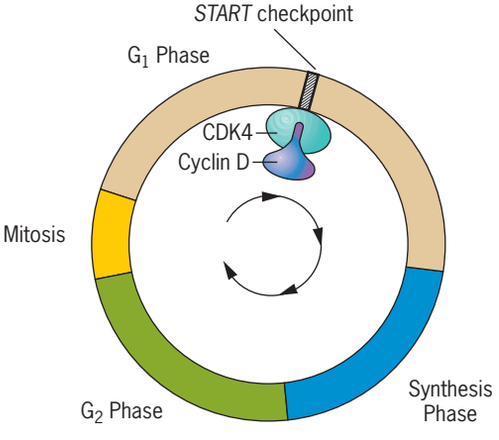
The cell cycle consists of periods of growth, DNA synthesis, and division. The length of this cycle and the duration of each of its components are controlled by external and internal chemical signals. The transition from each phase of the cycle requires the integration of specific chemical signals and precise responses to these signals. If the signals are incorrectly sensed or if the cell is not properly prepared to respond, the cell could become cancerous.

The current view of cell-cycle control is that transitions between different phases of the cycle (G_1, S, G_2, and M; see Chapter 2) are regulated at "checkpoints." A **checkpoint** is a mechanism that halts progression through the cycle until a critical process such as DNA synthesis is completed, or until damaged DNA is repaired. When a checkpoint is satisfied, the cell cycle can progress. Two types of proteins play important roles in this progression: the *cyclins* and the *cyclin-dependent kinases*, often abbreviated CDKs. Complexes formed between the cyclins and the CDKs cause the cell cycle to advance.

The CDKs are the catalytically active components of the cell-cycling mechanism. These proteins regulate the activities of other proteins by transferring phosphate groups to them. However, the phosphorylation activity of the CDKs depends on the presence of the cyclins. The cyclins enable the CDKs to carry out their function by forming cyclin/CDK complexes. When the cyclins are absent, these complexes cannot form, and the CDKs are inactive. Cell cycling therefore requires the alternate formation and degradation of cyclin/CDK complexes.

One of the most important cell-cycle checkpoints, called *START*, is in mid-G_1 (■ **Figure 21.2**). The cell receives both external and internal signals at this checkpoint to determine when it is appropriate to move into the S phase. This checkpoint is regulated by D-type cyclins in conjunction with CDK4. If a cell is driven past the *START* checkpoint by the cyclin D/CDK4 complex, it becomes committed to another round of DNA replication. Inhibitory proteins with the capability of sensing problems in the late G_1 phase, such as low levels of nutrients or DNA damage, can put a brake on the cyclin/CDK complex and prevent the cell from entering the S phase. In the absence of such problems, the cyclin D/CDK4 complex drives the cell through the end of the G_1 phase and into the S phase, thereby initiating the DNA replication that is a prelude to cell division.

In tumor cells, checkpoints in the cell cycle are typically deregulated. This deregulation is due to genetic defects in the machinery that alternately raises and lowers the abundance of the cyclin/CDK complexes. For example, the genes encoding the cyclins or the CDKs may be mutated, or the genes encoding the proteins that respond to specific cyclin/CDK complexes or that regulate the abundance of these complexes may be mutated. Many different types of genetic defects can deregulate the cell cycle, with the ultimate consequence that the cells may become cancerous.



■ **FIGURE 21.2** A schematic view of the *START* checkpoint in the mammalian cell cycle. Passage through the checkpoint depends on the activity of the cyclin D/CDK4 protein complex.

Cells in which the *START* checkpoint is dysfunctional are especially prone to become cancerous. The *START* checkpoint controls entry into the S phase of the cell cycle. If DNA within a cell has been damaged, it is important that entry into the S phase be delayed to allow for the damaged DNA to be repaired. Otherwise, the damaged DNA will be replicated and transmitted to all the cell's descendants. Normal cells are programmed to pause at the *START* checkpoint to ensure that repair is completed before DNA replication commences. By contrast, cells in which the *START* checkpoint is dysfunctional move into S phase without repairing their damaged DNA. Over a series of cell cycles, mutations that result from the replication of unrepaired DNA may accumulate and cause further deregulation of the cell cycle. A clone of cells with a dysfunctional *START* checkpoint may therefore become aggressively cancerous.

CANCER AND PROGRAMMED CELL DEATH

Every cancer involves the accumulation of unwanted cells. In many animals, superfluous cells can be disposed of by mechanisms that are programmed into the cells themselves. Programmed cell death is a fundamental and widespread phenomenon among animals. Without it, the formation and function of organs would be impaired by cells that simply "get in the way."

Programmed cell death is also important in preventing the occurrence of cancers. If a cell with an abnormal ability to replicate is killed, it cannot multiply to form a potentially dangerous tumor. Thus, programmed cell death is a check against renegade cells that could otherwise proliferate uncontrollably in an organism.

Programmed cell death is called **apoptosis**, from Greek roots that mean "falling away." The events that trigger cell death are only partially understood; we will investigate some of them later in this chapter. However, the actual killing events are known in some detail. A family of proteolytic enzymes called *caspases* plays a crucial role in the cell death phenomenon. The caspases remove small parts of other proteins by cleaving peptide bonds. Through this enzymatic trimming, the target proteins are inactivated. The caspases attack many different kinds of proteins, including the lamins, which make up the inner lining of the nuclear envelope, and several components of the cytoskeleton. The collective impact of this proteolytic cleavage is that cells in which it occurs lose their integrity; their chromatin becomes fragmented, blebs of cytoplasm form at their surfaces, and they begin to shrink. Cells undergoing this kind of disintegration are usually engulfed by phagocytes, which are scavenger cells of the immune system, and are then destroyed. If the apoptotic mechanism has been impaired or inactivated, a cell that should otherwise be killed can survive and proliferate. Such a cell has the potential to form a clone that could become cancerous if it acquires the ability to divide uncontrollably.

A GENETIC BASIS FOR CANCER

The recent great advances in understanding cancer have come through application of molecular genetic techniques. However, before these techniques were available to researchers, there was strong evidence that the underlying causes of cancer are genetic. First, it was known that the cancerous state is clonally inherited. When cancer cells are grown in culture, their descendants are all cancerous. The cancerous condition is therefore transmitted from each cell to its daughters at the time of division—a phenomenon indicating that cancer has a genetic (or epigenetic) basis. Second, it was known that certain types of viruses can induce the formation of tumors in experimental animals. The induction of cancer by viruses implies that the proteins encoded by viral genes are involved in the production of the cancerous state. Third, it was known that cancer can be induced by agents capable of causing mutations. Mutagenic chemicals and ionizing radiation had been shown to induce tumors in experimental animals. In addition, a wealth of epidemiological data had implicated these agents as the causes of cancer in humans. Fourth, it was known that certain types of cancer tend to run in families. In particular, susceptibility to retinoblastoma, a rare cancer of the eye, and susceptibility to some forms of colon cancer appeared to be inherited as simple dominant

conditions, albeit with incomplete penetrance and variable expressivity. Because susceptibility to these special types of cancer is inherited, it seemed plausible that all cancers might have their basis in genetic defects—either inherited mutations or somatic mutations acquired during a person's lifetime. Finally, it was known that certain types of white blood cell cancers (leukemias and lymphomas) are associated with particular chromosomal aberrations. Collectively, these diverse observations strongly suggested that cancer is caused by genetic malfunctions.

In the 1980s, when molecular genetic techniques were first used to study cancer cells, researchers discovered that the cancerous state is, indeed, traceable to specific genetic defects. Typically, however, not one but several such defects are required to convert a normal cell into a cancerous cell. Cancer researchers have identified two broad classes of genes that, when mutated, can contribute to the development of a cancerous state. In one of these classes, mutant genes actively promote cell division; in the other class, mutant genes fail to repress cell division. Genes in the first class are called **oncogenes,** from the Greek word for "tumor." Genes in the second class are called **tumor suppressor genes.** In the sections that follow, we discuss the discovery, characteristics, and significance of each of these classes of cancer-related genes.

KEY POINTS

- *Cancer is a group of diseases in which the cellular cycle of growth and division is unregulated.*
- *Cancers may develop if the mechanism for programmed cell death (apoptosis) is impaired.*
- *Cancers are due to the occurrence of mutations in genes whose protein products are involved in the control of the cell cycle.*

Oncogenes

Many cancers involve the overexpression of certain genes or the abnormal activity of their mutant protein products.

Oncogenes comprise a diverse group of genes whose products play important roles in the regulation of biochemical activities within cells, including those activities related to cell division. These genes were first discovered in the genomes of RNA viruses that are capable of inducing tumors in vertebrate hosts. Later, the cellular counterparts of these viral oncogenes were discovered in many different organisms, ranging from *Drosophila* to humans.

TUMOR-INDUCING RETROVIRUSES AND VIRAL ONCOGENES

Fundamental insights into the genetic basis of cancer have come from the study of tumor-inducing viruses. Many of these viruses have a genome composed of RNA instead of DNA. After entering a cell, the viral RNA is used as a template to synthesize complementary DNA, which is then inserted at one or more positions in the cell's chromosomes. The synthesis of DNA from RNA is catalyzed by the viral enzyme reverse transcriptase. This reversal of the normal flow of genetic information from DNA to RNA has prompted biologists to call these pathogens **retroviruses** (see Chapter 17).

The first tumor-inducing virus was discovered in 1910 by Peyton Rous; it caused a special kind of tumor, or sarcoma, in the connective tissue of chickens and has since been called the Rous sarcoma virus. Modern research has shown that the RNA genome of this retrovirus contains four genes: *gag*, which encodes the capsid protein of the virion; *pol*, which encodes the reverse transcriptase; *env*, which encodes a protein of the viral envelope; and *v-src*, which encodes a protein kinase that inserts into the plasma membranes of infected cells. The distinguishing feature of a kinase is that it can phosphorylate other proteins. Of these four genes, only the *v-src* gene is responsible for the virus's ability to form tumors. A virus in which the *v-src* gene has been deleted is infectious but unable to induce tumors. Genes such as *v-src* that cause cancer are called oncogenes.

TABLE 21.1

Retroviral Oncogenes

Oncogene	Virus	Host Species	Function of Gene Product
abl	Abelson murine leukemia virus	Mouse	Tyrosine-specific protein kinase
erbA	Avian erythroblastosis virus	Chicken	Analog of thyroid hormone receptor
erbB	Avian erythroblastosis virus	Chicken	Truncated version of epidermal growth-factor (EGF) receptor
fes	ST feline sarcoma virus	Cat	Tyrosine-specific protein kinase
fgr	Gardner-Rasheed feline sarcoma virus	Cat	Tyrosine-specific protein kinase
fms	McDonough feline sarcoma virus	Cat	Analog of colony stimulating growth-factor (CSF-1) receptor
fos	FJB osteosarcoma virus	Mouse	Transcriptional activator protein
fps	Fuginami sarcoma virus	Chicken	Tyrosine-specific protein kinase
jun	Avian sarcoma virus 17	Chicken	Transcriptional activator protein
mil (mht)	MH2 virus	Chicken	Serine/threonine protein kinase
mos	Moloney sarcoma virus	Mouse	Serine/threonine protein kinase
myb	Avian myeloblastosis virus	Chicken	Transcription factor
myc	MC29 myelocytomatosis virus	Chicken	Transcription factor
raf	3611 murine sarcoma virus	Mouse	Serine/threonine protein kinase
H-ras	Harvey murine sarcoma virus	Rat	GTP-binding protein
K-ras	Kirsten murine sarcoma virus	Rat	GTP-binding protein
rel	Reticuloendotheliosis virus	Turkey	Transcription factor
ros	URII avian sarcoma virus	Chicken	Tyrosine-specific protein kinase
sis	Simian sarcoma virus	Monkey	Analog of platelet-derived growth factor (PDGF)
src	Rous sarcoma virus	Chicken	Tyrosine-specific protein kinase
yes	Y73 sarcoma virus	Chicken	Tyrosine-specific protein kinase

Studies with other tumor-inducing retroviruses have uncovered at least 20 different viral oncogenes, usually denoted *v-onc* (Table 21.1). Each type of viral oncogene appears to encode a protein that could theoretically play a role in regulating the expression of cellular genes, including those involved in the processes of growth and division. Some of these proteins may act as signals to stimulate certain types of cellular activity; others may act as receptors to pick up these signals or as intracellular agents to convey them from the plasma membrane to the nucleus; yet another category of viral oncogene proteins may act as transcription factors to stimulate gene expression. To explore the functions of two of these proteins, use your research skills to answer the questions in Solve It: The *v-erbB* and *v-fms* Viral Oncogenes.

