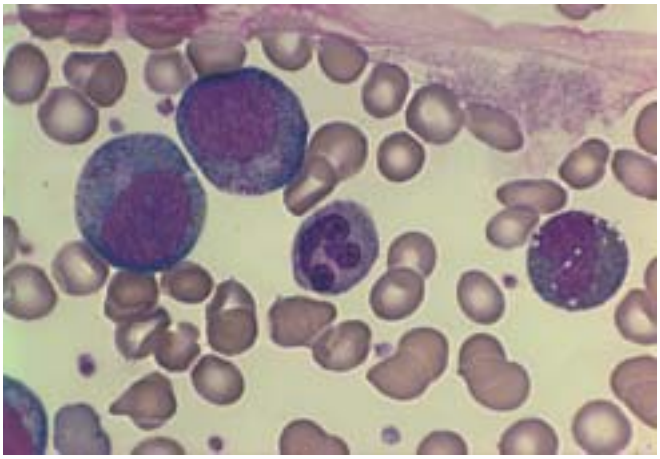
23

CANCER



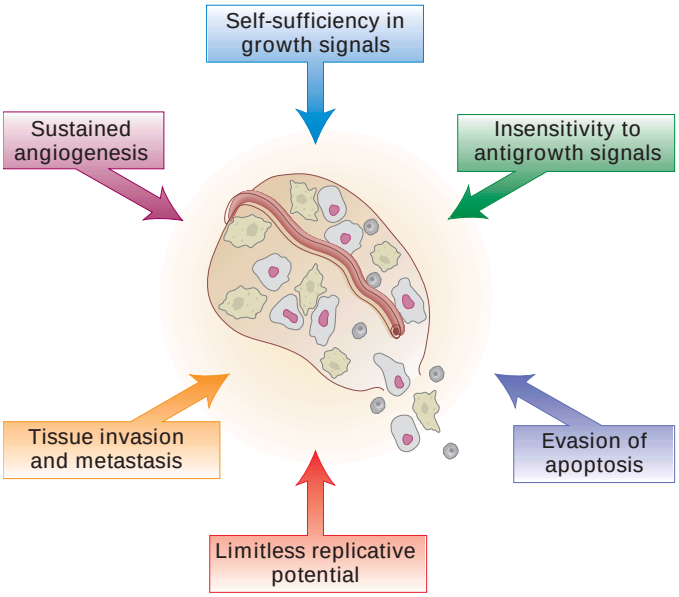
A blood smear from a person with acute myelogenous leukemia. The gigantic cells with irregularly shaped purple nuclei are leukemia cells. The small reddish-gray circular cells are normal red blood cells. [Margaret Cubberly/Phototake.]

Cancer causes about one-fifth of the deaths in the United States each year. Worldwide, between 100 and 350 of each 100,000 people die of cancer each year. Cancer is due to failures of the mechanisms that usually control the growth and proliferation of cells. During normal development and throughout adult life, intricate genetic control systems regulate the balance between cell birth and death in response to growth signals, growth-inhibiting signals, and death signals. Cell birth and death rates determine adult body size, and the rate of growth in reaching that size. In some adult tissues, cell proliferation occurs continuously as a constant tissue-renewal strategy. Intestinal epithelial cells, for instance, live for just a few days before they die and are replaced; certain white blood cells are replaced as rapidly, and skin cells commonly survive for only 2–4 weeks before being shed. The cells in many adult tissues, however, normally do not proliferate except during healing processes. Such stable cells (e.g., hepatocytes, heart muscle cells, neurons) can remain functional for long periods or even the entire lifetime of an organism.

The losses of cellular regulation that give rise to most or all cases of cancer are due to genetic damage (Figure 23-1). Mutations in two broad classes of genes have been implicated in the onset of cancer: **proto-oncogenes** and **tumor-suppressor genes**. Proto-oncogenes are activated to become oncogenes by mutations that cause the gene to be excessively active in growth promotion. Either increased gene expression or production of a hyperactive product will do it. Tumor-suppressor genes normally restrain growth, so damage to them allows inappropriate growth. Many of the genes in both classes encode proteins that help regulate cell birth (i.e., entry into and progression through the cell cycle) or cell death by **apoptosis;** others encode proteins that participate in repairing damaged DNA. Cancer commonly results from mutations that arise during a lifetime's exposure to **carcinogens,** which include certain chemicals and ultraviolet radiation. Cancer-causing mutations occur mostly in somatic cells, not in the germ-line cells, and somatic cell mutations are not passed on to the next generation. In contrast, certain inherited mutations, which are carried in the germ line, increase the probability that cancer will occur at some time. In a destructive partnership, somatic mutations can combine with inherited mutations to cause cancer.

Thus the cancer-forming process, called oncogenesis or tumorigenesis, is an interplay between genetics and the environment. Most cancers arise after genes are altered by carcinogens or by errors in the copying and repair of genes. Even if the genetic damage occurs only in one somatic cell, division of this cell will transmit the damage to the daughter cells, giving rise to a **clone** of altered cells. Rarely, however, does mutation in a single gene lead to the onset of cancer. More typically, a series of mutations in multiple genes creates a progressively more rapidly proliferating cell type that escapes normal growth restraints, creating an opportunity for additional mutations. Eventually the clone of cells grows into a **tumor.** In some cases cells from the primary tumor migrate to new sites (*metastasis*), forming secondary tumors that often have the greatest health impact.

OUTLINE



▲ FIGURE 23-1 **Overview of changes in cells that cause cancer.** During carcinogenesis, six fundamental cellular properties are altered, as shown here, to give rise to the complete, most destructive cancer phenotype. Less dangerous tumors arise when only some of these changes occur. In this chapter we examine the genetic changes that result in these altered cellular properties. [Adapted from D. Hanahan and R. A. Weinberg, 2000, *Cell* **100**:57.]

Metastasis is a complex process with many steps. Invasion of new tissues is nonrandom, depending on the nature of both the metastasizing cell and the invaded tissue. Metastasis is facilitated if the tumor cells produce growth and angiogenesis factors (blood vessel growth inducers). Motile, invasive, aggregating, deformable cells are most dangerous. Tissues under attack are most vulnerable if they produce growth factors and readily grow new vasculature. They are more resistant if they produce anti-proliferative factors, inhibitors of proteolytic enzymes, and anti-angiogenesis factors.

Research on the genetic foundations of a particular type of cancer often begins by identifying one or more genes that are mutationally altered in tumor cells. Subsequently it is important to learn whether an altered gene is a contributing cause for the tumor, or an irrelevant side event. Such investigations usually employ multiple approaches: epidemiological comparisons of the frequency with which the genetic change is associated with a type of tumor, tests of the growth properties of cells in culture that have the particular mutation, and the testing of mouse models of the disease to see if the mutation can be causally implicated. A more sophisticated analysis is possible when the altered gene is known to encode a component of a particular molecular pathway (e.g., an intracellular signaling pathway). In this case it is possible to alter other components of the same pathway and see whether the same type of cancer arises.

Because the multiple mutations that lead to formation of a tumor may require many years to accumulate, most cancers develop later in life. The occurrence of cancer after the age of reproduction may be one reason that evolutionary restraints have not done more to suppress cancer. The requirement for multiple mutations also lowers the frequency of cancer compared with what it would be if tumorigenesis were triggered by a single mutation. However, huge numbers of cells are, in essence, mutagenized and tested for altered growth during our lifetimes, a sort of evolutionary selection for cells that proliferate. Fortunately the tumor itself is not inherited.

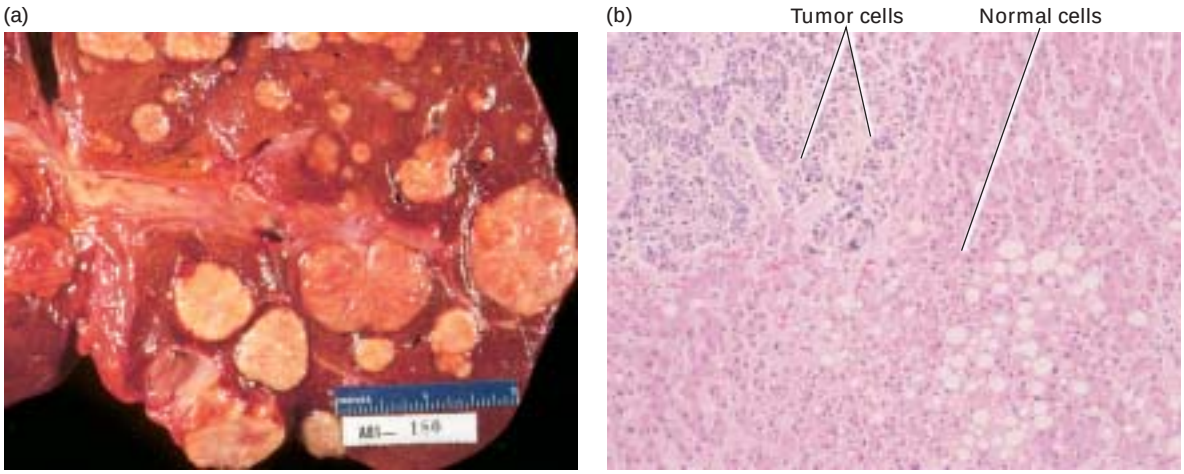
23.1 Tumor Cells and the Onset of Cancer

Before examining in detail the genetic basis of cancer, we consider the properties of tumor cells that distinguish them from normal cells and the general process of oncogenesis. The genetic changes that underlie oncogenesis alter several fundamental properties of cells, allowing cells to evade normal growth controls and ultimately conferring the full cancer phenotype (see Figure 23-1). Cancer cells acquire a drive to proliferate that does not require an external inducing signal. They fail to sense signals that restrict cell division and continue to live when they should die. They often change their attachment to surrounding cells or the extracellular matrix, breaking loose to divide more rapidly. A cancer cell may, up to a point, resemble a particular type of normal, rapidly dividing cell, but the cancer cell and its progeny will exhibit inappropriate immortality. To grow to more than a small size, tumors must obtain a blood supply, and they often do so by signaling to induce the growth of blood vessels into the tumor. As cancer progresses, tumors become an abnormal organ, increasingly well adapted to growth and invasion of surrounding tissues.

Metastatic Tumor Cells Are Invasive and Can Spread

Tumors arise with great frequency, especially in older individuals, but most pose little risk to their host because they are localized and of small size. We call such tumors **benign**; an example is warts, a benign skin tumor. The cells composing benign tumors closely resemble, and may function like, normal cells. The cell-adhesion molecules that hold tissues together keep benign tumor cells, like normal cells, localized to the tissues where they originate. A fibrous capsule usually delineates the extent of a benign tumor and makes it an easy target for a surgeon. Benign tumors become serious medical problems only if their sheer bulk interferes with normal functions or if they secrete excess amounts of biologically active substances like hormones. Acromegaly, the overgrowth of head, hands, and feet, for example, can occur when a benign pituitary tumor causes overproduction of growth hormone.

In contrast, cells composing a **malignant** tumor, or **cancer,** usually grow and divide more rapidly than normal, fail to die at the normal rate (e.g., chronic lymphocytic leukemia, a tumor of white blood cells), or invade nearby tissue without a significant change in their proliferation rate (e.g., less



▲ FIGURE 23-2 **Gross and microscopic views of a tumor invading normal liver tissue.** (a) The gross morphology of a human liver in which a metastatic lung tumor is growing. The white protrusions on the surface of the liver are the tumor masses. (b) A light micrograph of a section of the tumor in (a) showing areas of small, dark-staining tumor cells invading a region of larger, light-staining, normal liver cells. [Courtesy of J. Braun.]

harmful tumors of glial cells). Some malignant tumors, such as those in the ovary or breast, remain localized and encapsulated, at least for a time. When these tumors progress, the cells invade surrounding tissues, get into the body's circulatory system, and establish secondary areas of proliferation, a process called **metastasis.** Most malignant cells eventually acquire the ability to metastasize. Thus the major characteristics that differentiate metastatic (or malignant) tumors from benign ones are their invasiveness and spread.

Cancer cells can often be distinguished from normal cells by microscopic examination. They are usually less well differentiated than normal cells or benign tumor cells. In a specific tissue, malignant cells usually exhibit the characteristics of rapidly growing cells, that is, a high nucleus-to-cytoplasm ratio, prominent nucleoli, and relatively little specialized structure. The presence of invading cells in an otherwise normal tissue section is used to diagnose a malignancy (Figure 23-2).

Normal cells are restricted to their place in an organ or tissue by cell-cell adhesion and by physical barriers such as the **basal lamina,** which underlies layers of epithelial cells and also surrounds the endothelial cells of blood vessels (Chapter 6). Cancer cells have a complex relation to the extracellular matrix and basal lamina. The cells must degrade the basal lamina to penetrate it and metastasize, but in some cases cells may migrate along the lamina. Many tumor cells secrete a protein (plasminogen activator) that converts the serum protein plasminogen to the active protease plasmin. Increased plasmin activity promotes metastasis by digesting the basal lamina, thus allowing its penetration by tumor cells. As the basal lamina disintegrates, some tumor cells will enter the blood, but fewer than 1 in 10,000 cells that escape the primary tumor survive to colonize another tissue and form a secondary, metastatic tumor. In addition to escaping the original tumor and entering the blood, cells that will seed new tumors must then adhere to an endothelial cell lining a capillary and migrate across or through it into the underlying tissue. The multiple crossings of tissue layers that underlie malignancy often involve new or variant surface proteins made by malignant cells.

In addition to important changes in cell-surface proteins, drastic changes occur in the cytoskeleton during tumor-cell formation and metastasis. These alterations can result from changes in the expression of genes encoding Rho and other small GTPases that regulate the actin cytoskeleton (Chapter 19). For instance, tumor cells have been found to over-express the *RhoC* gene, and this increased activity stimulates metastasis.

Cancers Usually Originate in Proliferating Cells

In order for most oncogenic mutations to induce cancer, they must occur in dividing cells so that the mutation is passed on to many progeny cells. When such mutations occur in nondividing cells (e.g., neurons and muscle cells), they generally do not induce cancer, which is why tumors of muscle and nerve cells are rare in adults. Nonetheless, cancer can occur in tissues composed mainly of nondividing differentiated cells such as erythrocytes and most white blood cells, absorptive cells that line the small intestine, and keratinized cells that form the skin. The cells that initiate the tumors are not the differentiated cells, but rather their precursor cells. Fully differentiated cells usually do not divide. As they die or wear out, they are continually replaced by proliferation and differentiation of **stem cells,** and these cells are capable of transforming into tumor cells.

In Chapter 22, we learned that stem cells both perpetuate themselves and give rise to differentiating cells that can regenerate a particular tissue for the life of an organism (see Figure 22-2). For instance, many differentiated blood cells have short life spans and are continually replenished from hematopoietic (blood-forming) stem cells in the bone marrow (see Figure 22-5). Populations of stem cells in the intestine, liver, skin, bone, and other tissues likewise give rise to all or many of the cell types in these tissues, replacing aged

and dead cells, by pathways analogous to hematopoiesis in bone marrow. Similarly within a tumor there may be only certain cells with the ability to divide uncontrollably and generate new tumors; such cells are tumor stem cells.

Because stem cells can divide continually over the life of an organism, oncogenic mutations in their DNA can accumulate, eventually transforming them into cancer cells. Cells that have acquired these mutations have an abnormal proliferative capacity and generally cannot undergo normal processes of differentiation. Many oncogenic mutations, such as ones that prevent apoptosis or generate an inappropriate growth-promoting signal, also can occur in more differentiated, but still replicating, progenitor cells. Such mutations in hematopoietic progenitor cells can lead to various types of leukemia.

Normal animal cells are often classified according to their embryonic tissue of origin, and the naming of tumors has followed suit. Malignant tumors are classified as *carcinomas* if they derive from endoderm (gut epithelium) or ectoderm (skin and neural epithelia) and *sarcomas* if they derive from mesoderm (muscle, blood, and connective tissue precursors). The *leukemias*, a class of sarcomas, grow as individual cells in the blood, whereas most other tumors are solid masses. (The name *leukemia* is derived from the Latin for "white blood": the massive proliferation of leukemic cells can cause a patient's blood to appear milky.)

Tumor Growth Requires Formation of New Blood Vessels

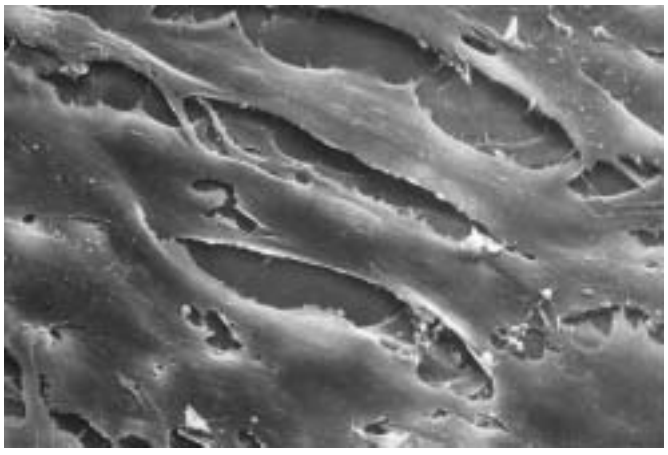
Tumors, whether primary or secondary, require recruitment of new blood vessels in order to grow to a large mass. In the absence of a blood supply, a tumor can grow into a mass of about 10^6 cells, roughly a sphere 2 mm in diameter. At this point, division of cells on the outside of the tumor mass is balanced by death of those in the center due to an inadequate supply of nutrients. Such tumors, unless they secrete hormones, cause few problems. However, most tumors induce the formation of new blood vessels that invade the tumor and nourish it, a process called *angiogenesis*. This complex process requires several discrete steps: degradation of the basal lamina that surrounds a nearby capillary, migration of endothelial cells lining the capillary into the tumor, division of these endothelial cells, and formation of a new basement membrane around the newly elongated capillary.

Many tumors produce growth factors that stimulate angiogenesis; other tumors somehow induce surrounding normal cells to synthesize and secrete such factors. Basic fibroblast growth factor (bFGF), transforming growth factor α (TGFα), and vascular endothelial growth factor (VEGF), which are secreted by many tumors, all have angiogenic properties. New blood vessels nourish the growing tumor, allowing it to increase in size and thus increase the probability that additional harmful mutations will occur. The presence of an adjacent blood vessel also facilitates the process of metastasis.

MEDICINE

Several natural proteins that inhibit angiogenesis (e.g., angiogenin and endostatin) or antagonists of the VEGF receptor have excited much interest as potential therapeutic agents. Although new blood vessels are constantly forming during embryonic development, few form normally in adults except after injury. Thus a specific inhibitor of angiogenesis not only might be effective against many kinds of tumors but also might have few adverse side effects. ▮

(a)



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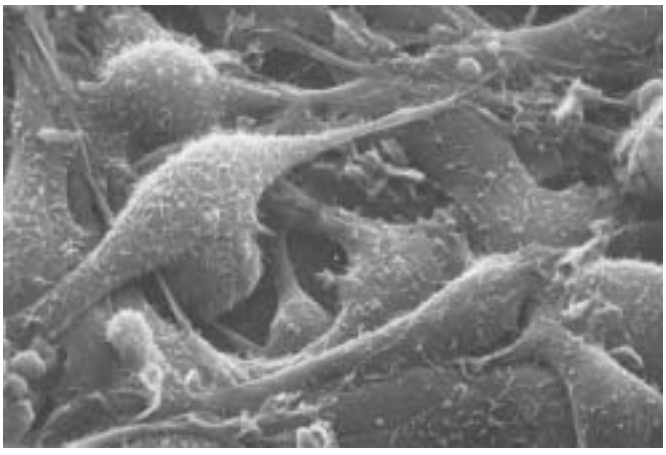


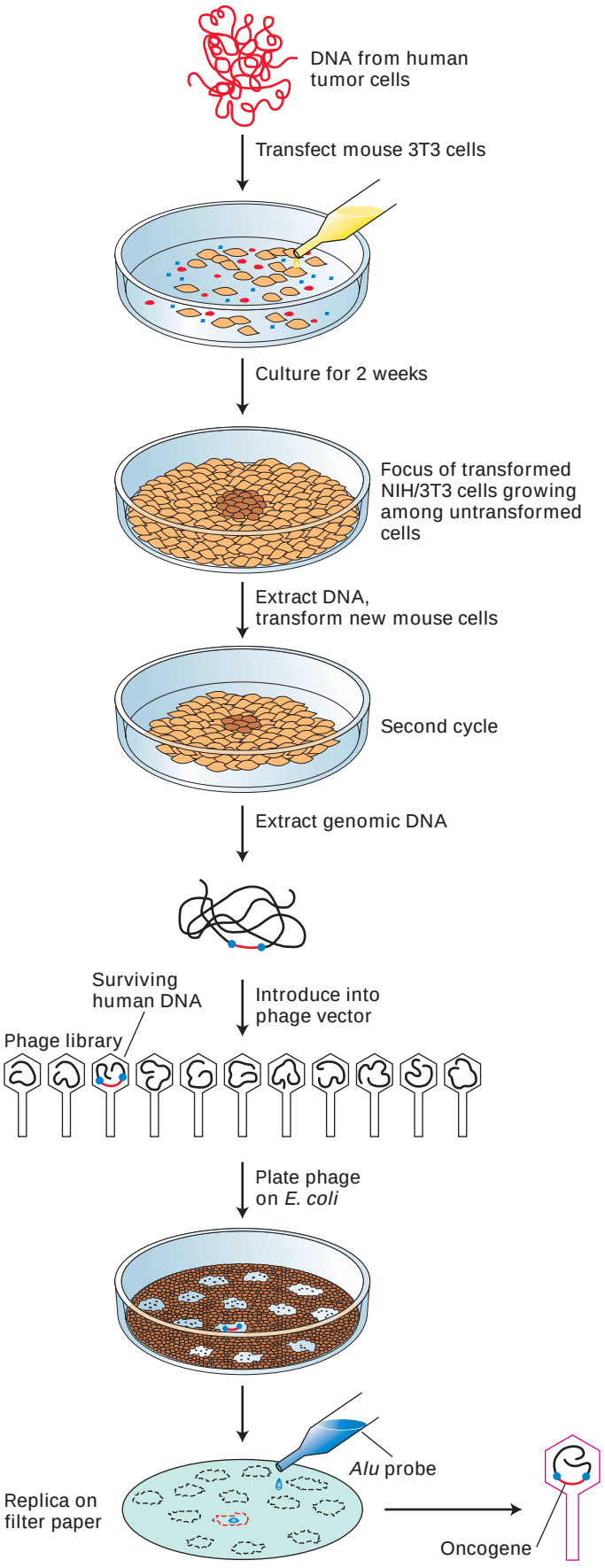
▲ EXPERIMENTAL FIGURE 23-3 **Scanning electron micrographs reveal the organizational and morphological differences between normal and transformed 3T3 cells.**
(a) Normal 3T3 cells are elongated and are aligned and closely packed in an orderly fashion. (b) 3T3 cells transformed by an oncogene encoded by Rous sarcoma virus are rounded and covered with small hairlike processes and bulbous projections. The transformed cells that grow have lost the side-by-side organization of the normal cells and grow one atop the other. These transformed cells have many of the same properties as malignant cells. Similar changes are seen in cells transfected with DNA from human cancers containing the *ras*^{D} oncogene. [Courtesy of L.-B. Chen.]

Cultured Cells Can Be Transformed into Tumor Cells

The morphology and growth properties of tumor cells clearly differ from those of their normal counterparts; some of these differences are also evident when cells are cultured. That mutations cause these differences was conclusively established by transfection experiments with a line of cultured mouse fibroblasts called 3T3 cells. These cells normally grow only when attached to the plastic surface of a culture dish and are maintained at a low cell density. Because 3T3 cells stop growing when they contact other cells, they eventually form a monolayer of well-ordered cells that have stopped proliferating and are in the quiescent G_0 phase of the cell cycle (Figure 23-3a).

When DNA from human bladder cancer cells is transfected into cultured 3T3 cells, about one cell in a million incorporates a particular segment of the exogenous DNA that causes a distinctive phenotypic change. The progeny of the affected cell are more rounded and less adherent to one another and to the dish than are the normal surrounding cells, forming a three-dimensional cluster of cells (a focus) that can be recognized under the microscope (Figure 23-3b). Such cells, which continue to grow when the normal cells have become quiescent, have undergone oncogenic **transformation**. The transformed cells have properties similar to those of malignant tumor cells, including changes in cell morphology, ability to grow unattached to an extracellular matrix, reduced requirement for growth factors, secretion of plasminogen activator, and loss of actin microfilaments.

Figure 23-4 outlines the procedure for transforming 3T3 cells with DNA from a human bladder cancer and cloning the specific DNA segment that causes transformation. It was remarkable to find a small piece of DNA with this capability; had more than one piece been needed, the experiment would



▶ EXPERIMENTAL FIGURE 23-4 **Transformation of mouse cells with DNA from a human cancer cell permits identification and molecular cloning of the *ras*^{D} oncogene.** Addition of DNA from a human bladder cancer to a culture of mouse 3T3 cells causes about one cell in a million to divide abnormally and form a focus, or clone, of transformed cells. To clone the oncogene responsible for transformation, advantage is taken of the fact that most human genes have nearby repetitive DNA sequences called *Alu* sequences. DNA from the initial focus of transformed mouse cells is isolated, and the oncogene is separated from adventitious human DNA by secondary transfer to mouse cells. The total DNA from a secondary transfected mouse cell is then cloned into bacteriophage λ; only the phage that receives human DNA hybridizes with an *Alu* probe. The hybridizing phage should contain part of or all the transforming oncogene. This expected result can be proved by showing either that the phage DNA can transform cells (if the oncogene has been completely cloned) or that the cloned piece of DNA is always present in cells transformed by DNA transfer from the original donor cell.

have failed. Subsequent studies showed that the cloned segment included a mutant version of the cellular *ras* gene, designated ras^D. Normal **Ras protein,** which participates in many intracellular signal-transduction pathways activated by growth factors, cycles between an inactive, "off" state with bound GDP and an active, "on" state with bound GTP. The mutated Ras^D protein hydrolyzes bound GTP very slowly and therefore accumulates in the active state, sending a growth-promoting signal to the nucleus even in the absence of the hormones normally required to activate its signaling function.

The production and **constitutive** activation of Ras^D protein are not sufficient to cause transformation of normal cells in a primary (fresh) culture of human, rat, or mouse fibroblasts. Unlike cells in a primary culture, however, cultured 3T3 cells have undergone a loss-of-function mutation in the *p16* gene, which encodes a cyclin-kinase inhibitor that restricts progression through the cell cycle. Such cells can grow for an unlimited time in culture if periodically diluted and supplied with nutrients, which normal cells cannot (see Figure 6-37b). These immortal 3T3 cells are transformed into full-blown tumor cells only when they produce a constitutively active Ras protein. For this reason, transfection with the ras^D gene can transform 3T3 cells, but not normal cultured primary fibroblast cells, into tumor cells.