Solve It!

The *v-erbB* and *v-fms* Viral Oncogenes

The *v-erbB* gene encodes a truncated version of the receptor for epidermal growth factor (EGF), and the *v-fms* gene encodes an analog of the receptor for colony stimulating growth factor (CSF-1). Both of these receptors are transmembrane proteins with a growth-factor-binding domain on the outside of the cell and a protein kinase domain on the inside. How might these proteins transfer a signal from outside the cell to inside the cell?

To see a solution to this problem, visit the Student Companion site.

CELLULAR HOMOLOGUES OF VIRAL ONCOGENES: THE PROTO-ONCOGENES

The proteins encoded by viral oncogenes are similar to cellular proteins with important regulatory functions. Many of these cellular proteins were identified by isolating the cellular homologue of the viral oncogene. For example, the cellular homologue of the *v-src* gene was obtained by screening a genomic DNA library made from uninfected chicken cells. For this screening, the *v-src* gene was used as a hybridization probe to detect recombinant DNA clones that could base-pair with it. Analysis of these clones established that chicken cells contain a gene that is similar to *v-src*—indeed, that is related to it in an evolutionary sense. However, this gene is not associated with an integrated sarcoma virus, and it differs from the *v-src* gene in a very important respect: it contains introns. There are, in fact, 11 introns in the chicken homologue of *v-src*, compared to zero in the *v-src* gene itself. This startling discovery suggested that perhaps *v-src* had evolved from a normal cellular gene and that, concomitantly, it had lost its introns.

The cellular homologues of viral oncogenes are called **proto-oncogenes,** or sometimes, *normal cellular oncogenes*, denoted *c-onc*. The cellular homologue of *v-src* is therefore *c-src*. The coding sequences of these two genes are very similar, differing only in 18 nucleotides; *v-src* encodes a protein of 526 amino acids, and *c-src* encodes a protein of 533 amino acids. By using *v-onc* genes as probes, other *c-onc* genes have been isolated from many different organisms, including humans. As a rule, these cellular oncogenes show considerable conservation in structure. *Drosophila*, for example, carries very similar homologues of the vertebrate cellular oncogenes *c-abl*, *c-erbB*, *c-fps*, *c-raf*, *c-ras*, and *c-myb*. The similarity of oncogenes from different species strongly suggests that the proteins they encode are involved in important cellular functions.

Why do *c-onc*s have introns whereas *v-onc*s do not? The most plausible answer is that *v-onc*s were derived from *c-onc*s by the insertion of a fully processed *c-onc* mRNA into the genome of a retrovirus. A virion that packaged such a recombinant molecule would then be able to transduce the *c-onc* gene whenever it infected another cell. During infection, the recombinant RNA would be reverse-transcribed into DNA and then integrated into the cell's chromosomes. What could be of greater value to a virus than to have a new gene that stimulates increased growth of its host, while its integrated genome goes along for the ride?

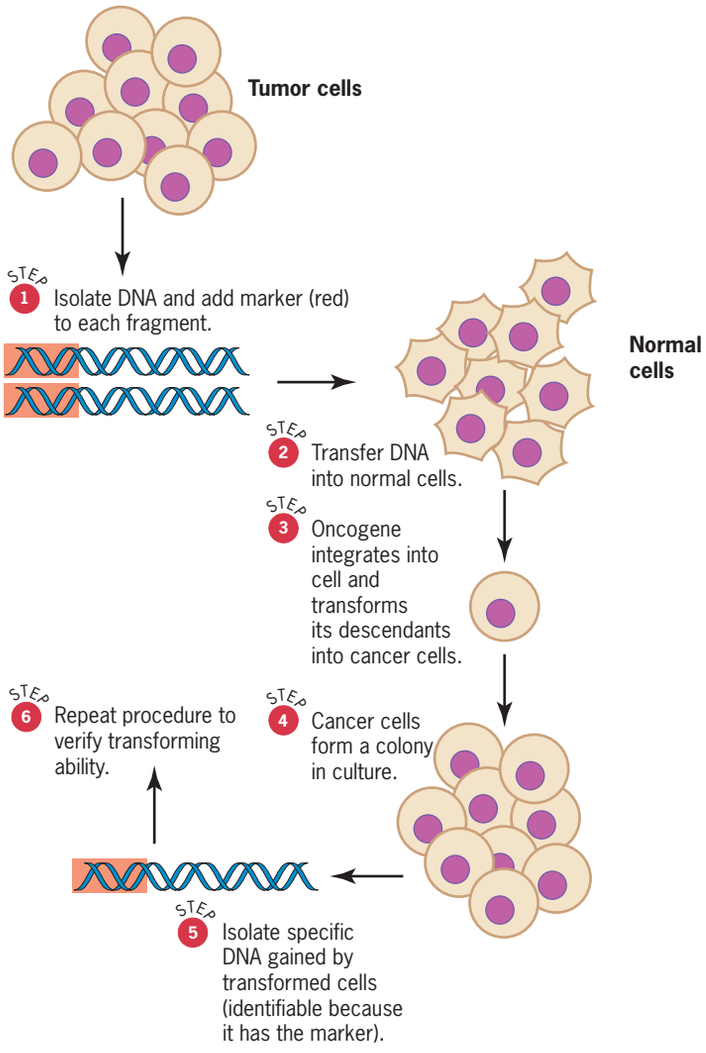
In many cases, the acquisition of an oncogene by a retrovirus has been accompanied by the loss of some viral genetic material. Because this lost material is needed for viral replication, these oncogenic viruses are able to reproduce only if a helper virus is present. In this respect, they resemble the defective transducing bacteriophages we discussed in Chapter 8.

Why do *v-onc*s induce tumors, whereas normal *c-onc*s do not? In some cases it appears that the viral oncogene produces much more protein than its cellular counterpart, perhaps because it has been transcriptionally activated by enhancers embedded in the viral genome. In chicken tumor cells, for example, the *v-src* gene produces 100 times as much tyrosine kinase as the *c-src* gene. This vast oversupply of the kinase evidently upsets the delicate signaling mechanisms that control cell division, causing unregulated growth. Other *v-onc* genes may induce tumors by expressing their proteins at inappropriate times, or by expressing altered—that is, mutant—forms of these proteins.

MUTANT CELLULAR ONCOGENES AND CANCER

The products of the *c-onc*s play key roles in regulating cellular activities. Consequently, a mutation in one of these genes can upset the biochemical balance within a cell and put it on the track to becoming cancerous. Studies of many different types of human cancer have demonstrated that mutant cellular oncogenes are associated with the development of a cancerous state.

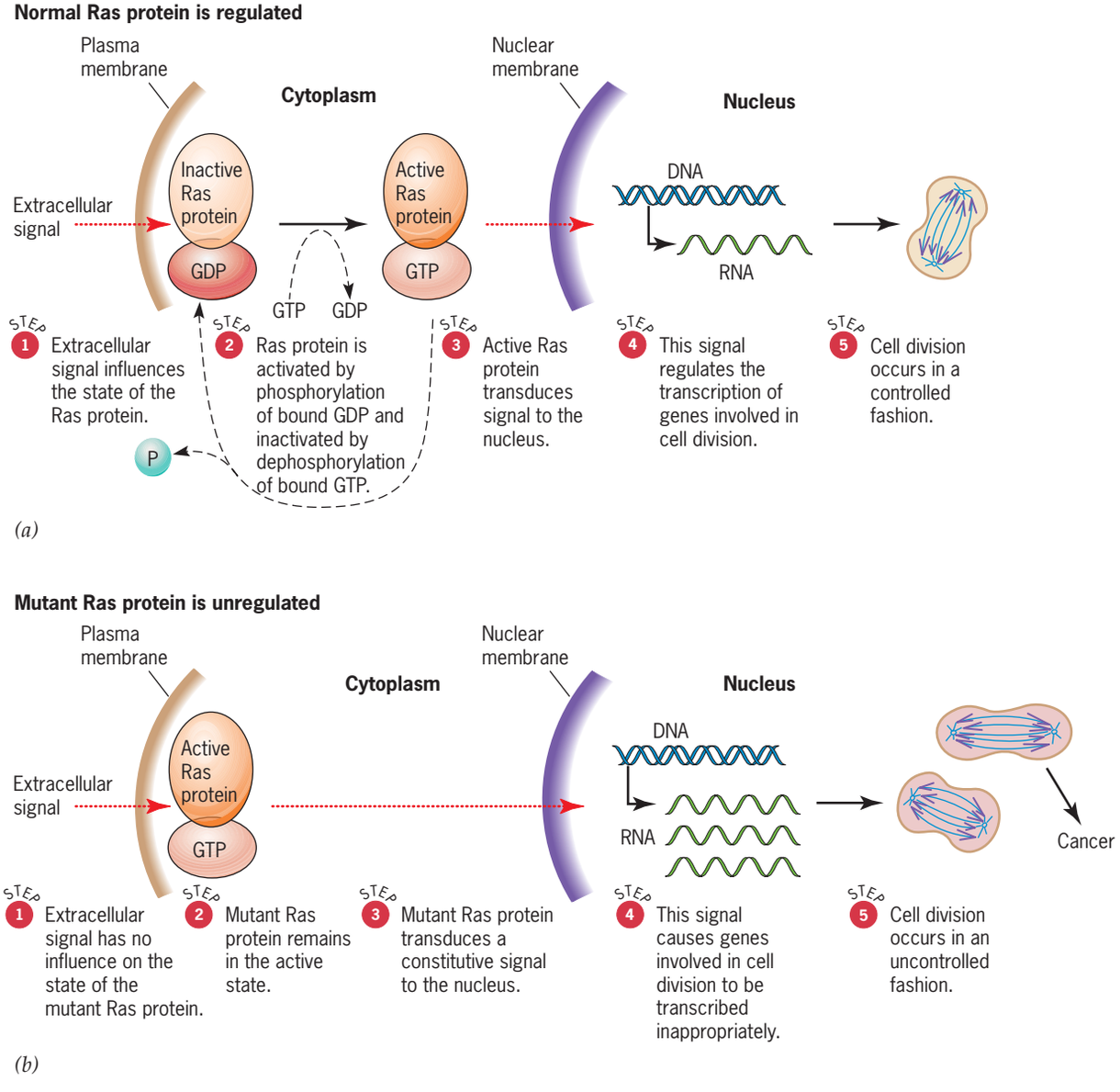
The first evidence linking cancer to a mutant *c-onc* came from the study of a human bladder cancer. The mutation responsible for this bladder cancer was isolated by Robert Weinberg and colleagues using a *transfection test* (■ **Figure 21.3**). DNA was extracted from the cancerous tissue and fragmented into small pieces; then each of these pieces was joined to a segment of bacterial DNA, which served as a molecular marker. The marked DNA fragments were then introduced, or transfected, into cells growing in culture to determine if any of them could transform the cells into a cancerous state. This state could be recognized by the tendency of the cancer cells to form small clumps, or foci, when grown on soft agar plates. The DNA from such cells was extracted and screened to see if it carried the molecular marker that was linked to the original transfecting fragments. If it did, this DNA was retested for its ability to induce the cancerous state. After several



■ **FIGURE 21.3** The transfection test to identify DNA sequences capable of transforming normal cells into cancer cells.

tests, Weinberg's research team identified a DNA fragment from the original bladder cancer that reproducibly transformed cultured cells into cancer cells. This fragment carried an allele of the *c-H-ras* oncogene, a homologue of an oncogene in the Harvey strain of the rat sarcoma virus. DNA sequence analysis subsequently showed that a nucleotide in codon 12 of this allele had been mutated, with a substitution of a valine for the glycine normally found at this position in the c-H-ras protein.

Geneticists now have some understanding of how this mutation causes cells to become cancerous. Unlike viral oncogenes, the mutant c-H-ras gene does not synthesize abnormally large amounts of protein. Instead, the valine-for-glycine substitution at position 12 impairs the ability of the mutant c-H-ras protein to hydrolyze one of its substrates, guanosine triphosphate (GTP). Because of this impairment, the mutant protein is kept in an active signaling mode, transmitting information that ultimately stimulates the cells to divide in an uncontrolled way (■ **Figure 21.4**).



■ **FIGURE 21.4** Ras protein signaling and cancer. (*a*) The normal protein product of the *ras* gene alternates between inactive and active states, depending on whether it is bound to GDP or GTP. Extracellular signals such as growth factors stimulate the conversion of inactive Ras to active Ras. Through active Ras, these signals are transmitted to other proteins and eventually to the nucleus, where they induce the expression of genes involved in cell division. Because this signaling is intermittent and regulated, cell division occurs in a controlled manner. (*b*) Mutant Ras proteins exist mainly in the active state. These proteins transmit their signals more or less constantly, leading to uncontrolled cell division, the hallmark of cancer.

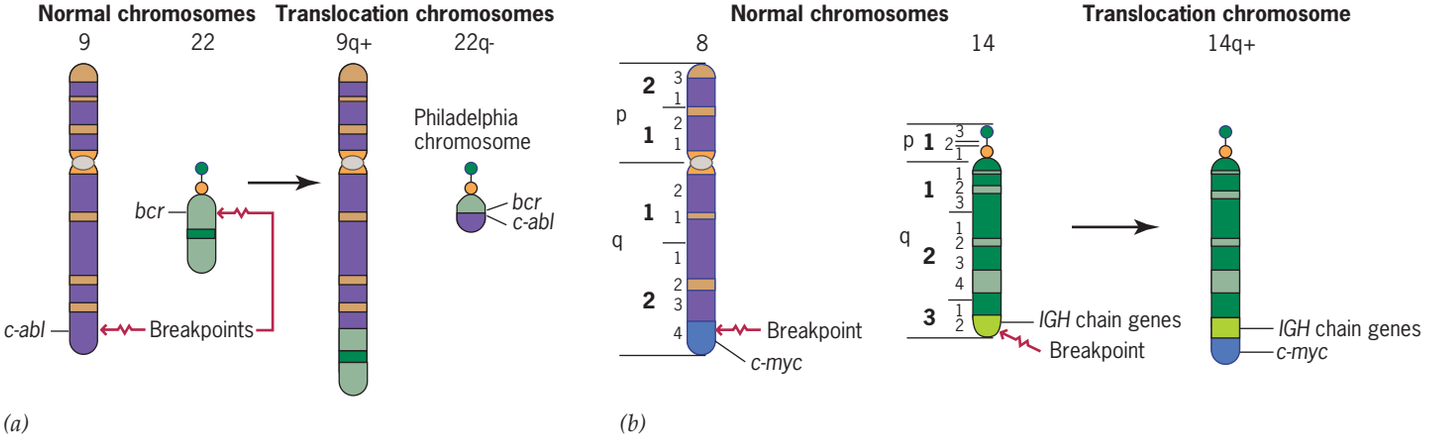
Mutant versions of the *c-ras* oncogenes have now been found in a large number of different human tumors, including lung, colon, mammary, prostate, and bladder tumors, as well as neuroblastomas (nerve cell cancers), fibrosarcomas (cancers of the connective tissues), and teratocarcinomas (cancers that contain different embryonic cell types). In all cases, the mutations involve amino acid changes in one of three positions—12, 59, or 61. Each of these amino acid changes impairs the ability of the mutant Ras protein to switch out of its active signaling mode. These types of mutations therefore stimulate cells to grow and divide.

In these types of cancer, only one of the two copies of the *c-ras* gene has been mutated. The single mutant allele is dominant in its ability to bring about the cancerous state. Mutations in *c-ras* and other cellular oncogenes that lead to cancer in this way are therefore *dominant activators* of uncontrolled cell growth.

Dominant activating mutations in cellular oncogenes are seldom inherited through the germ line; rather, the vast majority of them occur spontaneously in the soma during the course of cell division. Because the number of cell divisions in a human life is very large—more than 10^{16}—thousands of potentially oncogenic mutations are bound to occur, and if each one functioned as a dominant activator of uncontrolled cell growth, the development of a tumor would be inevitable. However, many people lead long lives without developing tumors. The explanation for this paradox is that each individual oncogene mutation is, by itself, seldom able to induce a cancerous state. However, when several different growth-regulating genes have been mutated, the cell cannot compensate for their separate effects, its growth becomes unregulated, and cancer ensues. In many tumors, at least one of these deleterious mutations is in a cellular oncogene. Thus, this group of genes plays an important role in the etiology of human cancer.

CHROMOSOME REARRANGEMENTS AND CANCER

Certain types of human cancer are associated with chromosome rearrangements. For example, chronic myelogenous leukemia (CML) is associated with an aberration of chromosome 22. This abnormal chromosome was originally discovered in the city of Philadelphia and thus is called the *Philadelphia chromosome*. Initially it was thought to have a simple deletion in its long arm; however, subsequent analysis using molecular techniques has shown that the Philadelphia chromosome is actually the result of a reciprocal translocation between chromosomes 9 and 22. (For a general discussion of translocations, see Chapter 6.) In the Philadelphia translocation, the tip of the long arm of chromosome 9 has been joined to the body of chromosome 22, and the distal portion of the long arm of chromosome 22 has been joined to the body of chromosome 9 (■ **Figure 21.5***a*). The translocation breakpoint on chromosome 9 is in the *c-abl*



■ **FIGURE 21.5** Translocations implicated in human cancers. (*a*) The reciprocal translocation involved in the Philadelphia chromosome that is associated with chronic myelogenous leukemia. (*b*) A reciprocal translocation involved in Burkitt's lymphoma. Only the translocation chromosome (14q+) that carries both the *c-myc* oncogene and the immunoglobulin heavy chain genes (*IGH*) is shown.

oncogene, which encodes a tyrosine kinase, and the breakpoint on chromosome 22 is in a gene called *bcr*. Through the translocation, the *bcr* and *c-abl* genes have been physically joined, creating a fusion gene whose polypeptide product has the amino terminus of the Bcr protein and the carboxy terminus of the c-Abl protein. Although it is not understood precisely why, this fusion polypeptide causes white blood cells to become cancerous. The mechanism may involve the tyrosine kinase activity of the c-Abl protein, which is tightly controlled in normal cells but is deregulated in cells that produce the fusion polypeptide. In effect, the tyrosine kinase function of the c-Abl protein has been constitutively activated by the *bcr/c-abl* gene fusion. This fusion is therefore a dominant activator of the c-Abl tyrosine kinase. Deregulation of the c-Abl tyrosine kinase leads to abnormal phosphorylation of other proteins, including some that are involved in controlling the cell cycle. In their phosphorylated state, these proteins cause cells to grow and divide uncontrollably.

Burkitt's lymphoma is another example of a white blood cell cancer associated with reciprocal translocations. These translocations invariably involve chromosome 8 and one of the three chromosomes (2, 14, and 22) that carry genes encoding the polypeptides that form immunoglobulins (also known as antibodies; see Chapter 20). Translocations involving chromosomes 8 and 14 are the most common (■ **Figure 21.5*b***). In these translocations, the *c-myc* oncogene on chromosome 8 is juxtaposed to the genes for the immunoglobulin heavy chains (*IGH*) on chromosome 14. This rearrangement results in the overexpression of the *c-myc* oncogene in cells that produce immunoglobulin heavy chains—that is, in the B cells of the immune system. The *c-myc* gene encodes a transcription factor that activates genes involved in promoting cell division. Consequently, the overexpression of *c-myc* that occurs in cells that carry the *IGH/c-myc* fusion created by the t8;14 translocation causes those cells to become cancerous.

KEY POINTS

- *Some viruses carry genes (oncogenes) that can induce the formation of tumors in animals.*
- *Viral oncogenes are homologous to cellular genes (proto-oncogenes), which can induce tumors when they are overexpressed or when they are mutated to produce abnormally active protein products.*
- *Mutations in proto-oncogenes actively promote cell proliferation.*
- *Some cancers are associated with chromosome rearrangements that enhance the expression of proto-oncogenes or that alter the nature of their protein products.*

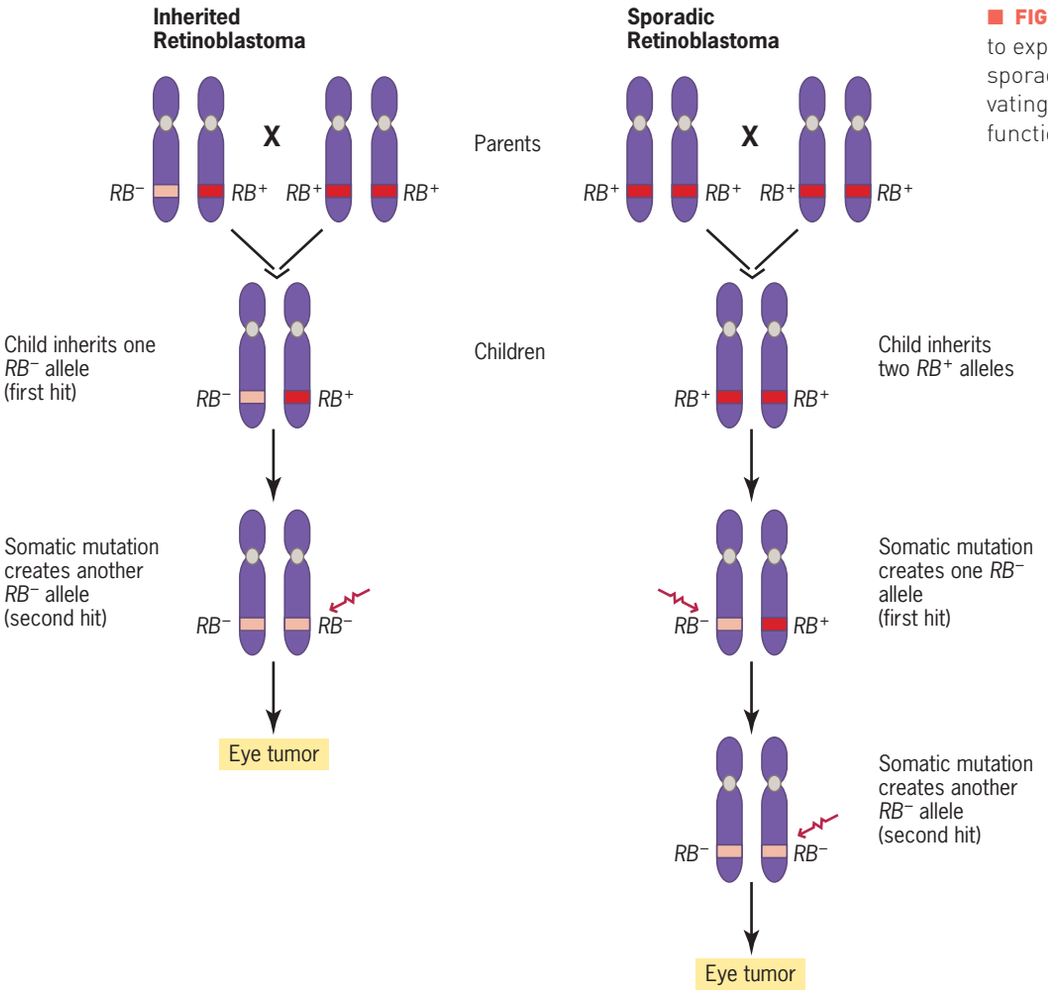
Tumor Suppressor Genes

Many cancers involve the inactivation of genes whose products play important roles in regulating the cell cycle.

The normal alleles of genes such as *c-ras* and *c-myc* produce proteins that regulate the cell cycle. When these genes are overexpressed, or when they produce proteins that function as dominant activators, the cell is predisposed to become cancerous. However, the full development of a cancerous state usually requires additional mutations, and typically these mutations affect genes that are normally involved in the restraint of cell growth. These mutations therefore define a second class of cancer-related genes—the anti-oncogenes, or, as they are more often called, the tumor suppressor genes.