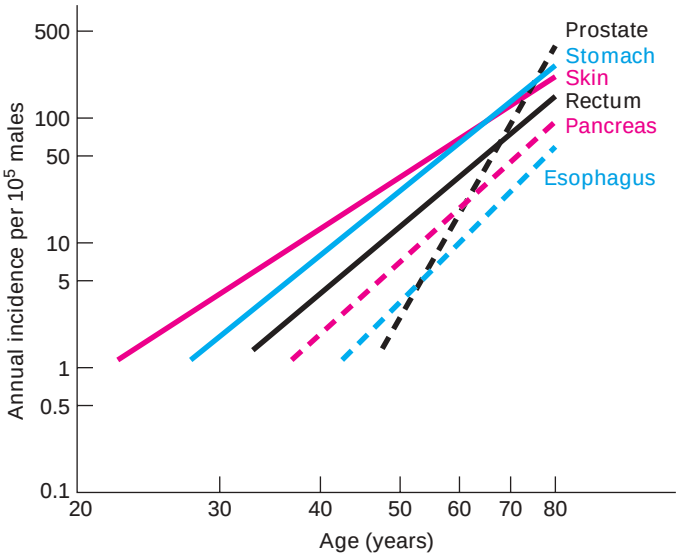
A mutant *ras* gene is found in most human colon, bladder, and other cancers, but not in normal human DNA; thus it must arise as the result of a somatic mutation in one of the tumor progenitor cells. Any gene, such as ras^D, that encodes a protein capable of transforming cells in culture or inducing cancer in animals is referred to as an **oncogene.** The normal cellular gene from which it arises is called a proto-oncogene. The oncogenes carried by viruses that cause tumors in animals are often derived from proto-oncogenes that were hijacked from the host genome and altered to be oncogenic. When this was first discovered, it was startling to find that these dangerous viruses were turning the animal's own genes against them.

A Multi-hit Model of Cancer Induction Is Supported by Several Lines of Evidence

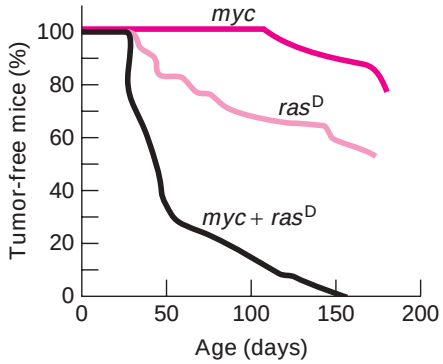
As noted earlier and illustrated by the oncogenic transformation of 3T3 cells, multiple mutations usually are required to convert a normal body cell into a malignant one. According to this "multi-hit" model, evolutionary (or "survival of the fittest") cancers arise by a process of clonal selection not unlike the selection of individual animals in a large population. A mutation in one cell would give it a slight growth advantage. One of the progeny cells would then undergo a second mutation that would allow its descendants to grow more uncontrollably and form a small benign tumor; a third mutation in a cell within this tumor would allow it to outgrow the others and overcome constraints imposed by the tumor microenvironment, and its progeny would form a mass of cells, each of which would have these three mutations. An additional mutation in one of these cells would allow its progeny to escape into the blood and establish daughter colonies at other sites, the hallmark of metastatic cancer. This model makes two easily testable predictions.

First, all the cells in a given tumor should contain at least some genetic alterations in common. Systematic analysis of cells from individual human tumors supports the prediction that all the cells are derived from a single progenitor. Recall that during the fetal life of a human female each cell inactivates one of the two X chromosomes. A woman is a genetic mosaic; half the cells have one X inactivated, and the remainder have the other X inactivated. If a tumor did not arise from a single progenitor, it would be composed of a mix of cells with one or the other X inactivated. In fact, the cells from a woman's tumor have the same inactive X chromosome. Different tumors can be composed of cells with either the maternal or the paternal X inactive. Second, cancer incidence should increase with age because it can take decades for the required multiple mutations to occur. Assuming that the rate of mutation is roughly constant during a lifetime, then the incidence of most types of cancer would be independent of age if only one mutation were required to convert a normal cell into a malignant one. As the data in Figure 23-5 show, the incidence of many types of human cancer does indeed increase drastically with age.

More direct evidence that multiple mutations are required for tumor induction comes from transgenic mice



▲ EXPERIMENTAL FIGURE 23-5 **The incidence of human cancers increases as a function of age.** The marked increase in the incidence with age is consistent with the multi-hit model of cancer induction. Note that the logarithm of annual incidence is plotted versus the logarithm of age. [From B. Vogelstein and K. Kinzler, 1993, *Trends Genet.* **9:**101.]



▲ EXPERIMENTAL FIGURE 23-6 **The kinetics of tumor appearance in female mice carrying either one or two oncogenic transgenes shows the cooperative nature of multiple mutations in cancer induction.** The transgenes were driven by the mouse mammary tumor virus (MMTV) breast-specific promoter. The hormonal stimulation associated with pregnancy activated overexpression of the transgenes. The graph shows the time course of tumorigenesis in mice carrying either *myc* or *ras*^{D} transgenes as well as in the progeny of a cross of *myc* carriers with *ras*^{D} carriers that contain both transgenes. The results clearly demonstrate the cooperative effects of multiple mutations in cancer induction. [See E. Sinn et al., 1987, *Cell* **49**:465.]

carrying both the mutant *ras*^{D} oncogene and the c-*myc* proto-oncogene controlled by a mammary cell–specific promoter/enhancer from a retrovirus. When linked to this promoter, the normal c-*myc* gene is overexpressed in breast tissue because the promoter is induced by endogenous hormone levels and tissue-specific regulators. This heightened transcription of c-*myc* mimics oncogenic mutations that turn up c-*myc* transcription, converting the proto-oncogene into an oncogene. By itself, the c-*myc* transgene causes tumors only after 100 days, and then in only a few mice; clearly only a minute fraction of the mammary cells that overproduce the Myc protein become malignant. Similarly, production of the mutant Ras^{D} protein alone causes tumors earlier but still slowly and with about 50 percent efficiency over 150 days. When the c-*myc* and *ras*^{D} transgenics are crossed, however, such that all mammary cells produce both Myc and Ras^{D}, tumors arise much more rapidly and all animals succumb to cancer (Figure 23-6). Such experiments emphasize the synergistic effects of multiple oncogenes. They also suggest that the long latency of tumor formation, even in the double-transgenic mice, is due to the need to acquire additional somatic mutations.

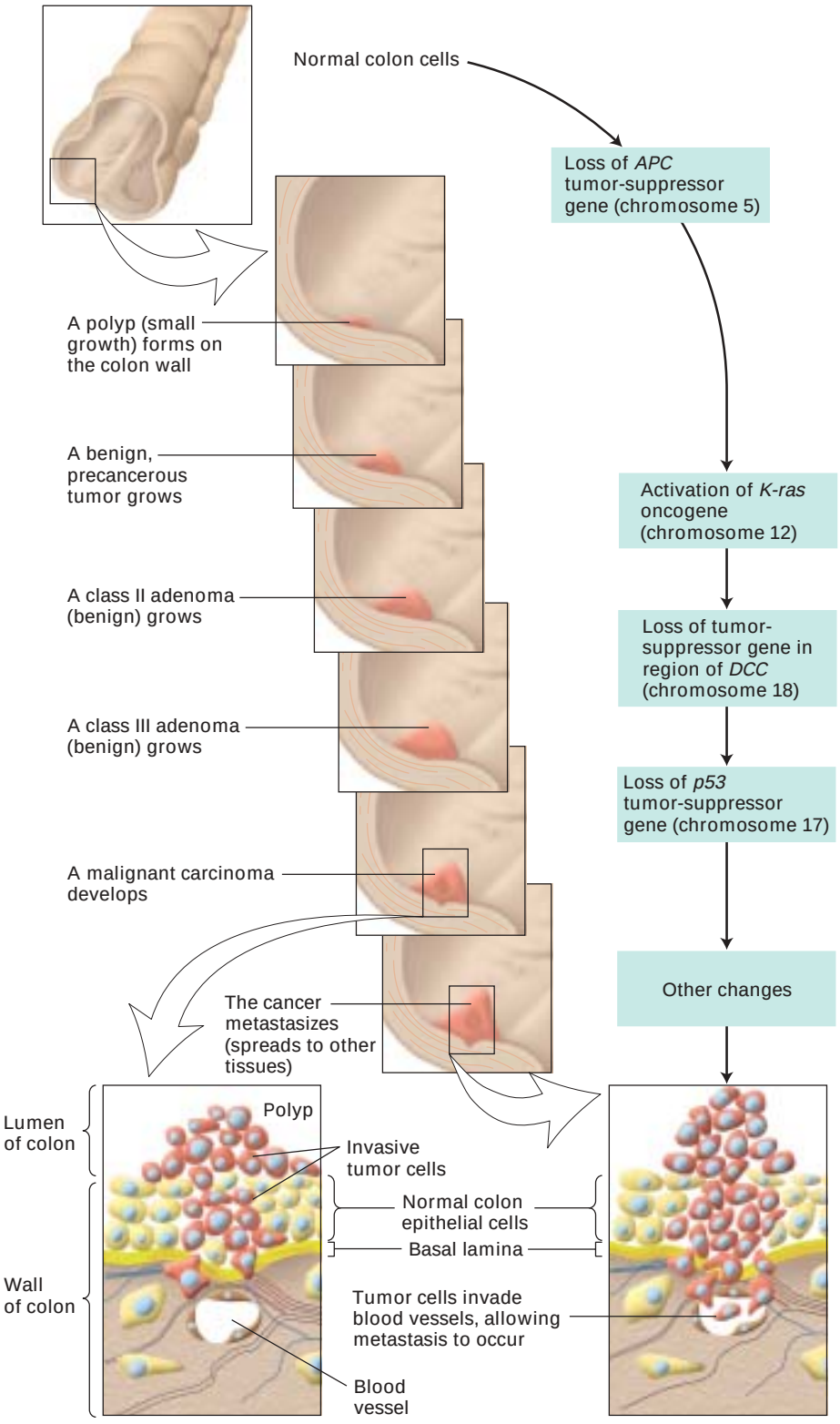
Similar cooperative effects between oncogenes can be seen in cultured cells. Transfection of normal fibroblasts with either c-*myc* or activated *ras*^{D} is not sufficient for oncogenic transformation, whereas when transfected together, the two genes cooperate to transform the cells. Deregulated levels of c-*myc* alone induce proliferation but also sensitize fibroblasts to apoptosis, and overexpression of activated *ras*^{D} alone induces senescence. When the two oncogenes are expressed in the same cell, these negative cellular responses are neutralized and the cells undergo transformation.

Successive Oncogenic Mutations Can Be Traced in Colon Cancers

Studies on colon cancer provide the most compelling evidence to date for the multi-hit model of cancer induction. Surgeons can obtain fairly pure samples of many human cancers, but generally the exact stage of tumor progression cannot be identified and analyzed. An exception is colon cancer, which evolves through distinct, well-characterized morphological stages. These intermediate stages—polyps, benign adenomas, and carcinomas—can be isolated by a surgeon, allowing mutations that occur in each of the morphological stages to be identified. Numerous studies show that colon cancer arises from a series of mutations that commonly occur in a well-defined order, providing strong support for the multi-hit model.

Invariably the first step in colon carcinogenesis involves loss of a functional *APC* gene, resulting in formation of polyps (precancerous growths) on the inside of the colon wall. Not every colon cancer, however, acquires all the later mutations or acquires them in the order depicted in Figure 23-7. Thus different combinations of mutations may result in the same phenotype. Most of the cells in a polyp contain the same one or two mutations in the *APC* gene that result in its loss or inactivation; thus they are clones of the cell in which the original mutation occurred. *APC* is a tumor-suppressor gene, and both alleles of the *APC* gene must carry an inactivating mutation for polyps to form because cells with one wild-type *APC* gene express enough APC protein to function normally. Like most tumor-suppressor genes, *APC* encodes a protein that inhibits the progression of certain types of cells through the cell cycle. The APC protein does so by preventing the Wnt signal-transduction pathway from activating expression of proto-oncogenes including the c-*myc* gene. The absence of functional APC protein thus leads to inappropriate production of Myc, a transcription factor that induces expression of many genes required for the transition from the G_1 to the S phase of the cell cycle. Cells homozygous for *APC* mutations proliferate at a rate higher than normal and form polyps.

If one of the cells in a polyp undergoes another mutation, this time an activating mutation in the *ras* gene, its progeny divide in an even more uncontrolled fashion, forming a larger adenoma (see Figure 23-7). Mutational loss of a particular chromosomal region (the relevant gene is not yet known), followed by inactivation of the *p53* gene, results in the gradual loss of normal regulation and the consequent formation of a malignant carcinoma. About half of all human tumors carry mutations in *p53*, which encodes a transcriptional regulator.



▲ FIGURE 23-7 **The development and metastasis of human colorectal cancer and its genetic basis.** A mutation in the APC tumor-suppressor gene in a single epithelial cell causes the cell to divide, although surrounding cells do not, forming a mass of localized benign tumor cells, or polyp. Subsequent mutations leading to expression of a constitutively active Ras protein and loss of two tumor-suppressor genes—an unidentified gene in the vicinity of *DCC* and *p53*—generate a malignant cell carrying all four mutations. This cell continues to divide, and the progeny invade the basal lamina that surrounds the tissue. Some tumor cells spread into blood vessels that will distribute them to other sites in the body. Additional mutations permit the tumor cells to exit from the blood vessels and proliferate at distant sites; a patient with such a tumor is said to have cancer. [Adapted from B. Vogelstein and K. Kinzler, 1993, *Trends Genet.* **9**:101]

DNA from different human colon carcinomas generally contains mutations in all these genes—loss-of-function mutations in the tumor suppressors *APC* and *p53*, the as yet mysterious gene, and an activating (gain-of-function) mutation in the dominant oncogene *K-ras*—establishing that multiple mutations in the same cell are needed for the cancer to form. Some of these mutations appear to confer growth advantages at an early stage of tumor development, whereas other mutations promote the later stages, including invasion and metastasis, which are required for the malignant phenotype. The number of mutations needed for colon cancer progression may at first seem surprising, seemingly an effective barrier to tumorigenesis. Our genomes, however, are under constant assault. Recent estimates indicate that sporadically arising polyps have about 11,000 genetic alterations in each cell, though very likely only a few of these are relevant to oncogenesis.