INHERITED CANCERS AND KNUDSON'S TWO-HIT HYPOTHESIS

Many of the tumor suppressor genes were initially discovered through the analysis of rare cancers in which a predisposition to develop the cancer follows a dominant pattern of inheritance. This predisposition is due to heterozygosity for an inherited loss-of-function mutation in the tumor suppressor gene. A cancer develops only if a second



■ **FIGURE 21.6** Knudson's two-hit hypothesis to explain the occurrence of inherited and sporadic cases of retinoblastoma. Two inactivating mutations are required to eliminate the function of the *RB* gene.

mutation occurs in the somatic cells and if this mutation knocks out the function of the wild-type allele of the tumor suppressor gene. Thus, development of the cancer requires two loss-of-function mutations—that is, two inactivating "hits," one in each of the two copies of the tumor suppressor gene.

In 1971 Alfred Knudson proposed this explanation for the occurrence of *retinoblastoma*, a rare childhood cancer of the eye. In most human populations, the incidence of retinoblastoma is about 5 in 100,000 children. Pedigree analysis indicates that approximately 40 percent of the cases involve an inherited mutation that predisposes the individual to develop the cancer. The other 60 percent of the cases cannot be traced to a specific inherited mutation. These noninherited cases are said to be *sporadic*. On the basis of statistical analyses, Knudson proposed that both the inherited and sporadic cases of retinoblastoma occur because the two copies of a particular gene have been inactivated (■ **Figure 21.6**). In the inherited cases, one of the inactivating mutations has been transmitted through the germ line, and the other occurs during the development of the somatic tissues of the eye. In the sporadic cases, both of the inactivating mutations occur during eye development. Thus, in either type of retinoblastoma, two mutational "hits" are required to knock out a gene that normally functions to suppress tumor formation in the eye.

Subsequent research findings have verified the correctness of Knudson's two-hit hypothesis. First, several cases of retinoblastoma were found to be associated with a small deletion in the long arm of chromosome 13. The gene that normally prevents retinoblastoma—symbolized *RB*—must therefore be located in the region defined by this deletion. More refined cytogenetic mapping subsequently placed the *RB* gene in locus 13q14.2. Second, positional cloning techniques were used to isolate a

TABLE 21.2

Inherited Cancer Syndromes

Syndrome	Primary Tumor	Gene	Chromosomal Location	Proposed Protein Function
Familial retinoblastoma	Retinoblastoma	*RB*	13q14.3	Cell cycle and transcriptional regulation
Li-Fraumeni syndrome	Sarcomas, breast cancer	*TP53*	17p13.1	Transcription factor
Familial adenomatous polyposis (FAP)	Colorectal cancer	*APC*	5q21	Regulation of β-catenin
Hereditary nonpolyposis colorectal cancer (HNPCC)	Colorectal cancer	*MSH2* *MLH1* *PMS1* *PMS2*	2p16 3p21 2q32 7p22	DNA mismatch repair
Neurofibromatosis type 1	Neurofibromas	*NF1*	17q11.2	Regulation of Ras-mediated signaling
Neurofibromatosis type 2	Acoustic neuromas, meningiomas	*NF2*	22q12.2	Linkage of membrane proteins to cytoskeleton
Wilms' tumor	Wilms' tumor	*WT1*	11p13	Transcriptional repressor
Familial breast cancer 1	Breast cancer	*BRCA1*	17q21	DNA repair
Familial breast cancer 2	Breast cancer	*BRCA2*	13q12	DNA repair
von Hippel-Lindau disease	Renal cancer	*VHL*	3p25	Regulation of transcriptional elongation
Familial melanoma	Melanoma	*p16*	9p21	Inhibitor of CDKs
Ataxia telangiectasia	Lymphoma	*ATM*	11q22	DNA repair
Bloom's syndrome	Solid tumors	*BLM*	15q26.1	DNA helicase

Source: Fearon, E. R. 1997. Human cancer syndromes: clues to the origin and nature of cancer. *Science* 278:1043–1050.

candidate *RB* gene. Once isolated, the gene's structure, sequence, and expression patterns were determined. Third, the structure of the candidate gene was examined in cells taken from tumorous eye tissue. As predicted by Knudson's two-hit hypothesis, both copies of this gene were inactivated in retinoblastoma cells. Thus, the candidate gene appeared to be the authentic *RB* gene. Finally, cell culture experiments demonstrated that a cDNA from the wild-type allele of the candidate gene could revert the cancerous properties of cultured tumor cells. These cancer reversion experiments proved beyond a doubt that the candidate gene was the authentic *RB* tumor suppressor gene. The protein product of this gene—denoted pRB—was subsequently found to be a ubiquitously expressed protein that interacts with a family of transcription factors involved in regulating the cell cycle.

Knudson's two-hit hypothesis has since been applied to other inherited cancers, including Wilms' tumor, Li-Fraumeni syndrome, neurofibromatosis, von Hippel-Lindau disease, and certain types of colon and breast cancer (Table 21.2). In each case, a different tumor suppressor gene is involved. For example, in Wilms' tumor, a cancer of the urogenital system, the relevant tumor suppressor gene is the *WT1* gene located in the short arm of chromosome 11; in neurofibromatosis, a disease characterized by benign tumors and skin lesions, it is the *NF1* gene located in the long arm of chromosome 17; and in familial adenomatous polyposis, a condition characterized by the occurrence of numerous tumors in the colon, it is the *APC* gene located in the long arm of chromosome 5. Like retinoblastoma, these three diseases are rare, and only a fraction of the observed cases involve an inherited mutation in the relevant tumor suppressor gene. The other cases are caused either by two independent somatic mutations in that gene or by mutations in other, as-yet-unidentified tumor suppressor genes. To explore the genetic dimensions of the two-hit hypothesis, work through Problem-Solving Skills: Estimating Mutation Rates in Retinoblastoma.

PROBLEM-SOLVING SKILLS

Estimating Mutation Rates in Retinoblastoma

THE PROBLEM

Alfred Knudson based his two-hit hypothesis of cancer on a statistical analysis of retinoblastoma. Patients with retinoblastoma (RB) may have tumors in one eye (unilateral RB) or in both eyes (bilateral RB), and within each eye, there may be more than one tumor. Among patients that had inherited an *RB* gene mutation from a parent, Knudson found that the average total number of tumors that formed was 3. Furthermore, he estimated that the total number of retinoblasts—the cells that form the embryonic retina—was about 2 million in each eye. If each tumor in this group of patients is due to the occurrence of another *RB* gene mutation within the first two years of life—the second hit in Knudson's hypothesis—what is the somatic mutation rate for the *RB* gene per year?

FACTS AND CONCEPTS

1. Retinoblastoma occurs when both *RB* genes have been inactivated by mutations.
2. One of these inactivating mutations may be inherited from a parent.
3. Sporadic cases of retinoblastoma occur when both of the inactivating mutations arise during eye development.
4. When two events are independent, we multiply their probabilities to obtain the probability that they will both occur.

ANALYSIS AND SOLUTION

To estimate the somatic mutation rate, we need to count the number of mutational events in comparison to the total number of chances for such events. The average number of tumors (3) is an estimate of the average number of mutational events. The number of chances for such events is a function of the total number of genes that can mutate to produce a tumor: 1 RB^+ gene per cell in a patient that has already inherited one RB^- mutation from a parent $\times$ 2×10^6 cells per eye $\times$ 2 eyes per patient $= 4 \times 10^6$ chances for a mutational event. Thus, the mutation rate is $3/(4 \times 10^6) = 7.5 \times 10^{-7}$ mutations, or, on an annualized basis, 7.5×10^{-7} mutations/2 years $= 3.7 \times 10^{-7}$ mutations/year.

For further discussion visit the Student Companion site.

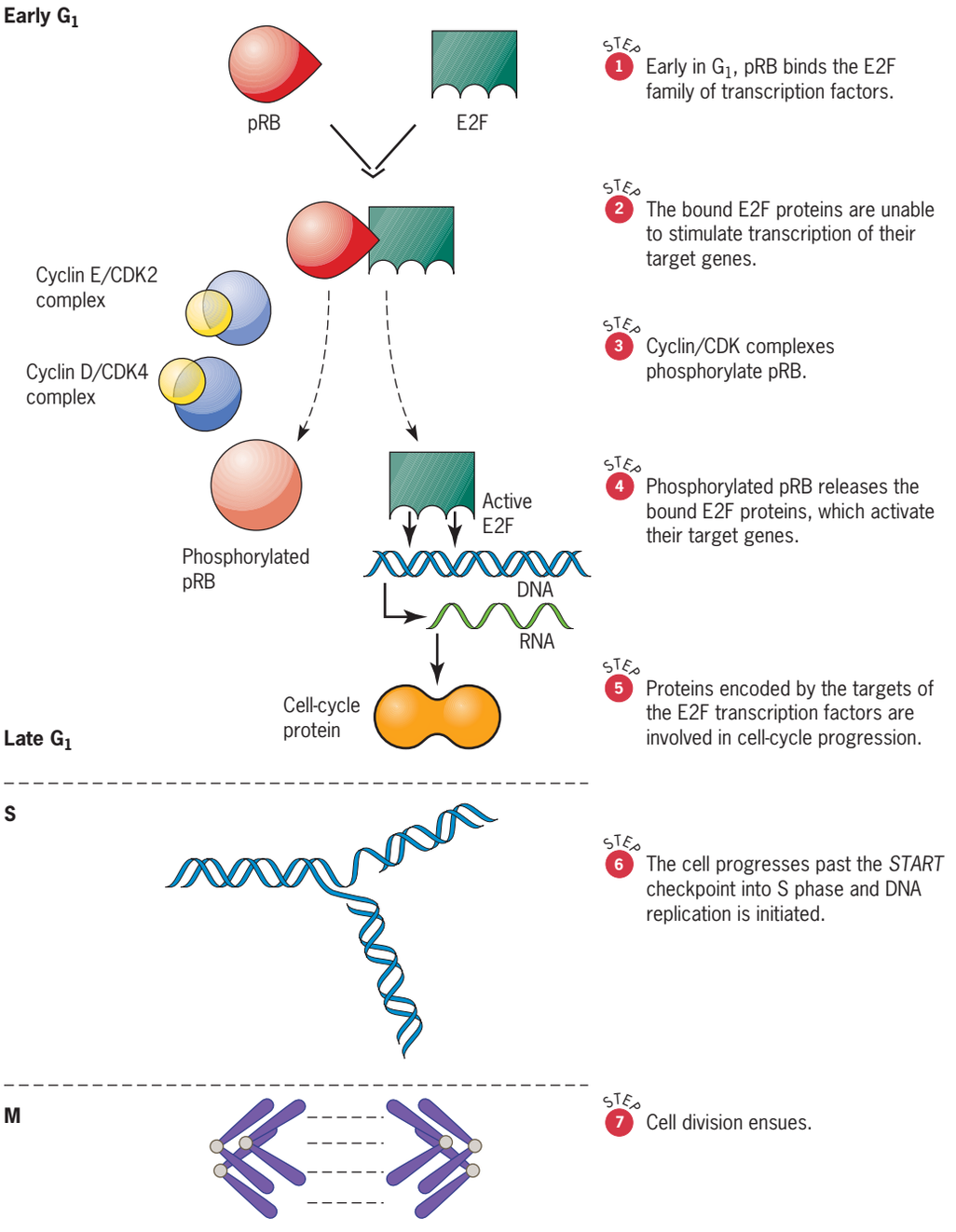
CELLULAR ROLES OF TUMOR SUPPRESSOR PROTEINS

Only about 1 percent of all cancers are hereditary. However, more than 20 different inherited cancer syndromes have been identified, and in nearly all of them the underlying defect is in a tumor suppressor gene rather than in an oncogene. The proteins encoded by these tumor suppressor genes function in a diverse array of cellular processes, including division, differentiation, programmed cell death, and DNA repair. In the following sections, we discuss some of the tumor suppressor proteins that have been studied intensively.

pRB

Recent research has revealed that the RB tumor suppressor protein plays a key role in regulation of the cell cycle. Although the *RB* gene was discovered through its association with retinoblastoma, mutations in this gene are also associated with other types of cancer, including small-cell lung carcinomas, osteosarcomas, and bladder, cervical, and prostate carcinomas. Furthermore, mice that are homozygous for an *RB* knockout mutation die during embryonic development. Thus, the RB gene product is essential for life.