Colon carcinoma provides an excellent example of the multi-hit mode of cancer. The degree to which this model applies to cancer is only now being learned, but it is clear that multiple types of cancer involve multiple mutations. The advent of **DNA microarray** technology is allowing more detailed examination of tumor properties by monitoring the spectrum of mRNA molecules from tens of thousands of genes, and recent data have provided some challenges to the multi-hit model. Not surprisingly, primary tumors can often be distinguishable from metastatic tumors by the pattern of gene expression. More interestingly, a subset of solid primary tumors has been found to have characteristics more typical of metastatic tumors, suggesting that it may be possible to identify primary tumors that have a greater probability of becoming metastatic. This also raises the possibility that, at least for some types of cancer, the initiating events of the primary tumor may set a course toward metastasis. This hypothesis can be distinguished from the emergence of a rare subset of cells within the primary tumor acquiring a necessary series of further mutations.

KEY CONCEPTS OF SECTION 23.1

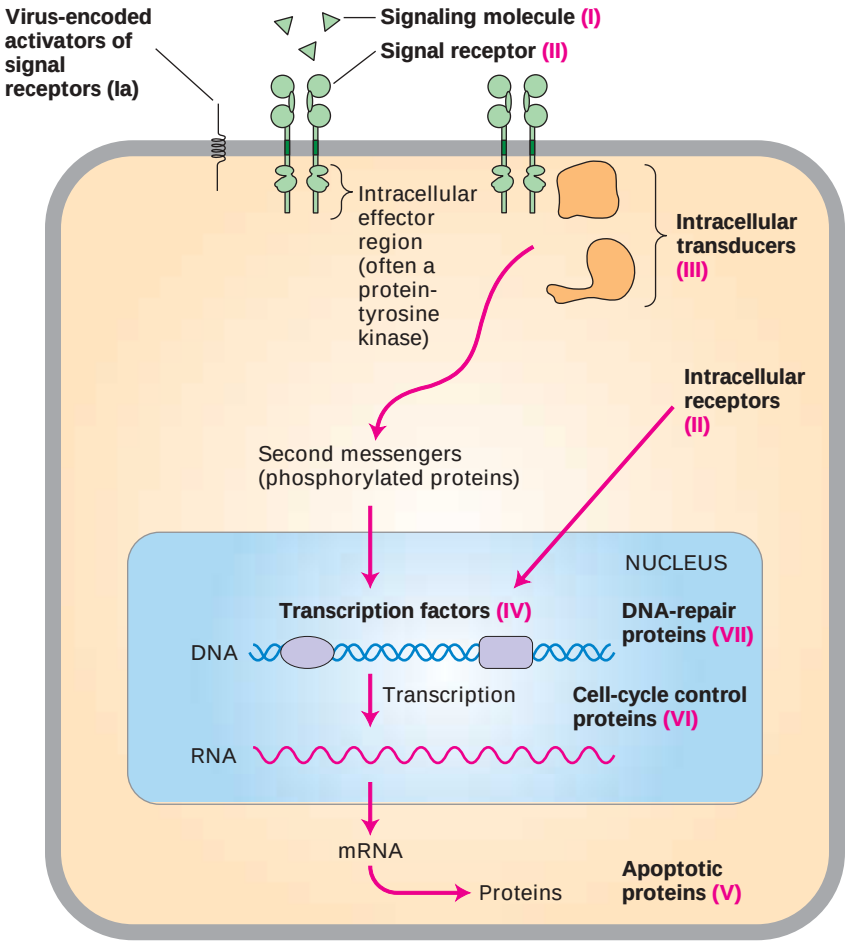
Tumor Cells and the Onset of Cancer

- Cancer is a fundamental aberration in cellular behavior, touching on many aspects of molecular cell biology. Most cell types of the body can give rise to malignant tumor (cancer) cells.
- Cancer cells usually arise from stem cells and other proliferating cells and bear more resemblance to these cells than to more mature differentiated cell types.
- Cancer cells can multiply in the absence of at least some of the growth-promoting factors required for proliferation of normal cells and are resistant to signals that normally program cell death (apoptosis).
- Certain cultured cells transfected with tumor-cell DNA undergo transformation (see Figure 23-4). Such transformed cells share certain properties with tumor cells.
- Cancer cells sometimes invade surrounding tissues, often breaking through the basal laminae that define the boundaries of tissues and spreading through the body to establish secondary areas of growth, a process called metastasis. Metastatic tumors often secrete proteases, which degrade the surrounding extracellular matrix.
- Both primary and secondary tumors require angiogenesis, the formation of new blood vessels, in order to grow to a large mass.
- The multi-hit model, which proposes that multiple mutations are needed to cause cancer, is consistent with the genetic homogeneity of cells from a given tumor, the observed increase in the incidence of human cancers with advancing age, and the cooperative effect of oncogenic transgenes on tumor formation in mice.
- Most oncogenic mutations occur in somatic cells and are not carried in the germ-line DNA.
- Colon cancer develops through distinct morphological stages that commonly are associated with mutations in specific tumor-suppressor genes and proto-oncogenes (see Figure 23-7).

23.2 The Genetic Basis of Cancer

As we have seen, mutations in two broad classes of genes—proto-oncogenes (e.g., *ras*) and tumor-suppressor genes (e.g., *APC*)—play key roles in cancer induction. These genes encode many kinds of proteins that help control cell growth and proliferation (Figure 23-8). Virtually all human tumors have inactivating mutations in genes that normally act at various cell-cycle **checkpoints** to stop a cell's progress through the cell cycle if a previous step has occurred incorrectly or if DNA has been damaged. For example, most cancers have inactivating mutations in the genes coding for one or more proteins that normally restrict progression through the G_1 stage of the cell cycle. Likewise, a constitutively active Ras or other activated signal-transduction protein is found in several kinds of human tumor that have different origins. Thus malignancy and the intricate processes for controlling the cell cycle discussed in Chapter 21 are two faces of the same coin. In the series of events leading to growth of a tumor, oncogenes combine with tumor-suppressor mutations to give rise to the full spectrum of tumor cell properties described in the previous section (see Figure 23-7).

In this section, we consider the general types of mutations that are oncogenic and see how certain viruses can cause cancer. We also explain why some inherited mutations increase the risk for particular cancers and consider the relation between cancer and developmentally important genes. We conclude this section with a brief discussion of how genomics methods are being used to characterize and classify tumors.



▶ FIGURE 23-8 **Seven types of proteins that participate in controlling cell growth and proliferation.** Cancer can result from expression of mutant forms of these proteins. Mutations changing the structure or expression of proteins that normally promote cell growth generally give rise to dominantly active oncogenes. Many, but not all, extracellular signaling molecules (I), signal receptors (II), signal-transduction proteins (III), and transcription factors (IV) are in this category. Cell-cycle control proteins (VI) that function to restrain cell proliferation and DNA-repair proteins (VII) are encoded by tumor-suppressor genes. Mutations in these genes act recessively, greatly increasing the probability that the mutant cells will become tumor cells or that mutations will occur in other classes. Apoptotic proteins (V) include tumor suppressors that promote apoptosis and oncoproteins that promote cell survival. Virus-encoded proteins that activate signal receptors (Ia) also can induce cancer.

Gain-of-Function Mutations Convert Proto-oncogenes into Oncogenes

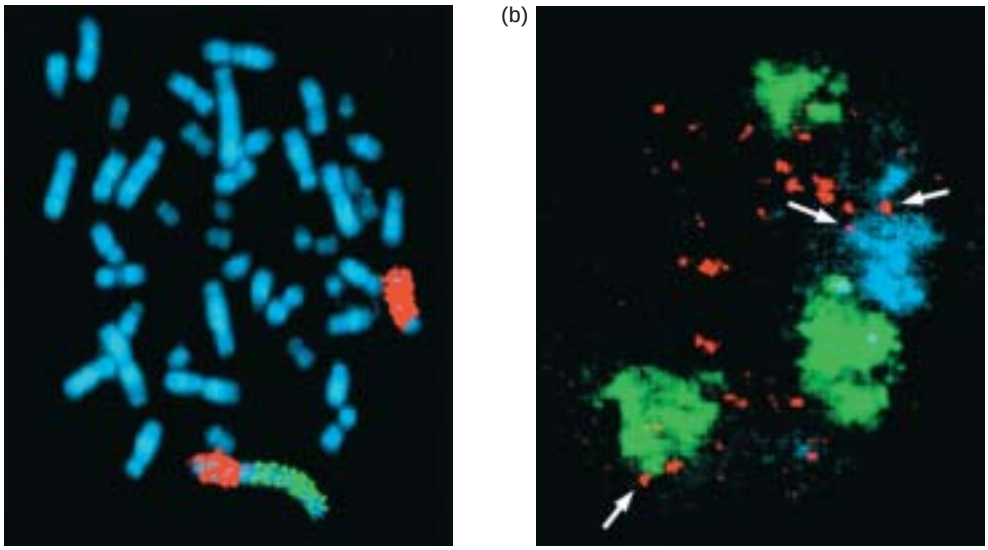
Recall that an oncogene is any gene that encodes a protein able to transform cells in culture or to induce cancer in animals. Of the many known oncogenes, all but a few are derived from normal cellular genes (i.e., proto-oncogenes) whose products promote cell proliferation. For example, the *ras* gene discussed previously is a proto-oncogene that encodes an intracellular signal-transduction protein; the mutant ras^D gene derived from *ras* is an oncogene, whose encoded protein provides an excessive or uncontrolled growth-promoting signal. Other proto-oncogenes encode growth-promoting signal molecules and their receptors, anti-apoptotic (cell-survival) proteins, and some transcription factors.

Conversion, or activation, of a proto-oncogene into an oncogene generally involves a *gain-of-function* mutation. At least four mechanisms can produce oncogenes from the corresponding proto-oncogenes:

- *Point mutation* (i.e., change in a single base pair) in a proto-oncogene that results in a constitutively active protein product
- *Chromosomal translocation* that fuses two genes together to produce a hybrid gene encoding a chimeric protein whose activity, unlike that of the parent proteins, often is constitutive
- *Chromosomal translocation* that brings a growth-regulatory gene under the control of a different promoter that causes inappropriate expression of the gene
- *Amplification* (i.e., abnormal DNA replication) of a DNA segment including a proto-oncogene, so that numerous copies exist, leading to overproduction of the encoded protein

An **oncogene** formed by either of the first two mechanisms encodes an "oncoprotein" that differs from the normal protein encoded by the corresponding proto-oncogene. In contrast, the other two mechanisms generate oncogenes whose protein products are identical with the normal proteins; their oncogenic effect is due to production at higher-than-normal levels or in cells where they normally are not produced.

The localized amplification of DNA to produce as many as 100 copies of a given region (usually a region spanning hundreds of kilobases) is a common genetic change seen in tumors. This anomaly may take either of two forms: the duplicated DNA may be tandemly organized at a single site on a chromosome, or it may exist as small, independent



▲ EXPERIMENTAL FIGURE 23-9 **DNA amplifications in stained chromosomes take two forms, visible under the light microscope.** (a) Homogeneously staining regions (HSRs) in a human chromosome from a neuroblastoma cell. The chromosomes are uniformly stained with a blue dye so that all can be seen. Specific DNA sequences were detected using fluorescent in situ hybridization (FISH) in which fluorescently labeled DNA clones are hybridized to denatured DNA in the chromosomes. The chromosome 4 pair is marked (red) by in situ hybridization with a large DNA cosmid clone containing the N-*myc* oncogene. On one of the chromosome 4's an HSR is visible (green) after staining for a sequence enriched in the HSR. (b) Optical sections through nuclei from a human neuroblastoma cell that contain double minute chromosomes. The normal chromosomes are the green and blue structures; the double minute chromosomes are the many small red dots. Arrows indicate double minutes associated with the surface or interior of the normal chromosomes. [Parts (a) and (b) from I. Solovei et al., 2000, *Genes Chromosomes Cancer* **29**:297–308, Figures 4 and 17.]

mini-chromosome-like structures. The former case leads to a homogeneously staining region (HSR) that is visible in the light microscope at the site of the amplification; the latter case causes extra "minute" chromosomes, separate from the normal chromosomes that pepper a stained chromosomal preparation (Figure 23-9).

Gene amplification may involve a small number of genes, such as the N-*myc* gene and its neighbor *DDX1* that are amplified in neuroblastoma, or a chromosome region containing many genes. It can be difficult to determine which genes are amplified, a first step in determining which gene caused the tumor. DNA microarrays offer a powerful approach for finding amplified regions of chromosomes. Rather than look at gene expression, the application of microarrays we described earlier, these experiments involve looking for abnormally abundant DNA sequences. Genomic DNA from cancer cells is used to probe arrays containing fragments of genomic DNA and spots with amplified DNA give stronger signals than control spots. Among the amplified genes, the strongest candidates for the relevant ones can be identified by also measuring gene expression. A breast carcinoma cell line, with four known amplified chromosome regions, was screened for amplified genes, and the expression levels of those genes were also studied on microarrays. Fifty genes were found to be amplified, but only five were also highly expressed. These five are new candidates as oncogenes. ▮

However they arise, the gain-of-function mutations that convert proto-oncogenes to oncogenes are genetically **dominant;** that is, mutation in only one of the two alleles is sufficient for induction of cancer.