The RB gene product, symbolized pRB, is a 105-kilodalton nuclear protein that is involved in cell-cycle regulation. Two genes homologous to *RB* have been found in mammalian genomes, and their protein products, p107 and p130 (each named for its mass in kilodaltons), may also play key roles in cell-cycle regulation. No human tumors are known to have inactivating mutations in either of these two genes, and mice homozygous for a knockout mutation in either of them do not show abnormal phenotypes. However, mice that are homozygous for knockout mutations in both of these genes die shortly after birth. Thus, together the p107 and p130 members of the RB family of proteins are involved in important cellular processes.



■ **FIGURE 21.7** Role of pRB in progression of the cell cycle. Through its negative interaction with E2F transcription factors, pRB stalls the cell cycle in the G_1 phase. Phosphorylation of pRB by the cyclin/CDK complexes frees E2F proteins to activate their target genes, which encode proteins that are instrumental in moving the cell past the *START* checkpoint into the S phase.

Molecular and biochemical analyses have elucidated the role of pRB in cell-cycle regulation (■ **Figure 21.7**). Early in the G_1 phase of the cell cycle, pRB binds to the E2F proteins, a family of transcription factors that control the expression of several genes whose products move the cell through its cycle. When E2F transcription factors are bound to pRB, they cannot bind to specific enhancer sequences in their target genes. Consequently, the cell-cycle factors encoded by these genes are not produced, and the machinery for DNA synthesis and cell division remains quiescent. Later in G_1, pRB is phosphorylated through the action of cyclin-dependent kinases. In this changed state, pRB releases the E2F transcription factors that have bound to it. These released transcription factors are then free to activate their target genes, which encode proteins that induce the cell to progress through S phase and into mitosis. After mitosis, pRB is dephosphorylated, and each of the daughter cells enters the quiescent phase of a new cell cycle.

This orderly and rhythmic progression through the cell cycle is disrupted in cancer cells. In many types of cancer—not just retinoblastoma—both copies of the *RB* gene have been inactivated, either by deletions or by mutations that impair or abolish the ability of the RB protein to bind E2F transcription factors. The inability of pRB to bind to these transcription factors leaves them free to activate their target genes, thereby setting in motion the machinery for DNA synthesis and cell division. In effect, one of the natural brakes on the process of cell division has been released. In the absence of this brake, cells have a tendency to move through their cycle quickly. If other cell-cycle brakes fail, the cells divide ceaselessly to form tumors.

p53

The 53-kilodalton tumor suppressor protein p53 was discovered through its role in the induction of cancers by certain DNA viruses. This protein is encoded by a tumor suppressor gene called *TP53*. Inherited mutations in *TP53* are associated with the Li-Fraumeni syndrome, a rare dominant condition in which any of several different types of cancer may develop. Somatic mutations that inactivate both copies of the *TP53* gene are also associated with a variety of cancers. In fact, such mutations are found in a majority of all human tumors. Loss of p53 function is therefore a key step in carcinogenesis.

The p53 protein is a 393-amino-acid-long transcription factor that consists of three distinct domains: an N-terminal transcription-activation domain (TAD), a central DNA-binding core domain (DBD), and a C-terminal homo-oligomerization domain (OD) (■ **Figure 21.8*a***). Most of the mutations that inactivate p53 are located in the DBD. These mutations evidently impair or abolish the ability of p53 to bind to specific DNA sequences that are embedded in its target genes, thereby preventing the transcriptional activation of these genes. Thus, mutations in the DBD are typically recessive loss-of-function mutations. Other types of mutations are found in the OD portion of the polypeptide. Molecules of p53 with these types of mutations dimerize with wild-type p53 polypeptides and prevent the wild-type polypeptides from functioning as transcriptional activators. Thus, mutations in the OD have a *dominant negative* effect on p53 function.

The p53 protein plays a key role in cellular responses to stress (■ **Figure 21.8*b***). In normal cells the level of p53 is low, but when the cells are treated with a DNA-damaging agent such as radiation, the level of p53 increases dramatically. This response to DNA damage is mediated by a pathway that decreases the degradation of p53. In response to DNA damage, p53 is phosphorylated, converting it into a stable and active form. Once activated, p53 either stimulates the transcription of genes whose products arrest the cell cycle, thereby allowing the damaged DNA to be repaired, or it activates another set of genes whose products ultimately cause the damaged cell to die.

One prominent factor in the response that arrests the cell cycle is p21, a protein encoded by a gene that is activated by the p53 transcription factor. The p21 protein is an inhibitor of cyclin/CDK protein complexes. When p21 is synthesized in response to cell stress, the cyclin/CDK complexes are inactivated and the cell cycle is arrested. During this timeout, the cell's damaged DNA can be repaired. Thus, p53 is responsible for activating a brake on the cell cycle, and this brake allows the cell to maintain its genetic integrity. Cells that lack functional p53 have difficulty applying this brake. If these cells progress through the cell cycle and proceed into subsequent divisions, additional mutations that cause them to be unregulated may accumulate. Mutational inactivation of p53 is therefore often a key step in the pathway to cancer. Solve It: Downstream of p53 challenges you to consider what might happen if p21 were inactivated by mutations.

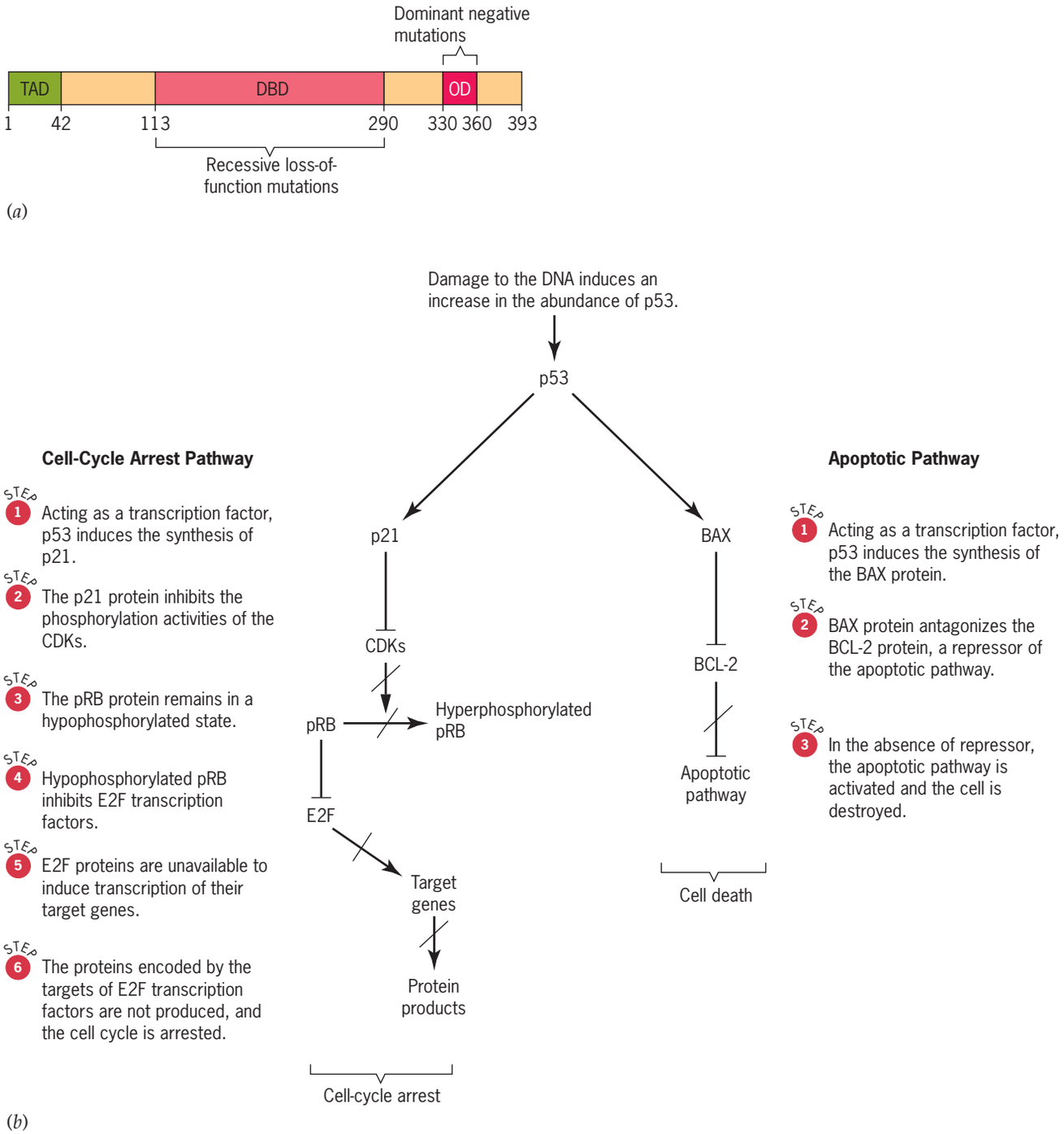
The p53 protein can also mediate another response to cell stress. Instead of orchestrating efforts to repair damage within a cell, p53 may trigger a suicidal response in which the damaged cell is programmed for destruction. The way in which p53 programs cell death is not well understood. One mechanism seems to involve the protein product of the *BAX* gene. The BAX protein is an antagonist of another protein called BCL-2, which normally suppresses the apoptotic, or cell-death, pathway. When the *BAX* gene is activated by p53, its protein product releases the BCL-2 protein from its

Solve It!

Downstream of p53

The p53 protein controls two pathways that respond to damage in a cell's DNA. One pathway arrests the cell cycle to permit repair of the damaged DNA. This pathway is triggered when p53 activates the gene for p21, a protein that inhibits the phosphorylation activities of the cyclin-dependent kinases (CDKs). Would this pathway operate in a cell that has loss-of-function mutations in both of its *p21* genes? Explain your answer. Would you classify the *p21* gene as a tumor suppressor gene?

▶ *To see a solution to this problem, visit the Student Companion site.*



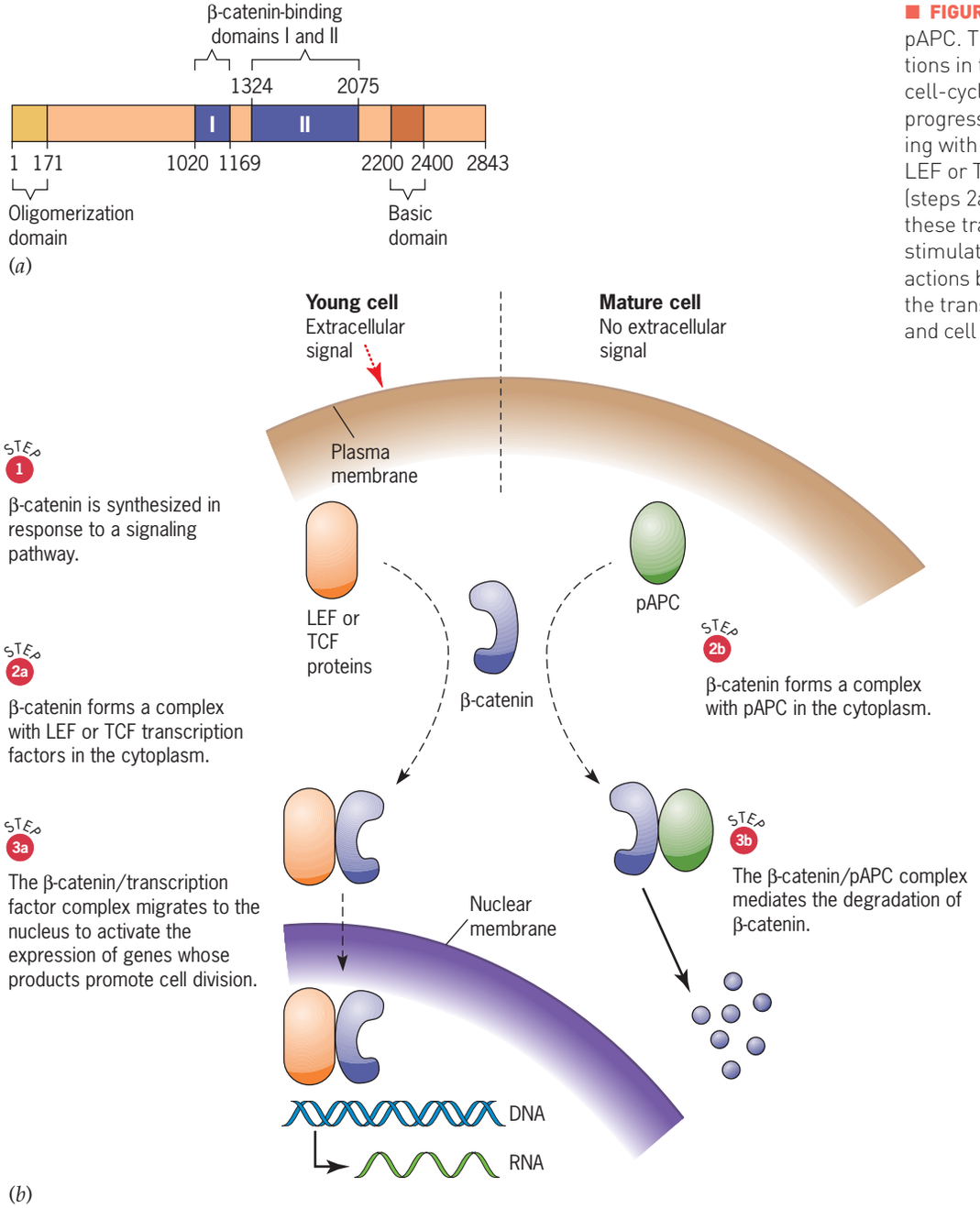
■ **FIGURE 21.8** (*a*) Principal domains within p53. TAD = transcription-activation domain; DBD = DNA-binding domain; OD = oligomerization domain. The numbers refer to amino acid positions in the polypeptide. (*b*) Role of p53 in the cellular response to DNA damage. Two response pathways have been identified. Within each pathway, a pointed arrow indicates a positive influence or a directional change (e.g., a protein is synthesized or phosphorylated, a protein catalyzes a reaction, or a gene is expressed), and a blunted arrow indicates a negative influence (e.g., repression of protein synthesis or protein activity, or repression of a pathway). A slash through an arrow indicates that the influence—positive or negative—is blocked.

suppressing mode. This release then opens the apoptotic pathway, and the cell proceeds to its own destruction.

Curiously, the p53 protein does not seem to play a significant role in the programmed cell death that occurs during embryogenesis. Mice that are homozygous for knockout mutations in *TP53* develop normally, although they have a tendency to develop tumors as they age. Thus, despite its pivotal role in regulating cellular responses to stress, p53 does not seem to influence the course of embryonic development.

pAPC

The 310-kilodalton pAPC protein was discovered through the study of *adenomatous polyposis coli*, an inherited condition that often leads to colorectal cancer. This large protein, 2843 amino acids long (■ **Figure 21.9a**), plays a key role in regulating the renewal of cells in the lining, or epithelium, of the large intestine. Although



■ **FIGURE 21.9** (*a*) Principal domains within pAPC. The numbers refer to amino acid positions in the polypeptide. (*b*) Role of pAPC in cell-cycle control. The pAPC protein influences progression through the cell cycle by interacting with β-catenin, a protein that can activate LEF or TCF transcription factors. In young cells (steps 2a, 3a), an extracellular signal activates these transcription factors and cell division is stimulated. In mature cells (steps 2b, 3b), interactions between pAPC and β-catenin prevent the transcription factors from being activated and cell division is inhibited.

the mechanisms that regulate this process are not fully understood, current information suggests that pAPC controls the proliferation and differentiation of cells in the epithelium of the intestine. When pAPC function is lost, the cells that generate the fingerlike projections on the intestinal epithelium remain in an undifferentiated state. As these cells continue to divide, they produce more of their own kind, and the resulting increase in cell number causes many small, benign tumors to form in the intestinal epithelium. These tumors are called *polyps* or *adenomas*, and the predisposition to form them is inherited as a rare autosomal dominant condition called *familial adenomatous polyposis (FAP)*. In Western countries, its population frequency is about 1 in 7000.

Patients with FAP develop multiple adenomas during their teens and early twenties. Although the adenomas are initially benign, there is a high probability that at least one of them will become a malignant tumor. Thus, at a relatively early age—in the United States, the median is 42—carriers of an FAP mutation develop full-fledged colorectal cancer.

Multiple adenomas develop in the intestines of people who are heterozygous for an FAP mutation because the wild-type *APC* allele they carry mutates multiple times during the natural regeneration of the intestinal epithelium. When such mutations occur, the cells lose their ability to synthesize functional pAPC protein. The absence of this protein releases an important brake on cell proliferation, and cell division proceeds unchecked. Thus, the formation of numerous benign tumors in the intestines of FAP heterozygotes results from the independent occurrence of second mutational "hits" in the cells of the intestinal epithelium. Individuals who do not carry an FAP mutation seldom form multiple adenomas. However, they may produce one or a few adenomas if by chance both of their *APC* genes are inactivated by somatic mutations.

The pAPC protein appears to regulate cell division through its ability to bind β-catenin, a protein that is present inside cells. β-catenin naturally binds to other proteins as well, including certain transcription factors that stimulate the expression of genes whose protein products promote cell division. The interactions with these transcription factors are favored when signals impinging on the cell surface cue the cell to divide (■ **Figure 21.9*b***). Signal-induced cell proliferation is a necessary process in the intestinal epithelium because this tissue loses an enormous number of cells every day—in humans, about 10^{11}—and the lost cells must be replaced by fresh cells generated by division. Normally, the newly created cells lose their ability to divide as they move away from the generative part of the epithelium and assume their roles in the mature part of the epithelium. This shift from a dividing to a nondividing state occurs because the mature epithelial cells do not receive the extracellular signals that stimulate cells to divide. In the absence of these signals, pAPC forms a complex with the β-catenin in the cells' cytoplasm, and the complexed β-catenin is targeted for degradation. Because pAPC keeps β-catenin levels low in the mature cells of the intestinal epithelium, there is little chance for β-catenin to combine with and activate the transcription factors that stimulate cell division. Cells with mutations in pAPC lose their ability to control β-catenin levels. Without this control, they retain their vigor for division and fail to differentiate properly into mature epithelial cells. The result is that a tumor begins to form in the intestinal lining. Thus, normal pAPC molecules play an important role in suppressing tumor formation in the intestine.

phMSH2

The phMSH2 protein is the human homologue of a DNA repair protein called MutS found in bacteria and yeast. Its involvement in human cancer was elucidated through the study of *hereditary nonpolyposis colorectal cancer (HNPCC)*, a dominant autosomal condition with a population frequency of about 1 in 500. Unlike FAP, HNPCC is characterized by the occurrence of a small number of adenomas, one of which eventually progresses to a cancerous condition. In the United States, the median age at

which the cancer occurs is 42, the same age at which malignant cancer occurs in FAP patients.

The *hMSH2* gene was implicated in the inheritance of HNPCC after researchers found that cells in HNPCC tumors suffer from a general genetic instability. In these cells, di- and trinucleotide microsatellite repeat sequences (see Chapter 13) throughout the genome exhibit frequent changes in length. This instability is reminiscent of the types of DNA sequence changes observed in bacteria with mutations in the genes that control DNA mismatch repair (see Chapter 13). The human homologue of one of these bacterial genes maps to the short arm of chromosome 2, a chromosome that had previously been implicated in HNPCC by linkage analysis. Sequence analysis of this gene—denoted *hMSH2*—indicated that it was inactivated in tumors removed from some HNPCC patients. Thus, loss of *hMSH2* function was causally connected to the genome-wide instability observed in HNPCC tumors. Subsequent analysis has demonstrated that germ-line mutations in *hMSH2*, or in three other human homologues of bacterial mismatch repair genes, account for the inherited cases of HNPCC.

pBRCA1 AND pBRCA2

Mutant versions of the tumor suppressor genes *BRCA1* and *BRCA2* genes have been implicated in hereditary breast and ovarian cancer. *BRCA1* was mapped to chromosome 17 in 1990 and isolated in 1994 (see A Milestone in Genetics: The Identification of the *BRCA1* Gene on the Student Companion site), and *BRCA2* was mapped to chromosome 13 in 1994 and isolated in 1995. Both genes encode large proteins; pBRCA1 is a 220-kilodalton polypeptide, and pBRCA2 is a 384-kilodalton polypeptide. Cellular and biochemical studies have shown that each of these proteins is located within the nuclei of normal cells and that each contains a transcriptional activation domain. The pBRCA1 and pBRCA2 proteins also contain a domain that allows them to interact physically with other proteins, in particular with pRAD51, a eukaryotic homologue of the bacterial DNA repair protein known as RecA. Thus, pBRCA1 and pBRCA2 likely participate in one of the many systems that repair damaged DNA in human cells.

Both pBRCA1 and pBRCA2 carry out important functions within cells. Mice that are homozygous for a knockout mutation in either gene die early during embryogenesis. In the etiology of human cancers, mutant pBRCA1 and pBRCA2 proteins appear to compromise a cell's ability to detect or repair damaged DNA.

Mutations in the *BRCA1* and *BRCA2* genes account for about 7 percent of all cases of breast cancer and about 10 percent of all cases of ovarian cancer in the United States. For each gene, the predisposition to develop these cancers is inherited as a dominant allele with high penetrance. Carriers have a 10- to 25-fold greater risk than noncarriers of developing breast or ovarian cancer, and in some families, the risk of developing colon or prostate cancer is also increased. Because many different inactivating mutations in *BRCA1* and *BRCA2* are found in the human population, genetic counseling for families that are segregating these mutations can be difficult (see the Focus on Cancer and Genetic Counseling).

KEY POINTS

- *Tumor suppressor genes were discovered through their association with rare, inherited cancers such as retinoblastoma.*
- *Mutational inactivation of various tumor suppressor genes is characteristic of most forms of cancer.*
- *Two mutational hits are required to eliminate both functional copies of a tumor suppressor gene within a cell.*
- *The proteins encoded by tumor suppressor genes play key roles in regulating the cell cycle.*

FOCUS ON

CANCER AND GENETIC COUNSELING[1]

The identification of inherited mutations in tumor suppressor genes has opened a new era in genetic counseling. The carriers of such mutations are often at high risk to develop potentially life-threatening tumors, sometimes at a relatively early age. If molecular tests reveal that an individual carries a mutant tumor suppressor gene, medical treatment can be given to reduce the chance that he or she will develop a lethal cancer. For example, a child who carries a mutation in the *APC* gene could be checked periodically by endoscopy and suspicious lesions in the intestine could be removed, or a woman who carries a mutation in either of the *BRCA* genes could undergo a prophylactic mastectomy (removal of the breasts) or oophorectomy (removal of the ovaries).

A negative result from a test for a mutant tumor suppressor gene would, of course, be a cause for celebration—at least to the extent that the test can be trusted. For a large gene with many different mutant alleles segregating in the population, it is difficult to design a cost-effective test to detect mutations located anywhere in the gene. Typically, these tests are based on the polymerase chain reaction, and most of them are designed to detect specific mutant alleles. An individual who is at risk to carry a mutant tumor suppressor gene can be tested for the known mutations—at least the most frequent ones. However, a negative result is not definitive because that individual could carry a *"private"* mutation—that is, one that has not previously been identified in the population.

The existence of private alleles makes counseling for inherited cancers difficult. For example, over 300 different mutations have been identified in the *BRCA1* gene, and about 50 percent of them are private. If an individual with a family history of breast cancer comes to a genetic counselor for evaluation, which mutations should the counselor look for? Sometimes data from other family members or information collected from the individual's ethnic group can provide clues. If other individuals in the family have been found to carry a particular mutant allele, then the counselor should test for that allele. If certain mutant alleles are characteristic of the individual's ethnic group, then the counselor should test for them. In Ashkenazi Jewish populations, for example, some *BRCA1* and *BRCA2* mutant alleles have frequencies as high as 2.5 percent. By comparison, the combined frequency of all mutant alleles in non-Jewish Caucasian populations is only 0.1 percent. Thus, an Ashkenazi Jew at risk for inherited breast or ovarian cancer should be tested for the mutant alleles that are likely to be segregating in Ashkenazi Jewish families.