Cancer-Causing Viruses Contain Oncogenes or Activate Cellular Proto-oncogenes

Pioneering studies by Peyton Rous beginning in 1911 led to the initial recognition that a virus could cause cancer when injected into a suitable host animal. Many years later molecular biologists showed that Rous sarcoma virus (RSV) is a **retrovirus** whose RNA genome is reverse-transcribed into DNA, which is incorporated into the host-cell genome (see Figure 4-43). In addition to the "normal" genes present in all retroviruses, oncogenic transforming viruses like RSV contain the v-*src* gene. Subsequent studies with mutant forms of RSV demonstrated that only the v-*src* gene, not the other viral genes, was required for cancer induction.

In the late 1970s, scientists were surprised to find that normal cells from chickens and other species contain a gene that is closely related to the RSV v-*src* gene. This normal cellular gene, a proto-oncogene, commonly is distinguished from the viral gene by the prefix "c" (c-*src*). RSV and other oncogene-carrying viruses are thought to have arisen by incorporating, or transducing, a normal cellular proto-oncogene into their genome. Subsequent mutation in the transduced gene then converted it into a dominantly acting

oncogene, which can induce cell transformation in the presence of the normal c-*src* proto-oncogene. Such viruses are called *transducing retroviruses* because their genomes contain an oncogene derived from a transduced cellular proto-oncogene.

Because its genome carries the potent v-*src* oncogene, the transducing RSV induces tumors within days. In contrast, most oncogenic retroviruses induce cancer only after a period of months or years. The genomes of these *slow-acting retroviruses* differ from those of transducing viruses in one crucial respect: they lack an oncogene. All slow-acting, or "long latency," retroviruses appear to cause cancer by integrating into the host-cell DNA near a cellular proto-oncogene and activating its expression. The long terminal repeat (LTR) sequences in integrated retroviral DNA can act as an enhancer or promoter of a nearby cellular gene, thereby stimulating its transcription. For example, in the cells from tumors caused by avian leukosis virus (ALV), the retroviral DNA is inserted near the c-*myc* gene. These cells overproduce c-Myc protein; as noted earlier, overproduction of c-Myc causes abnormally rapid proliferation of cells. Slow-acting viruses act slowly for two reasons: integration near a cellular proto-oncogene (e.g., c-*myc*) is a random, rare event, and additional mutations have to occur before a full-fledged tumor becomes evident.

In natural bird and mouse populations, slow-acting retroviruses are much more common than oncogene-containing retroviruses such as Rous sarcoma virus. Thus, insertional proto-oncogene activation is probably the major mechanism by which retroviruses cause cancer. Although few human tumors have been associated with any retrovirus, the huge investment in studying retroviruses as a model for human cancer paid off both in the discovery of cellular oncogenes and in the sophisticated understanding of retroviruses, which later accelerated progress on the HIV virus that causes AIDS.

A few DNA viruses also are oncogenic. Unlike most DNA viruses that infect animal cells, oncogenic DNA viruses integrate their viral DNA into the host-cell genome. The viral DNA contains one or more oncogenes, which permanently transform infected cells. For example, many warts and other benign tumors of epithelial cells are caused by the DNA-containing papillomaviruses. Unlike retroviral oncogenes, which are derived from normal cellular genes and have no function for the virus except to allow their proliferation in tumors, the known oncogenes of DNA viruses are integral parts of the viral genome and are required for viral replication. As discussed later, the oncoproteins expressed from integrated viral DNA in infected cells act in various ways to stimulate cell growth and proliferation.

Loss-of-Function Mutations in Tumor-Suppressor Genes Are Oncogenic

Tumor-suppressor genes generally encode proteins that in one way or another inhibit cell proliferation. *Loss-of-function* mutations in one or more of these "brakes" contribute to the development of many cancers. Five broad classes of proteins are generally recognized as being encoded by tumor-suppressor genes:

- Intracellular proteins that regulate or inhibit progression through a specific stage of the cell cycle (e.g., p16 and Rb)
- Receptors or signal transducers for secreted hormones or developmental signals that inhibit cell proliferation (e.g., TGFβ, the hedgehog receptor patched)
- Checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal (e.g., p53)
- Proteins that promote apoptosis
- Enzymes that participate in DNA repair

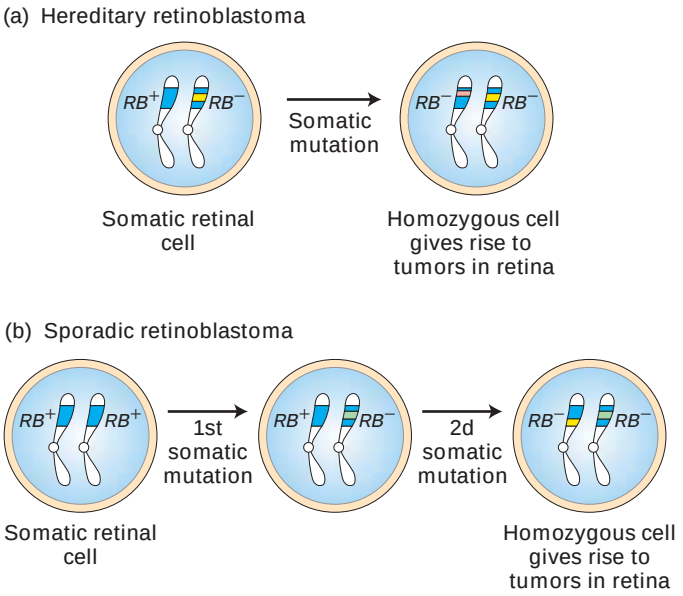
Although DNA-repair enzymes do not directly inhibit cell proliferation, cells that have lost the ability to repair errors, gaps, or broken ends in DNA accumulate mutations in many genes, including those that are critical in controlling cell growth and proliferation. Thus loss-of-function mutations in the genes encoding DNA-repair enzymes prevent cells from correcting mutations that inactivate tumor-suppressor genes or activate oncogenes.

Since generally one copy of a tumor-suppressor gene suffices to control cell proliferation, both alleles of a tumor-suppressor gene must be lost or inactivated in order to promote tumor development. Thus oncogenic loss-of-function mutations in tumor-suppressor genes are genetically **recessive.** In many cancers, tumor-suppressor genes have deletions or point mutations that prevent production of any protein or lead to production of a nonfunctional protein. Another mechanism for inactivating tumor-suppressor genes is methylation of cytosine residues in the promoter or other control elements. Such methylation is commonly found in nontranscribed regions of DNA.

Inherited Mutations in Tumor-Suppressor Genes Increase Cancer Risk

Individuals with inherited mutations in tumor-suppressor genes have a hereditary predisposition for certain cancers. Such individuals generally inherit a germ-line mutation in one allele of the gene; somatic mutation of the second allele facilitates tumor progression. A classic case is retinoblastoma, which is caused by loss of function of *RB*, the first tumor-suppressor gene to be identified. As we discuss later, the protein encoded by *RB* helps regulate progress through the cell cycle.

Hereditary versus Sporadic Retinoblastoma Children with hereditary retinoblastoma inherit a single defective copy of the *RB* gene, sometimes seen as a small deletion on one of the copies of chromosome 13. The children develop retinal tumors early in life and generally in both eyes. One essential event in tumor development is the deletion or



▲ FIGURE 23-10 **Role of spontaneous somatic mutation in retinoblastoma.** This disease is marked by retinal tumors that arise from cells carrying two mutant RB^- alleles. (a) In hereditary (familial) retinoblastoma, a child inherits a normal RB^+ allele from one parent and a mutant RB^- allele from the other parent. A single mutation in a heterozygous somatic retinal cell that inactivates the normal allele will produce a cell homozygous for two mutant alleles. (b) In sporadic retinoblastoma, a child inherits two normal RB^+ alleles. Two separate somatic mutations in a particular retinal cell or its progeny are required to produce a homozygous RB^-/RB^- cell.

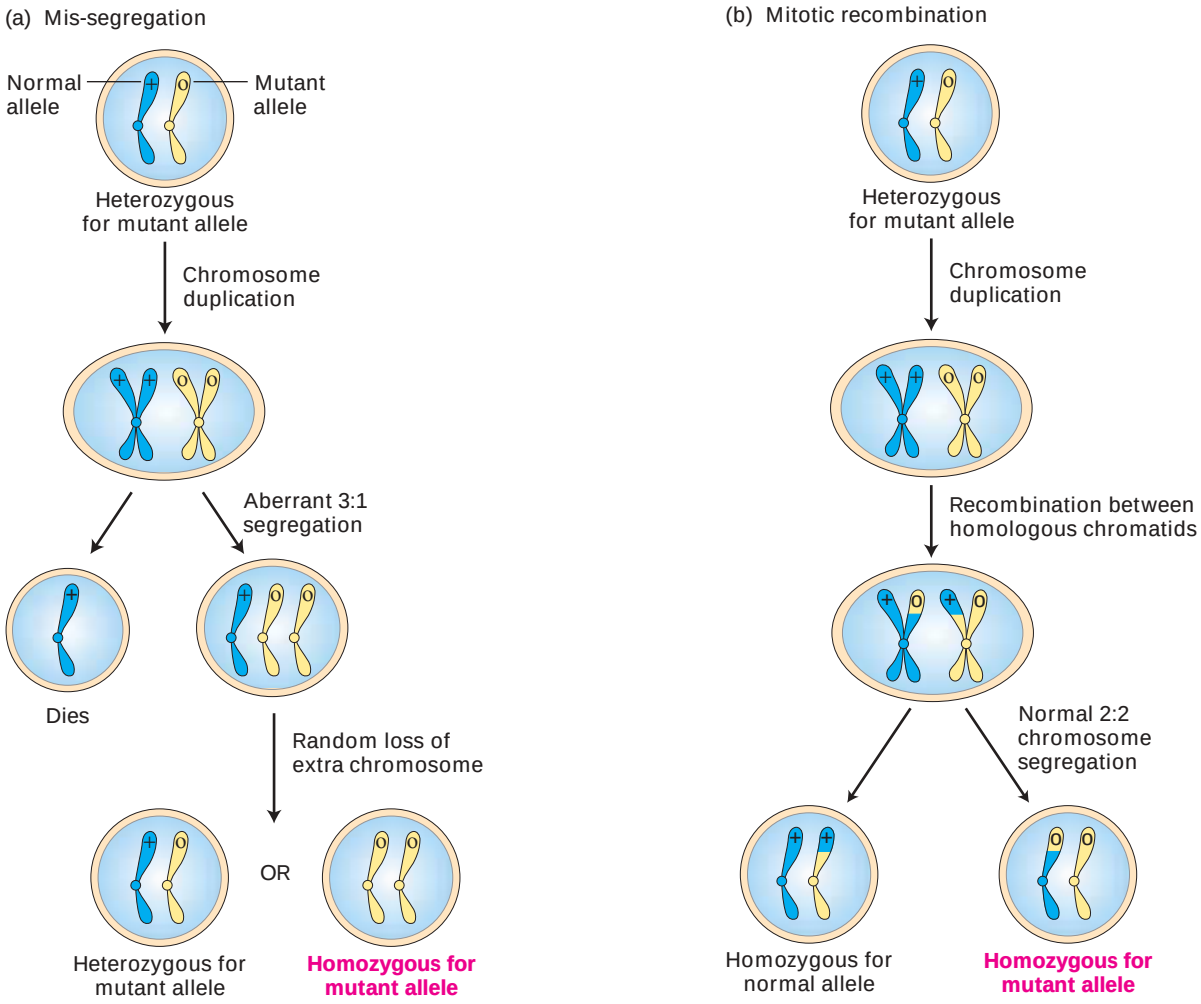
mutation of the normal *RB* gene on the other chromosome, giving rise to a cell that produces no functional Rb protein (Figure 23-10). Individuals with sporadic retinoblastoma, in contrast, inherit two normal *RB* alleles, each of which has undergone a loss-of-function somatic mutation in a single retinal cell. Because losing two copies of the *RB* gene is far less likely than losing one, sporadic retinoblastoma is rare, develops late in life, and usually affects only one eye.

If retinal tumors are removed before they become malignant, children with hereditary retinoblastoma often survive until adulthood and produce children. Because their germ cells contain one normal and one mutant *RB* allele, these individuals will, on average, pass on the mutant allele to half their children and the normal allele to the other half. Children who inherit the normal allele are normal if their other parent has two normal *RB* alleles. However, those who inherit the mutant allele have the same enhanced predisposition to develop retinal tumors as their affected parent, even though they inherit a normal *RB* allele from their other, normal parent. Thus the tendency to develop retinoblastoma is inherited as a dominant trait. As discussed below, many human tumors (not just retinal tumors) contain mutant *RB* alleles; most of these arise as the result of somatic mutations.

Inherited Forms of Colon and Breast Cancer Similar hereditary predisposition for other cancers has been associated with inherited mutations in other tumor-suppressor genes. For example, individuals who inherit a germ-line mutation in one *APC* allele develop thousands of precancerous intestinal polyps (see Figure 23-7). Since there is a high probability that one or more of these polyps will progress to malignancy, such individuals have a greatly increased risk for developing colon cancer before the age of 50. Likewise, women who inherit one mutant allele of *BRCA1*, another tumor-suppressor gene, have a 60 percent probability of developing breast cancer by age 50, whereas those who inherit two normal *BRCA1* alleles have a 2 percent probability of doing so. In women with hereditary breast cancer, loss of the second *BRCA1* allele, together with other mutations, is required for a normal breast duct cell to become malignant. However, *BRCA1* generally is not mutated in sporadic, noninherited breast cancer.