Genetic testing for mutant tumor suppressor genes raises a host of psychological issues. In cases where therapeutic medical treatment is not available, an individual might choose not to be tested because the psychological burden of living with the knowledge that one carries a potentially lethal mutant gene could be overwhelming. Knowledge that one is a carrier might be expected to influence career plans and decisions about marriage and child-bearing. The prospect of an early death might dissuade an individual from seeking permanent commitments—to a spouse, to children, or to a vocation—and the chance of transmitting a mutant allele to children might deter the individual from reproducing. Knowledge that one is a carrier can also influence other people—family members, friends, and coworkers. A young daughter whose mother has tested positively for a *BRCA1* mutation must herself begin to grapple with the prospect of being a carrier, and a husband whose wife carries a *BRCA1* mutation must share in the decision of whether or not she should undergo a prophylactic oophorectomy and preclude the couple from ever having children of their own.

Testing for mutant tumor suppressor genes raises many ethical issues. To whom should the test results be revealed? the patient? the patient's family? parents? children? employer? landlord? insurance agent? What measures should society take to safeguard the privacy of genetic test results? What policies should governments adopt to protect individuals from discrimination on the basis of their genotypes? How should insurance and employment policies be modified? Should the reproductive rights of individuals who carry harmful mutations be limited? As with any technological advance, the ability to detect mutations in tumor suppressor genes leaves us with many questions about how we should proceed. Currently, the answers to these questions are far from clear.

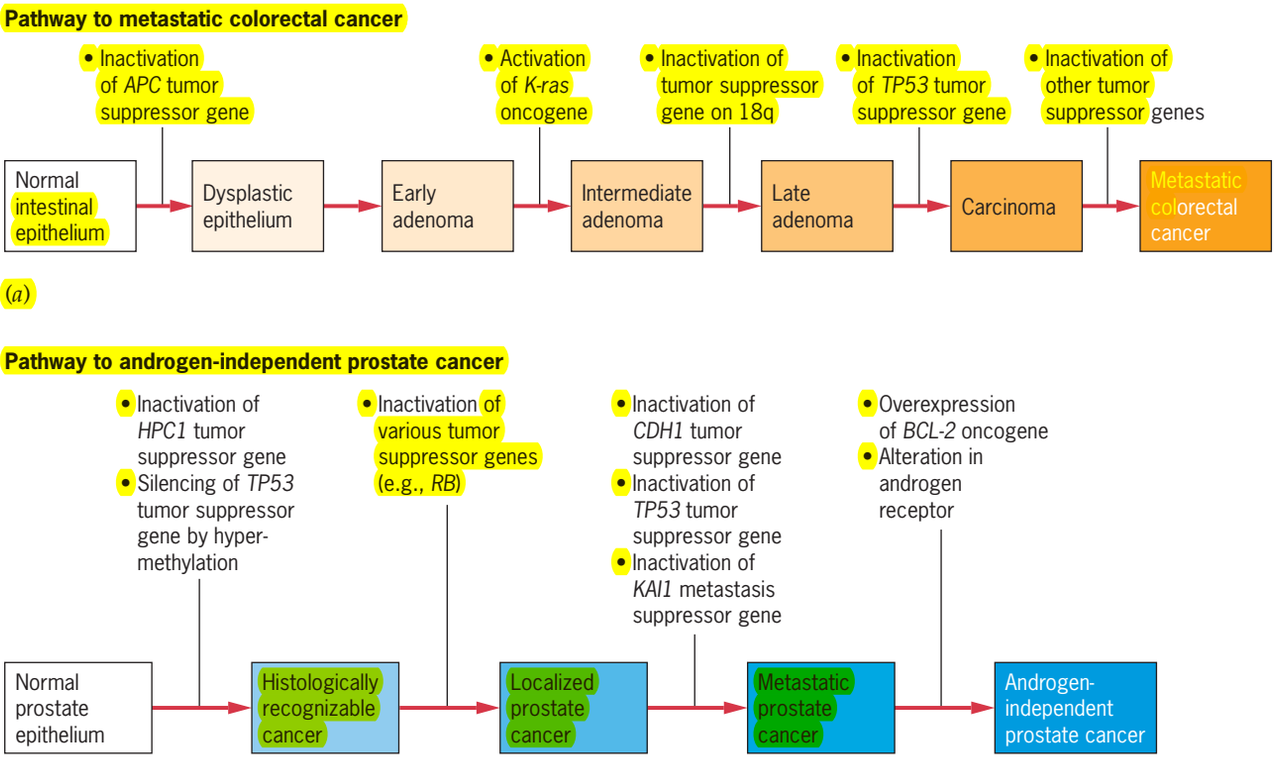
[1]Ponder, Bruce. 1997. Genetic testing for cancer risk. *Science* 278: 1050–1054.

Genetic Pathways to Cancer

Cancers develop through an accumulation of somatic mutations in proto-oncogenes and tumor suppressor genes.

In most cancer cases, the formation of a malignant tumor is not attributable to the uncontrolled activation of a single proto-oncogene or to the inactivation of a single tumor suppressor gene. Rather, tumor formation, growth, and metastasis usually depend on the accumulation of mutations in several different genes. Thus, the genetic pathways to cancer are diverse and complex.

We can see this diversity and complexity in the formation and development of different types of tumors. For example, benign tumors of the large intestine develop in individuals with inactivating mutations in the *APC* gene. However, the progression of these tumors to potentially lethal cancers requires mutations in several other genes. This mutational pathway is summarized in ■ **Figure 21.10*a***. Inactivating mutations



■ **FIGURE 21.10** Genetic pathways to cancer.

in the *APC* gene initiate the process of tumor formation by causing the development of abnormal tissues within the intestinal epithelium. These abnormal tissues contain dysplastic cells—cells with unusual shapes and enlarged nuclei—that may grow into early-stage adenomas. If the *K-ras* proto-oncogene is activated in one of these adenomas, that adenoma may grow and develop more fully. Inactivating mutations in any of several tumor suppressor genes located in the long arm of chromosome 18 may then induce the adenoma to progress further, and inactivating mutations in the *TP53* tumor suppressor gene on chromosome 17 may transform it into a vigorously growing carcinoma. Additional tumor suppressor gene mutations may allow carcinoma cells to break away and invade other tissues. Thus, no less than seven independent mutations (two inactivating hits in the *APC* gene, one activating mutation in the *K-ras* gene, two inactivating hits in a tumor suppressor gene on chromosome 18, and two inactivating hits in the *TP53* gene) are required for the development of an intestinal carcinoma, and still more mutations are probably required for the metastasis of that carcinoma to other parts of the body.

The genetic pathways to prostate cancer has also been elucidated (■ **Figure 21.10*b***). Mutations in *HPC1*, a gene for hereditary prostate cancer located in the long arm of chromosome 1, have been implicated in the origin of prostate tumors. Mutations in other tumor suppressor genes located in chromosomes 13, 16, 17, and 18 can transform prostate tumors into metastatic cancers, and overexpression of the *BCL-2* proto-oncogene gene can make these cancers immune to androgen deprivation therapy, a standard technique for the treatment of prostate cancer. The steroid hormone androgen is required for the proliferation of cells in the prostate epithelium. In the absence of androgen, these cells are programmed to die. However, prostate tumor cells may acquire the ability to survive in the absence of androgen, probably because an excess of the *BCL-2* gene product represses the programmed cell death pathway. Prostate cancers that have progressed to the stage of androgen independence are almost always fatal.

Douglas Hanahan and Robert Weinberg have proposed six hallmarks of the pathways leading to malignant cancer:

1. *Cancer cells acquire self-sufficiency in the signalling processes that stimulate division and growth*. This self-sufficiency may arise from changes in the extracellular factors that cue cells to divide, or from changes in any part of the system that transduces these cues or translates their instructions into action inside the cell. In the most extreme case, self-sufficiency occurs when cells respond to growth factors that they themselves produce, thereby creating a positive feedback loop that stimulates ceaseless cell division.
2. *Cancer cells are abnormally insensitive to signals that inhibit growth*. Cell division is stimulated by a variety of biochemical signals; however, other signals inhibit cell division. In normal cells, these countervailing factors balance each other with the result that growth occurs in a regulated manner. In cancer cells, growth is unregulated because the stimulatory signals have the upper hand. During the progression to malignancy, cancer cells lose their ability to respond appropriately to signals that inhibit growth. For example, cells in intestinal adenomas often no longer respond to TGFβ, a protein that instructs pRB to block progression through the cell cycle. When this block fails, the cells advance from G_1 into S, replicate their DNA, and divide. These cells are then on their way to forming a malignant tumor.
3. *Cancer cells can evade programmed cell death*. As we have seen, p53 plays a key role in protecting an organism from the accumulation of damaged cells that could endanger its life. Through mechanisms that are still incompletely understood, p53 sends damaged cells into an autodestruct pathway that clears them from the organism. When p53 malfunctions, this autodestruct pathway is blocked, and the damaged cells survive and multiply. Such cells are likely to produce descendants that are even more abnormal than they are. Consequently, lineages derived from damaged cells are prone to advance to a cancerous state. The ability to evade programmed cell death is therefore a key characteristic in the progression to malignant cancer.
4. *Cancer cells acquire limitless replicative potential*. Normal cells are able to divide around 60 to 70 times. This limitation arises from the minute, but inexorable, loss of DNA from the ends of chromosomes every time the DNA is replicated (Chapter 10). The cumulative effect of this loss enforces a finite reproductive ability on every cell lineage. Cells that go past the reproductive limit become genetically unstable and die. Cancer cells manage to transcend this limit by replenishing their lost DNA. They do so by increasing the activity of the enzyme telomerase, which adds DNA sequences to the ends of chromosomes. When cells have acquired limitless replicative potential by overcoming the loss of DNA at the ends of chromosomes, they are said to be *immortalized.*
5. *Cancer cells develop ways to nourish themselves*. Any tissue in a complex, multicellular organism needs a vascular system to bring nutrients to it. In humans and other vertebrate animals, the circulatory system provides this function. The cells in premalignant tumors fail to grow aggressively because they are not directly fed by the circulatory system. However, when blood vessels are induced to grow among these cells—through a process called *angiogenesis*—the tumor is nourished and can then expand. Thus, a key step in the progression to malignant cancer is the induction of blood vessel growth by the cells of the tumor. Many factors that induce or inhibit angiogenesis are known. In normal tissues, these factors are kept in balance so that blood vessels grow appropriately in the body; in cancerous tissues, the balance is tipped in favor of the inducing factors, which act to stimulate blood vessel development. Once capillaries have grown into a tumor, a reliable means of nourishment is at hand. The tumor can then feed itself and grow to a size where it becomes a danger to the organism.
6. *Cancer cells acquire the ability to invade other tissues and colonize them*. More than 90 percent of all cancer deaths are caused by metastasis of the cancer to other parts of the body. When tumors metastasize, the cancer cells detach from the primary tumor and travel through the bloodstream to another location, where they estab-

lish a new, lasting, and, in the end, lethal, relationship with the surrounding cells. Profound changes must take place on the surfaces of the cancer cells for this process to occur. When it does, secondary tumors may develop in tissues far removed from the primary tumor. Cancers that have spread in this fashion are extremely difficult to control and eradicate. Metastasis is therefore the most serious occurrence in the progression of a cancer.

Numerous studies have established that somatic mutation is the basis for the development and progression of all types of cancer. As a cancer progresses on the pathway to malignancy, its cells become increasingly unregulated. Mutations accumulate, and whole chromosomes or chromosome segments may be lost. This genetic instability increases the likelihood that the cancer will develop each of the hallmarks discussed above.

Because of the importance of somatic mutations in the etiology of cancer, factors that increase the mutation rate are bound to increase the incidence of cancer. Today many countries maintain research programs to identify mutagenic and carcinogenic agents (see Chapter 13 for a discussion of the Ames test to identify chemical mutagens). When such agents are identified, public health authorities devise policies to minimize human exposure to them. However, no environment is carcinogen-free, and human behaviors that contribute to the risk of cancer such as smoking, excessive exposure to sunlight, and consumption of fatty foods that contain little fiber are difficult to change. Understanding of the processes that cause cancer has advanced significantly. In the future, we can expect this understanding to lead to more effective strategies for cancer prevention and treatment.