Loss of Heterozygosity Clearly, then, we can inherit a propensity to cancer by receiving a damaged allele of a tumor-suppressor gene from one of our parents; that is, we are heterozygous for the mutation. That in itself will not cause cancer, since the remaining normal allele prevents aberrant growth; the cancer is recessive. Subsequent loss or inactivation of the normal allele in a somatic cell, referred to as *loss of heterozygosity (LOH)*, is a prerequisite for cancer to develop. One common mechanism for LOH involves mis-segregation during mitosis of the chromosomes bearing the affected tumor-suppressor gene (Figure 23-11a). This process, also referred to as *nondisjunction*, is caused by failure of the spindle-assembly checkpoint, which normally prevents a metaphase cell with an abnormal mitotic spindle from completing mitosis (see Figure 21-32, 2). Another possible mechanism for LOH is mitotic recombination between a chromatid bearing the wild-type allele and a homologous chromatid bearing a mutant allele. As illustrated in Figure 23-11b, subsequent chromosome segregation can generate a daughter cell that is homozygous for the mutant tumor-suppressor allele. A third mechanism is the deletion or mutation of the normal copy of the tumor-suppressor gene; such a deletion can encompass a large chromosomal region and need not be a precise deletion of just the tumor-suppressor gene.

Hereditary cancers constitute about 10 percent of human cancers. It is important to remember, however, that the inherited, germ-line mutation alone is not sufficient to cause tumor development. In all cases, not only must the inherited normal tumor-suppressor allele be lost or inactivated, but mutations affecting other genes also are necessary for cancer to develop. Thus a person with a recessive tumor-suppressor-gene mutation can be exceptionally susceptible to environmental mutagens such as radiation.

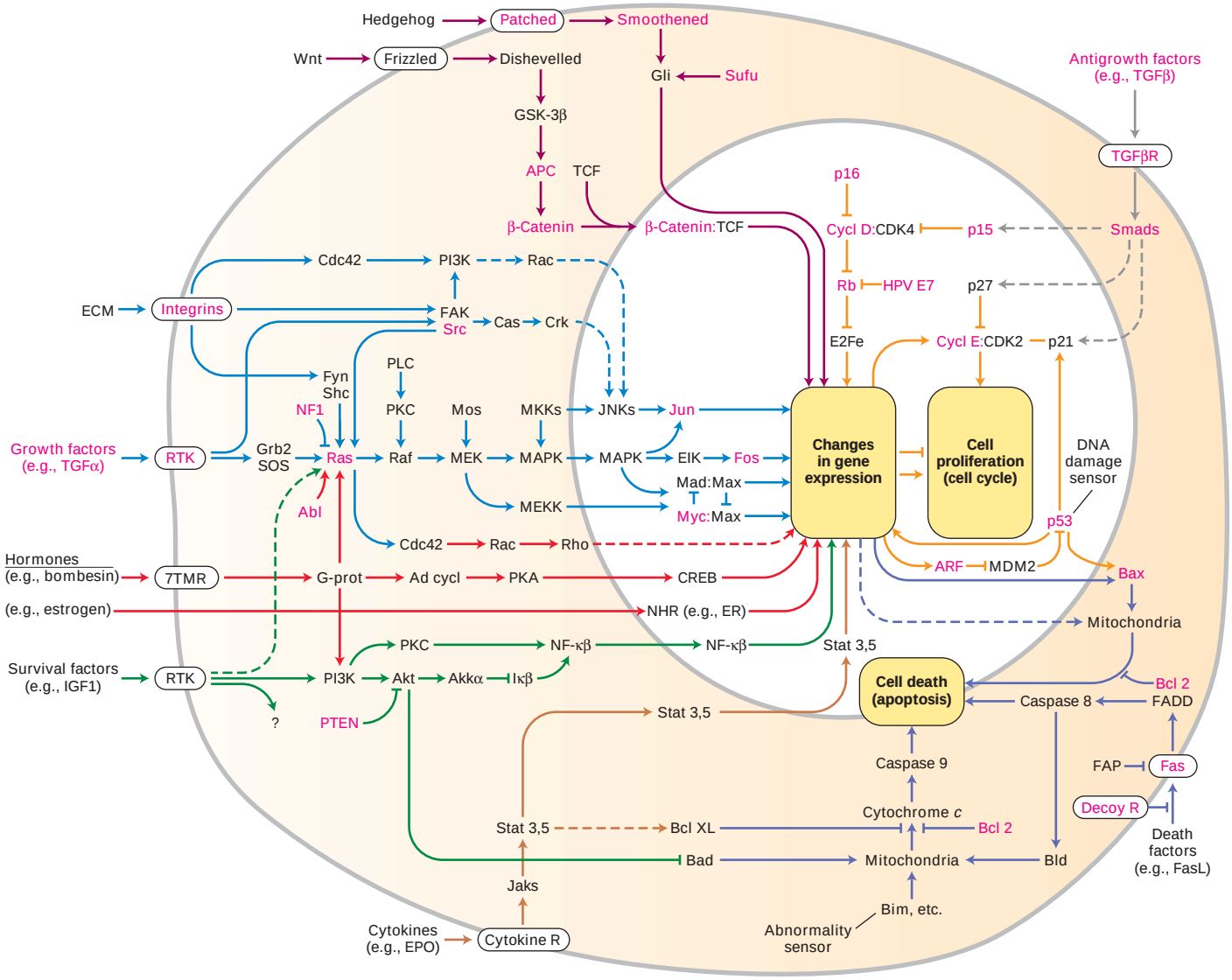


▲ FIGURE 23-11 **Two mechanisms for loss of heterozygosity (LOH) of tumor-suppressor genes.** A cell containing one normal and one mutant allele of a tumor-suppressor gene is generally phenotypically normal. (a) If formation of the mitotic spindle is defective, then the duplicated chromosomes bearing the normal and mutant alleles may segregate in an aberrant 3:1 ratio. A daughter cell that receives three chromosomes of a type will generally lose one, restoring the normal 2*n* chromosome number. Sometimes the resultant cell will contain one normal and one mutant allele, but sometimes it will be homozygous for the mutant allele. Note that such aneuploidy (abnormal chromosome constitution) is generally damaging or lethal to cells that have to develop into the many complex structures of an organism, but can often be tolerated in clones of cells that have limited fates and duties. (b) Mitotic recombination between a chromosome with a wild-type and a mutant allele, followed by chromosome segregation, can produce a cell that contains two copies of the mutant allele.

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

During normal development secreted signals such as Wnt, TGFβ, and Hedgehog (Hh) are frequently used to direct cells to particular developmental fates, which may include the property of rapid mitosis. The effects of such signals must be regulated so that growth is limited to the right time and place. Among the mechanisms available for reining in the effects of powerful developmental signals are inducible intracellular antagonists, receptor blockers, and competing signals (Chapter 15). Mutations that prevent such restraining mechanisms from operating are likely to be oncogenic, causing inappropriate or cancerous growth.

Hh signaling, which is used repeatedly during development to control cell fates, is a good example of a signaling pathway implicated in cancer induction. In the skin and cerebellum one of the human Hh proteins, Sonic hedgehog, stimulates cell division by binding to and inactivating a membrane protein called Patched1 (Ptc1) (see Figure 15-31). Loss-of-function mutations in *ptc1* permit cell proliferation in the absence of an Hh signal; thus *ptc1* is a tumor-suppressor gene. Not surprisingly, mutations in *ptc1* have been found in tumors of the skin and cerebellum in mice and



▲ FIGURE 23-12 **Cell circuitry that is affected by cancer-causing mutations.** Growth control and the cell cycle, the heart of cancer, are influenced by many types of signals, and the external inputs become integrated as the cell makes its decision about whether to divide or to continue dividing. Developmental pathways give cells their identity, and along with that identity often comes a commitment to proliferate or not. Genes known to be mutated in cancer cells are highlighted in red. Less firmly established pathways are shown with dashed lines. [From D. Hanahan and R. A. Weinberg, 2000, *Cell* **100**:57.]

humans. Mutations in other genes in the Hh signaling pathway are also associated with cancer. Some such mutations create oncogenes that turn on Hh target genes inappropriately; others are recessive mutations that affect negative regulators like Ptc1. As is the case for a number of other tumor-suppressor genes, complete loss of Ptc1 function would lead to early fetal death, since it is needed for development, so it is only the tumor cells that are homozygous *ptc1/ptc1*.

Many of the signaling pathways described in other chapters play roles in controlling embryonic development and cell proliferation in adult tissues. In recent years mutations affecting components of most of these signaling pathways have been linked to cancer (Figure 23-12). Indeed, once one gene in a developmental pathway has been linked to a type of human cancer, knowledge of the pathway gleaned from model organisms like worms, flies, or mice allows focused investigations of the possible involvement of additional pathway genes in other cases of the cancer. For example, *APC*, the critical first gene mutated on the path to colon carcinoma, is now known to be part of the Wnt signaling pathway, which led to the discovery of the involvement of *β-catenin* mutations in colon cancer. Mutations in tumor-suppressor developmental genes promote tumor formation

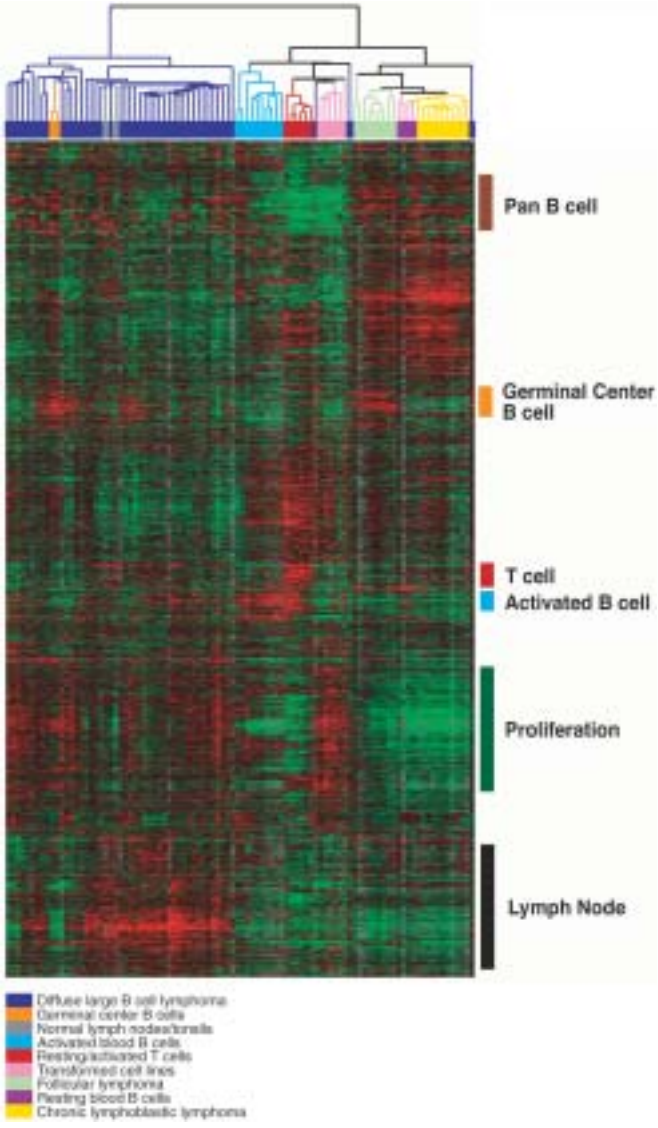
in tissues where the affected gene normally helps restrain growth, and not in cells where the primary role of the developmental regulator is to control cell fate but not growth. Mutations in developmental proto-oncogenes may induce tumor formation in tissues where an affected gene normally promotes growth or in another tissue where the gene has become aberrantly active.

DNA Microarray Analysis of Expression Patterns Can Reveal Subtle Differences Between Tumor Cells

Traditionally the properties of tumor and normal cells have been assessed by staining and microscopy. The prognosis for many tumors could be determined, within certain limits, from their histology. However the appearance of cells alone has limited information content, and better ways to discern the properties of cells are desirable both to understand tumorigenesis and to arrive at meaningful and accurate decisions about prognosis and therapy.

As we've seen, genetic studies can identify the single initiating mutation or series of mutations that cause transformation of normal cells into tumor cells, as in the case of colon cancer. After these initial events, however, the cells of a tumor undergo a cascade of changes reflecting the interplay between the initiating events and signals from outside. As a result, tumor cells can become quite different, even if they arise from the same initiating mutation or mutations. Although these differences may not be recognized from the appearance of cells, they can be detected from the cells' patterns of gene expression. DNA microarray analysis can determine the expression of thousands of genes simultaneously, permitting complex phenotypes to be defined at the molecular genetic level. (See Figures 9-35 and 9-36 for an explanation of this technique.)