KEY POINTS

- *Different types of cancer are associated with mutations in different genes.*
- *Cancer cells may stimulate their own growth and division.*
- *Cancer cells do not respond to factors that inhibit cell growth.*
- *Cancer cells can evade the natural mechanisms that kill abnormal cells.*
- *Immortalized cancer cells can divide endlessly.*
- *Tumors can expand when they induce the in-growth of blood vessels to nourish their cells.*
- *Metastatic cancer cells can invade other tissues and colonize them.*

Basic Exercises

Illustrate Basic Genetic Analysis

1. Which cell-cycle checkpoint prevents a cell from replicating damaged DNA?

Answer: The *START* checkpoint in mid-G_1 of the cell cycle.

2. (a) In which class of genes do dominant gain-of-function mutations cause cancer? (b) In which class of genes do recessive loss-of-function mutations cause cancer?

Answer: (a) Oncogenes. (b) Tumor suppressor genes.

3. Why do some chromosomal rearrangements lead to cancer?

Answer: The breakpoints of these rearrangements often juxtapose a cellular oncogene to a promoter that stimulates the vigorous expression of the oncogene. Overexpression of the gene product can lead to excessive cell division and growth.

4. Intestinal cancer occurs in individuals with inactivating mutations in the *APC* gene. Explain how it might also occur in individuals with mutations in the β-catenin gene.

Answer: A mutation that specifically prevented β-catenin from binding to pAPC might lead to cancer. β-catenin that cannot bind to pAPC would be available to bind to the transcription factors that stimulate the expression of genes whose products promote cell division and growth.

5. Which tumor suppressor gene is most frequently mutated in human cancers?

Answer: *TP53*, the gene that encodes p53.

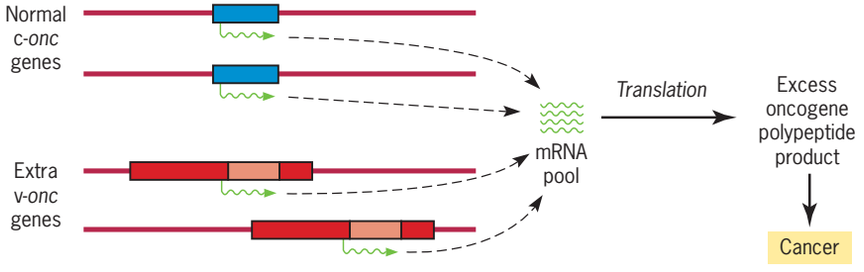
Testing Your Knowledge

Integrate Different Concepts and Techniques

1. An oncogene within the genome of a retrovirus has a high probability of causing cancer, but an oncogene in its normal chromosomal position does not. If these two oncogenes encode exactly the same polypeptide, how can we explain their different properties?

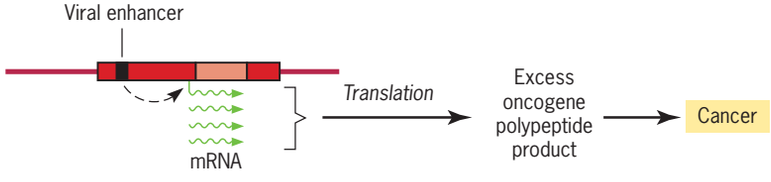
Answer: There are at least three possibilities. One (*a*) is that the virus simply adds extra copies of the oncogene to the cell and that collectively these produce too much of the polypeptide. An excess of polypeptide might cause uncontrolled cell division; that is, cancer. Another possibility (*b*) is that the viral oncogene is expressed inappropriately under the control of enhancers in the viral DNA. These enhancers might trigger the oncogene to be expressed at the wrong time or to be overexpressed constitutively. In either case, the polypeptide would be inappropriately produced and might thereby upset the normal controls on cell division. A third possibility (*c*) is that integration of the virus into the chromosomes of the infected cell might put the viral oncogene in the vicinity of an enhancer in the chromosomal DNA and that this enhancer might elicit inappropriate expression. All three explanations stress the idea that the expression of an oncogene must be correctly regulated. Misexpression or overexpression could lead to uncontrolled cell division.

The virus adds extra copies of the oncogene to the cell



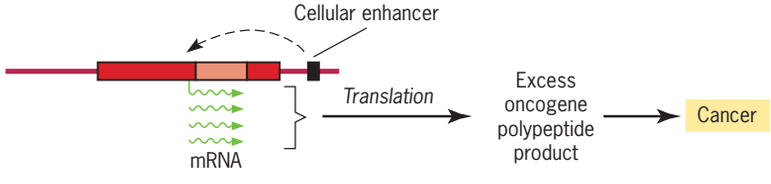
(a)

The viral oncogene is expressed inappropriately under the control of a viral enhancer



(b)

The viral oncogene is expressed inappropriately under the control of a cellular enhancer



(c)

Questions and Problems

Enhance Understanding and Develop Analytical Skills

21.1 Many cancers seem to involve environmental factors. Why, then, is cancer called a genetic disease?

21.2 Both embryonic cells and cancer cells divide quickly. How can these two types of cells be distinguished from each other?

21.3 Most cancer cells are aneuploid. Suggest how aneuploidy might contribute to deregulation of the cell cycle.

21.4 Would you ever expect to find a tumor-inducing retrovirus that carried a processed cellular tumor suppressor gene in its genome?

21.5 How do we know that normal cellular oncogenes are not simply integrated retroviral oncogenes that have acquired the proper regulation?

21.6 How might the absence of introns in a retroviral oncogene explain that gene's overexpression in the tissues of an infected animal?

21.7 When cellular oncogenes are isolated from different animals and compared, the amino acid sequences of the polypeptides they encode are found to be very similar. What does this suggest about the functions of these polypeptides?

21.8 The majority of the *c-ras* oncogenes obtained from cancerous tissues have mutations in codon 12, 59, or 61 in the coding sequence. Suggest an explanation.

21.9 When a mutant *c-H-ras* oncogene with a valine for glycine substitution in codon 12 is transfected into cultured NIH 3T3 cells, it transforms those cells into cancer cells. When the same mutant oncogene is transfected into cultured embryonic cells, it does not transform them. Why?

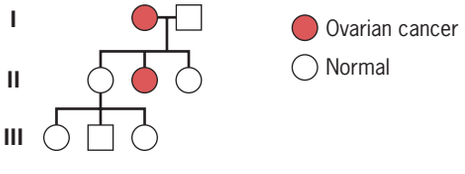
21.10 GO A mutation in the *ras* cellular oncogene can cause cancer when it is in heterozygous condition, but a mutation in the *RB* tumor suppressor gene can cause cancer only when it is in homozygous condition. What does this difference between dominant and recessive mutations imply about the roles that the *ras* and *RB* gene products play in normal cellular activities?

21.11 Explain why individuals who develop nonhereditary retinoblastoma usually have tumors in only one eye, whereas individuals with hereditary retinoblastoma usually develop tumors in both eyes.

21.12 Approximately 5 percent of the individuals who inherit an inactivated *RB* gene do not develop retinoblastoma. Use this statistic to estimate the number of cell divisions that form the retinal tissues of the eye. Assume that the rate at which somatic mutations inactivate the *RB* gene is 1 mutation per 10^6 cell divisions.

21.13 Inherited cancers like retinoblastoma show a dominant pattern of inheritance. However, the underlying genetic defect is a recessive loss-of-function mutation—often the result of a deletion. How can the dominant pattern of inheritance be reconciled with the recessive nature of the mutation?

21.14 The following pedigree shows the inheritance of familial ovarian cancer caused by a mutation in the *BRCA1* gene. Should II-1 be tested for the presence of the predisposing mutation? Discuss the advantages and disadvantages of testing.



21.15 In what sense is pRB a negative regulator of E2F transcription factors?

21.16 A particular E2F transcription factor recognizes the sequence TTTCGCGC in the promoter of its target gene. A temperature-sensitive mutation in the gene encoding this E2F transcription factor alters the ability of its protein product to activate transcription; at 25°C the mutant protein activates transcription normally, but at 35°C, it fails to activate transcription at all. However, the ability of the protein to recognize its target DNA sequence is not impaired at either temperature. Would cells heterozygous for this temperature-sensitive mutation be expected to divide normally at 25°C? at 35°C? Would your answers change if the E2F protein functions as a homodimer?

21.17 During the cell cycle, the p16 protein is an inhibitor of cyclin/CDK activity. Predict the phenotype of cells homozygous for a loss-of-function mutation in the gene that encodes p16. Would this gene be classified as a proto-oncogene or as a tumor suppressor gene?

21.18 The *BCL-2* gene encodes a protein that represses the pathway for programmed cell death. Predict the phenotype of cells heterozygous for a dominant activating mutation in this gene. Would the *BCL-2* gene be classified as a proto-oncogene or as a tumor suppressor gene?

21.19 The protein product of the *BAX* gene negatively regulates the protein product of the *BCL-2* gene—that is, BAX protein interferes with the function of the BCL-2 protein. Predict the phenotype of cells homozygous for a loss-of-function mutation in the *BAX* gene. Would this gene be classified as a proto-oncogene or as a tumor suppressor gene?

21.20 Cancer cells frequently are homozygous for loss-of-function mutations in the *TP53* gene, and many of these mutations map in the portion of *TP53* that encodes the DNA-binding domain of p53. Explain how these mutations contribute to the cancerous phenotype of the cells.

21.21 Suppose that a cell is heterozygous for a mutation that caused p53 to bind tightly and constitutively to the DNA of its target genes. How would this mutation affect the cell cycle? Would such a cell be expected to be more or less sensitive to the effects of ionizing radiation?

21.22 Mice homozygous for a knockout mutation of the *TP53* gene are viable. Would they be expected to be more or less sensitive to the killing effects of ionizing radiation?

21.23 Would cancer-causing mutations of the *APC* gene be expected to increase or decrease the ability of pAPC to bind β-catenin?

21.24 GO Mice that are heterozygous for a knockout mutation in the *RB* gene develop pituitary and thyroid tumors. Mice that are homozygous for this mutation die during embryonic development. Mice that are homozygous for a knockout mutation in the gene encoding the p130 homologue of RB and heterozygous for a knockout mutation

in the gene encoding the p107 homologue of RB do not have a tendency to develop tumors. However, homozygotes for knockout mutations in both of these genes die during embryonic development. What do these findings suggest about the roles of the *RB*, *p139*, and *p107* genes in embryos and adults?

21.25 It has been demonstrated that individuals with diets poor in fiber and rich in fatty foods have an increased risk to develop colorectal cancer. Fiber-poor, fat-rich diets may irritate the epithelial lining of the large intestine. How could such irritation contribute to the increased risk for colorectal cancer?

21.26 Messenger RNA from the *KAI1* gene is strongly expressed in normal prostate tissues but weakly expressed in cell lines derived from metastatic prostate cancers. What does this finding suggest about the role of the KAI1 gene product in the etiology of prostate cancer?

21.27 The p21 protein is strongly expressed in cells that have been irradiated. Researchers have thought that this strong expression is elicited by transcriptional activation of the *p21* gene by the p53 protein acting as a transcription factor. Does this hypothesis fit with the observation that p21 expression is induced by radiation treatment in mice homozygous for a knockout mutation in the *TP53* gene? Explain.

Genomics on the Web at http://www.ncbi.nlm.nih.gov

The von Hippel-Lindau syndrome is characterized by the occurrence of cancer in the kidney. Often the *VHL* tumor suppressor gene has been mutated in this type of cancer.

1. Search the NCBI databases for information on the *VHL* gene. Where is it located in the genome? How long is its polypeptide product? Are different isoforms of the VHL protein created by alternate splicing?
2. The VHL protein physically interacts with other proteins inside cells. One interactant is the von Hippel-Lindau binding protein, VBP1. Search the databases for the gene encoding this protein. Where is this gene located? How long is the VBP1 polypeptide? How is this polypeptide thought to function inside cells?
3. The VHL protein plays a role in biochemical pathways inside cells. Find the Pathways section on the *VHL* page and click on KEGG pathway: renal cell carcinoma to see where the VHL protein functions. What is its role in renal cells? What proteins does it interact with?
4. Homologues of the *VHL* gene exist in the genomes of the rat and mouse. Use the Map Viewer function under the Homology section on the *VHL* page to locate these homologues. What chromosomes are they on? Is the region around these homologues similar in all three organisms—rat, mouse, and human? What does the structure of this chromosomal region in these three organisms suggest about the evolutionary process?