Microarray analysis recently has been applied to diffuse large B cell lymphoma, a disease marked by the presence of abnormally large B lymphocytes throughout lymph nodes. Affected patients have highly variable outcomes, so the disease has long been suspected to be,



▶ EXPERIMENTAL FIGURE 23-13 **Differences in gene expression patterns determined by DNA microarray analysis can distinguish between otherwise phenotypically similar lymphomas.** Samples of mRNA were extracted from normal lymphocytes at different stages of differentiation and from malignant lymphocytes obtained from patients with three types of lymphoma. DNA microarray analysis of the extracted RNA determined the transcription of about 18,000 genes by each of the 96 experimental samples of normal and malignant lymphocytes relative to a control, reference sample. (See Figures 9-35 and 9-36 for description of microarray analysis.) The cluster diagram shown here includes the data from a selected set of genes whose expression differs the most in the various lymphocyte samples. An intense red color indicates that the experimental cells transcribe the gene represented by a particular DNA spot at a much higher level than the reference cells; intense green indicates the opposite. Black indicates similar transcript levels in the samples compared; gray indicates missing or excluded data. Each vertical column contains the data for a particular lymphocyte sample (see sample key). Each horizontal row contains data for a single gene. The genes were grouped according to their similar patterns of hybridization. For example, genes indicated by the green bar on the right are active in proliferating cells, such as transformed cultured cells (pink bar at top) or lymphoma cells (purple bar at top). The different cell samples (along the top of the diagram) also were grouped according to their similar expression patterns. The resulting dendrogram (tree diagram) shows that the samples from patients with diffuse large B cell lymphoma (purple samples) fall into two groups. One group is similar to relatively undifferentiated B lymphocytes in germinal centers (orange samples); the other is similar to more differentiated B cells (light-purple samples). [From A. A. Alizadeh et al., 2000, *Nature* **403**:505.]

in fact, multiple diseases. Microarray analysis of lymphomas from different patients revealed two groups distinguished by their patterns of gene expression (Figure 23-13). No morphological or visible criteria were found that could distinguish the two types of tumors. Patients with one tumor type defined by the microarray data survived much longer than those with the other type. Lymphomas whose gene expression is similar to that of B lymphocytes in the earliest stages of differentiation have a better prognosis; lymphomas whose gene expression is closer to that of more differentiated B lymphocytes have a worse prognosis. Similar analyses of the gene expression patterns, or "signatures," of other tumors are likely to improve classification and diagnosis, allowing informed decisions about treatments, and also provide insights into the properties of tumor cells. ▮

KEY CONCEPTS OF SECTION 23.2

The Genetic Basis of Cancer

- Dominant gain-of-function mutations in proto-oncogenes and recessive loss-of-function mutations in tumor-suppressor genes are oncogenic.
- Among the proteins encoded by proto-oncogenes are growth-promoting signaling proteins and their receptors, signal-transduction proteins, transcription factors, and apoptotic proteins (see Figure 23-8).
- An activating mutation of one of the two alleles of a proto-oncogene converts it to an oncogene. This can occur by point mutation, gene amplification, and gene translocation.
- The first human oncogene to be identified encodes a constitutively active form of Ras, a signal-transduction protein. This oncogene was isolated from a human bladder carcinoma (see Figure 23-4).
- Slow-acting retroviruses can cause cancer by integrating near a proto-oncogene in such a way that transcription of the cellular gene is activated continuously and inappropriately.
- Tumor-suppressor genes encode proteins that directly or indirectly slow progression through the cell cycle, checkpoint-control proteins that arrest the cell cycle, components of growth-inhibiting signaling pathways, pro-apoptotic proteins, and DNA-repair enzymes.
- The first tumor-suppressor gene to be recognized, *RB*, is mutated in retinoblastoma and some other tumors.
- Inheritance of a single mutant allele of *RB* greatly increases the probability that a specific kind of cancer will develop, as is the case for many other tumor-suppressor genes (e.g., *APC* and *BRCA1*).
- In individuals born heterozygous for a tumor-suppressor gene, a somatic cell can undergo loss of heterozygosity (LOH) by mitotic recombination, chromosome mis-segregation, mutation, or deletion (see Figure 23-11).
- Many genes that regulate normal developmental processes encode proteins that function in various signaling pathways (see Figure 23-12). Their normal roles in regulating where and when growth occurs are reflected in the character of the tumors that arise when the genes are mutated.
- DNA microarray analysis can identify differences in gene expression between types of tumor cells that are indistinguishable by traditional criteria. Some tumor cells appear to be related to specific types of normal cells at certain stages of development based on similarities in their expression patterns.

23.3 Oncogenic Mutations in Growth-Promoting Proteins

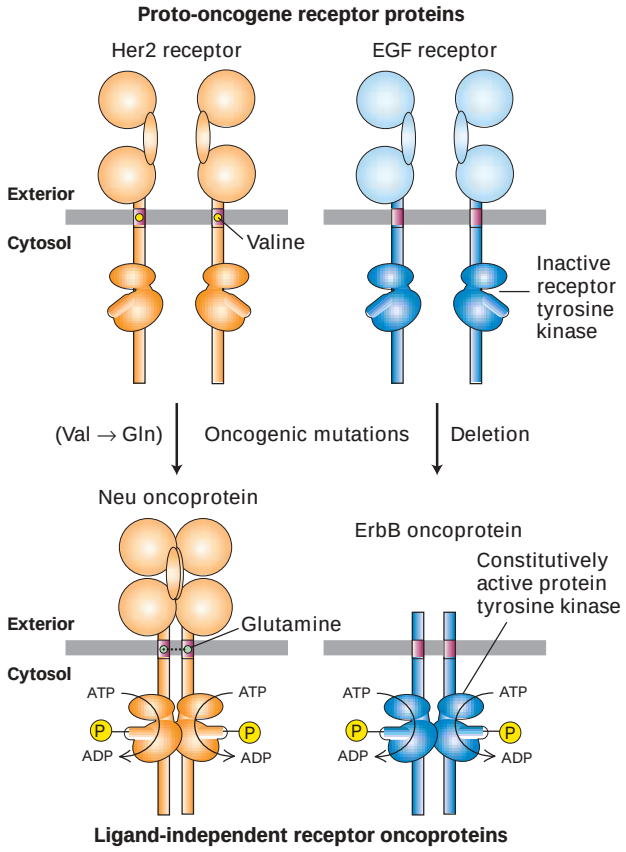
Genes encoding each class of cell regulatory protein depicted in Figure 23-8 have been identified as proto-oncogenes or tumor-suppressor genes. In this section we examine in more detail how mutations that result in the unregulated, constitutive activity of certain proteins or in their overproduction promote cell proliferation and transformation, thereby contributing to carcinogenesis. In each case we see how a rare cell that has undergone a very particular sort of mutation becomes abundant owing to its uncontrolled proliferation.

Oncogenic Receptors Can Promote Proliferation in the Absence of External Growth Factors

Although oncogenes theoretically could arise from mutations in genes encoding growth-promoting signaling molecules, this rarely occurs. In fact, only one such naturally occurring oncogene, *sis*, has been discovered. The *sis* oncogene, which encodes a type of platelet-derived growth factor (PDGF), can aberrantly autostimulate proliferation of cells that normally express the PDGF receptor.

In contrast, oncogenes encoding cell-surface receptors that transduce growth-promoting signals have been associated with several types of cancer. The receptors for many such growth factors have intrinsic protein-tyrosine kinase activity in their cytosolic domains, an activity that is quiescent until activated. Ligand binding to the external domains of these **receptor tyrosine kinases (RTKs)** leads to their dimerization and activation of their kinase activity, initiating an intracellular signaling pathway that ultimately promotes proliferation.

In some cases, a point mutation changes a normal RTK into one that dimerizes and is constitutively active in the absence of ligand. For instance, a single point mutation converts the normal Her2 receptor into the Neu oncoprotein, which is an initiator of certain mouse cancers (Figure 23-14, *left*). Similarly, human tumors called multiple endocrine neoplasia type 2 produce a constitutively active dimeric Glia-derived neurotrophic factor (GDNF) receptor that results



▲ FIGURE 23-14 **Effects of oncogenic mutations in proto-oncogenes that encode cell-surface receptors.** *Left:* A mutation that alters a single amino acid (valine to glutamine) in the transmembrane region of the Her2 receptor causes dimerization of the receptor, even in the absence of the normal EGF-related ligand, making the oncoprotein Neu a constitutively active kinase. *Right:* A deletion that causes loss of the extracellular ligand-binding domain in the EGF receptor leads, for unknown reasons, to constitutive activation of the kinase activity of the resulting oncoprotein ErbB.

from a point mutation in the extracellular domain. In other cases, deletion of much of the extracellular ligand-binding domain produces a constitutively active oncogenic receptor. For example, deletion of the extracellular domain of the normal EGF receptor converts it to the dimeric ErbB oncoprotein (Figure 23-14, *right*).

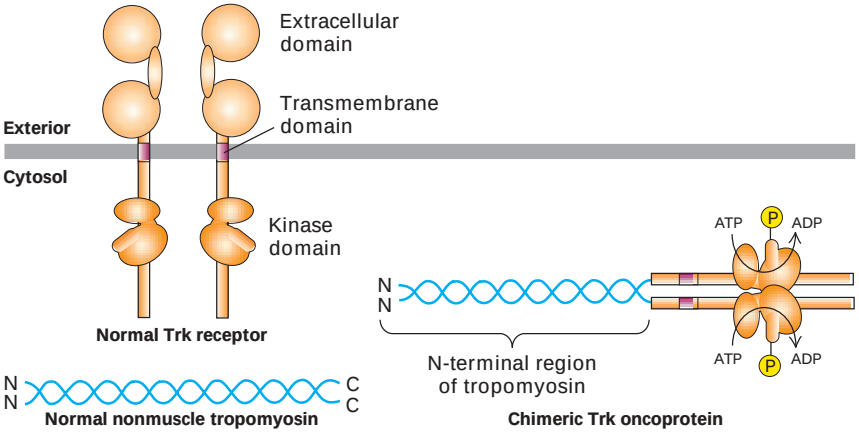
Mutations leading to overproduction of a normal RTK also can be oncogenic. For instance, many human breast cancers overproduce a normal Her2 receptor. As a result, the cells are stimulated to proliferate in the presence of very low concentrations of EGF and related hormones, concentrations too low to stimulate proliferation of normal cells.

MEDICINE

A monoclonal antibody specific for Her2 has been a strikingly successful new treatment for the subset of breast cancers that overproduce Her2. Her2 antibody injected into the blood recognizes Her2 and causes it to be internalized, selectively killing the cancer cells without any apparent effect on normal breast (and other) cells that produce moderate amounts of Her2. ▮

Another mechanism for generating an oncogenic receptor is illustrated by the human *trk* oncogene, which was isolated from a colon carcinoma. This oncogene encodes a chimeric protein as the result of a chromosomal translocation that replaced the sequences encoding most of the extracellular domain of the normal Trk receptor with the sequences encoding the N-terminal amino acids of nonmuscle tropomyosin (Figure 23-15). The translocated tropomyosin segment can mediate dimerization of the chimeric Trk receptor by forming a coiled-coil structure, leading to activation of the kinase domains in the absence of ligand. The normal Trk protein is a cell-surface receptor tyrosine kinase that binds a nerve growth factor (Chapter 22). In contrast, the constitutively active Trk oncoprotein is localized in the cytosol, since the N-terminal signal sequence directing it to the membrane has been deleted.

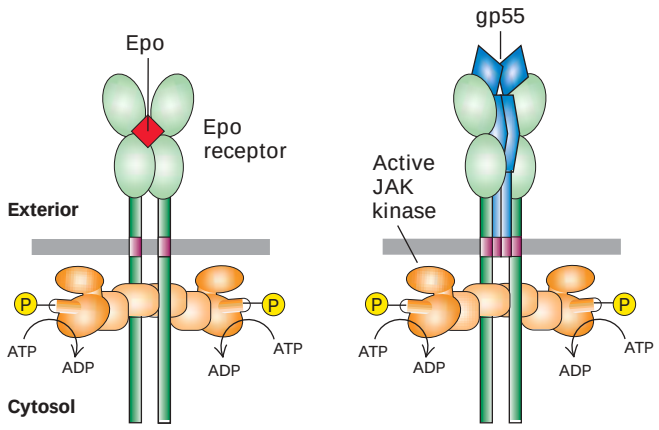
▶ FIGURE 23-15 **Domain structures of normal tropomyosin, the normal Trk receptor, and chimeric Trk oncoprotein.** A chromosomal translocation results in replacement of most of the extracellular domain of the normal human Trk protein, a receptor tyrosine kinase, with the N-terminal domain of nonmuscle tropomyosin. Dimerized by the tropomyosin segment, the Trk oncoprotein kinase is constitutively active. Unlike the normal Trk, which is localized to the plasma membrane, the Trk oncoprotein is found in the cytosol. [See F. Coulier et al., 1989, *Mol. Cell Biol.* **9**:15.]



Viral Activators of Growth-Factor Receptors Act as Oncoproteins

Viruses use their own tricks to cause cancer, presumably to increase the production of virus from the infected cancer cells. For example, a retrovirus called spleen focus-forming virus (SFFV) induces erythroleukemia (a tumor of erythroid progenitors) in adult mice by manipulating a normal developmental signal. The proliferation, survival, and differentiation of erythroid progenitors into mature red cells absolutely require erythropoietin (Epo) and the corresponding Epo receptor (see Figure 14-7). A mutant SFFV envelope glycoprotein, termed gp55, is responsible for the oncogenic effect of the virus. Although gp55 cannot function as a normal retrovirus envelope protein in virus budding and infection, it has acquired the remarkable ability to bind to and activate Epo receptors in the same cell (Figure 23-16). By inappropriately and continuously stimulating the proliferation of erythroid progenitors, gp55 induces formation of excessive numbers of erythrocytes. Malignant clones of erythroid progenitors emerge several weeks after SFFV infection as a result of further mutations in these aberrantly proliferating cells.

Another example of this phenomenon is provided by human papillomavirus (HPV), a DNA virus that causes genital warts. A papillomavirus protein designated E5, which contains only 44 amino acids, spans the plasma membrane and forms a dimer or trimer. Each E5 polypeptide can form a stable complex with one endogenous receptor for PDGF, thereby aggregating two or more PDGF receptors within the plane of the plasma membrane. This mimics hormone-mediated receptor dimerization, causing sustained receptor activation and eventually cell transformation.



▲ FIGURE 23-16 **Activation of the erythropoietin (Epo) receptor by the natural ligand, Epo, or a viral oncoprotein.** Binding of Epo dimerizes the receptor and induces formation of erythrocytes from erythroid progenitor cells. Normally cancers occur when progenitor cells infected by the spleen focus-forming virus produce the Epo receptor and viral gp55, both localized to the plasma membrane. The transmembrane domains of dimeric gp55 specifically bind the Epo receptor, dimerizing and activating the receptor in the absence of Epo. [See S. N. Constantinescu et al., 1999, *EMBO J.* **18**:3334.]

Many Oncogenes Encode Constitutively Active Signal-Transduction Proteins

A large number of oncogenes are derived from proto-oncogenes whose encoded proteins aid in transducing signals from an activated receptor to a cellular target. We describe several examples of such oncogenes; each is expressed in many types of tumor cells.

Ras Pathway Components Among the best-studied oncogenes in this category are the *ras*^{D} genes, which were the first nonviral oncogenes to be recognized. A point mutation that substitutes any amino acid for glycine at position 12 in the Ras sequence can convert the normal protein into a constitutively active oncoprotein. This simple mutation reduces the protein's GTPase activity, thus maintaining Ras in the active GTP-bound state. Constitutively active Ras oncoproteins are produced by many types of human tumors, including bladder, colon, mammary, skin, and lung carcinomas, neuroblastomas, and leukemias.

As we saw in Chapter 14, Ras is a key component in transducing signals from activated receptors to a cascade of protein kinases. In the first part of this pathway, a signal from an activated RTK is carried via two adapter proteins to Ras, converting it to the active GTP-bound form (see Figure 14-16). In the second part of the pathway, activated Ras transmits the signal via two intermediate protein kinases to MAP kinase. The activated MAP kinase then phosphorylates a number of transcription factors that induce synthesis of important cell-cycle and differentiation-specific proteins (see Figure 14-21). Activating Ras mutations short-circuit the first part of this pathway, making upstream activation triggered by ligand binding to the receptor unnecessary.

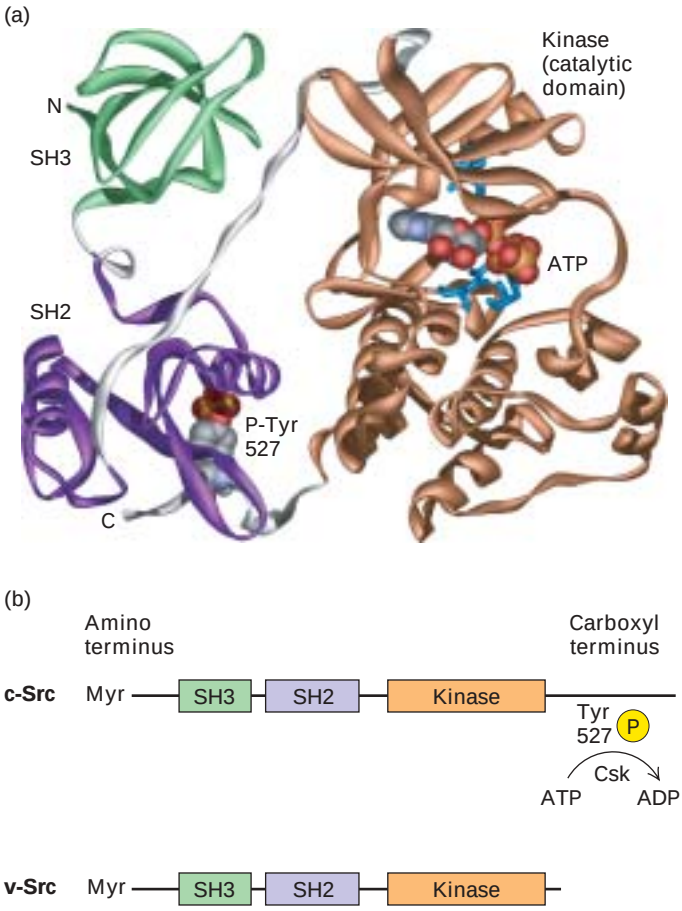
Oncogenes encoding other altered components of the RTK-Ras–MAP kinase pathway also have been identified. One example, found in certain transforming mouse retroviruses, encodes a constitutively activated Raf serine/threonine kinase, which is in the pathway between Ras and MAP kinase. Another is the *crk* (pronounced "crack") oncogene found in avian sarcoma virus, which causes certain tumors when overexpressed. The Crk protein, which contains one SH2 and two SH3 domains, is similar to the GRB2 adapter protein that functions between an RTK and Ras (see Figure 14-16). The SH2 and SH3 domains in GRB2 and other adapter proteins mediate formation of specific protein aggregates that normally serve as signaling units for cellular events. Overproduction of Crk leads to formation of protein aggregates that inappropriately transduce signals, thus

promoting the growth and metastatic abilities characteristic of cancer cells.

Constitutive Ras activation can also arise from a recessive loss-of-function mutation in a GTPase-accelerating protein (GAP). The normal GAP function is to accelerate hydrolysis of GTP and the conversion of active GTP-bound Ras to inactive GDP-bound Ras (see Figure 3-29). The loss of GAP leads to sustained Ras activation of downstream signal-transduction proteins. For example, neurofibromatosis, a benign tumor of the sheath cells that surround nerves, is caused by loss of both alleles of *NF1*, which encodes a GAP-type protein. Individuals with neurofibromatosis have inherited a single mutant *NF1* allele; subsequent somatic mutation in the other allele leads to formation of neurofibromas. Thus *NF1*, like *RB*, is a tumor-suppressor gene, and neurofibromatosis, like hereditary retinoblastoma, is inherited as an autosomal dominant trait.

Src Protein Kinase Several oncogenes, some initially identified in human tumors, others in transforming retroviruses, encode cytosolic protein kinases that normally transduce signals in a variety of intracellular signaling pathways. Indeed the first oncogene to be discovered, v-*src* from Rous sarcoma retrovirus, encodes a constitutively active protein-tyrosine kinase. At least eight mammalian proto-oncogenes encode a family of nonreceptor tyrosine kinases related to the v-Src protein. In addition to a catalytic domain, these kinases contain SH2 and SH3 protein-protein interaction domains. The kinase activity of cellular Src and related proteins normally is inactivated by phosphorylation of the tyrosine residue at position 527, which is six residues from the C-terminus (Figure 23-17a, b). Hydrolysis of phosphotyrosine 527 by a specific phosphatase enzyme normally activates c-Src. Tyrosine 527 is often missing or altered in Src oncoproteins that have constitutive kinase activity; that is, they do not require activation by a phosphatase. In Rous sarcoma virus, for instance, the *src* gene has suffered a deletion that eliminates the C-terminal 18 amino acids of c-Src; as a consequence the v-Src kinase is constitutively active (Figure 23-17b). Phosphorylation of target proteins by aberrant Src oncoproteins contributes to abnormal proliferation of many types of cells.

Abl Protein Kinase Another oncogene encoding a cytosolic nonreceptor protein kinase is generated by a chromosomal translocation that fuses a part of the c-*abl* gene, which encodes a tyrosine kinase, with part of the *bcr* gene, whose function is unknown. The normal c-Abl protein promotes branching of filamentous actin and extension of cell processes, so it may function primarily to control the cytoskeleton and cell shape. The chimeric oncoproteins encoded by the *bcr-abl* oncogene form a tetramer that exhibits unregulated and continuous Abl kinase activity. (This is similar to dimerization and activation of the chimeric Trk oncoprotein shown in Figure 23-15.) Bcr-Abl can phosphorylate and thereby activate many



▲ FIGURE 23-17 **Structure of Src tyrosine kinases and activation by an oncogenic mutation.** (a) Three-dimensional structure of Hck, one of several Src kinases in mammals. Binding of phosphotyrosine 527 to the SH2 domain induces conformational strains in the SH3 and kinase domains, distorting the kinase active site so it is catalytically inactive. The kinase activity of cellular Src proteins is normally activated by removing the phosphate on tyrosine 527. (b) Domain structure of c-Src and v-Src. Phosphorylation of tyrosine 527 by Csk, another cellular tyrosine kinase, inactivates the Src kinase activity. The transforming v-Src oncoprotein encoded by Rous sarcoma virus is missing the C-terminal 18 amino acids including tyrosine 527 and thus is constitutively active. [Part (a) from F. Sicheri et al., 1997, *Nature* **385**:602. See also T. Pawson, 1997, *Nature* **385**:582, and W. Xu et al., 1997, *Nature* **385**:595.]

intracellular signal-transduction proteins; at least some of these proteins are not normal substrates of Abl. For instance, Bcr-Abl can activate JAK2 kinase and STAT5 transcription factor, which normally are activated by binding of growth factors (e.g., erythropoietin) to cell-surface receptors (see Figure 14-12).

The chromosomal translocation that forms *bcr-abl* generates the diagnostic *Philadelphia chromosome*, discovered in 1960 (see Figure 10-29). The identity of the genes involved

was discovered by molecular cloning of the relevant translocation "joint," allowing biochemical study of the Bcr-Abl oncoprotein. If this translocation occurs in a hematopoietic cell in the bone marrow, the activity of the chimeric *bcr-abl* oncogene results in the initial phase of human chronic myelogenous leukemia (CML), characterized by an expansion in the number of white blood cells. A second mutation in a cell carrying *bcr-abl* (e.g., in *p53*) leads to acute leukemia, which often kills the patient. The CML chromosome translocation was only the first of a long series of distinctive, or "signature," chromosome translocations linked to particular forms of leukemia. Each one presents an opportunity for greater understanding of the disease and for new therapies. In the case of CML, that second step to successful therapy has already been taken.

After a painstaking search, an inhibitor of Abl kinase named STI-571 (Gleevec) was identified as a possible treatment for CML in the early 1990s. STI-571 is highly lethal to CML cells while sparing normal cells. After clinical trials showing STI-571 is remarkably effective in treating CML despite some side effects, it was approved by the FDA in 2001, the first cancer drug targeted to a signal-transduction protein unique to tumor cells. STI-571 inhibits several other tyrosine kinases that are implicated in different cancers and has been successful in trials for treating these diseases as well. There are 96 tyrosine kinases encoded in the human genome, so drugs related to Gleevec may be useful in controlling the activities of all these proteins. ▮

The Gleevec story illustrates how genetics—the discovery of the Philadelphia chromosome and the critical oncogene it creates—together with biochemistry—discovery of the molecular action of the Abl protein—can lead to a powerful new therapy. In general, each difference between cancer cells and normal cells provides a new opportunity to identify a specific drug that kills just the cancer cells or at least stops their uncontrolled growth.

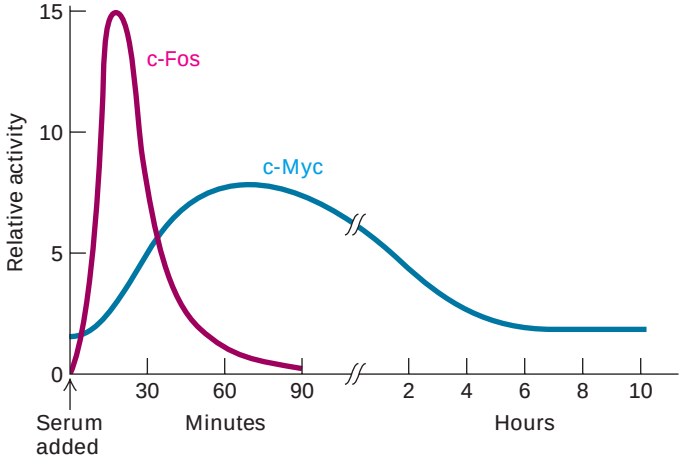
Inappropriate Production of Nuclear Transcription Factors Can Induce Transformation

By one mechanism or another, the proteins encoded by all proto-oncogenes and oncogenes eventually cause changes in gene expression. This is reflected in the differences in the proportions of different mRNAs in growing cells and quiescent cells, as well as similar differences between tumor cells and their normal counterparts. As discussed in the last section, we can now measure such differences in the expression of thousands of genes with DNA microarrays (see Figure 23-13).

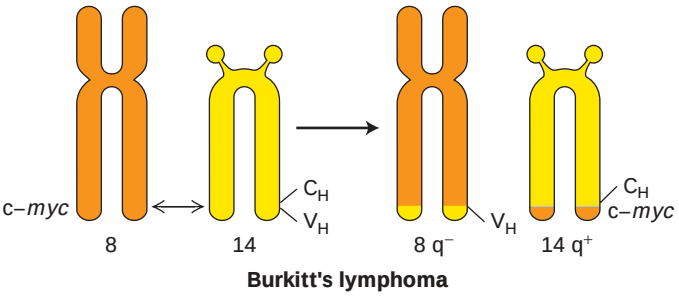
Since the most direct effect on gene expression is exerted by transcription factors, it is not surprising that many oncogenes encode transcription factors. Two examples are *jun* and *fos*, which initially were identified in transforming retroviruses and later found to be overexpressed in some human tumors. The c-*jun* and c-*fos* proto-oncogenes encode proteins that sometimes associate to form a heterodimeric transcription factor, called AP1, that binds to a sequence found in promoters and enhancers of many genes (see Figure 11-24). Both Fos and Jun also can act independently as transcription factors. They function as oncoproteins by activating transcription of key genes that encode growth-promoting proteins or by inhibiting transcription of growth-repressing genes.

Many nuclear proto-oncogene proteins are induced when normal cells are stimulated to grow, indicating their direct role in growth control. For example, PDGF treatment of quiescent 3T3 cells induces an ≈50-fold increase in the production of c-Fos and c-Myc, the normal products of the *fos* and *myc* proto-oncogenes. Initially there is a transient rise of c-Fos and later a more prolonged rise of c-Myc (Figure 23-18). The levels of both proteins decline within a few hours, a regulatory effect that may, in normal cells, help to avoid cancer. As discussed in Chapter 21, c-Fos and c-Myc stimulate transcription of genes encoding proteins that promote progression through the G_1 phase of the cell cycle and the G_1 to S transition. In tumors, the oncogenic forms of these or other transcription factors are frequently expressed at high and unregulated levels.

In normal cells, c-Fos and c-Myc mRNAs and the proteins they encode are intrinsically unstable, leading to their rapid loss after the genes are induced. Some of the changes



▲ EXPERIMENTAL FIGURE 23-18 **Addition of serum to quiescent 3T3 cells yields a marked increase in the activity of two proto-oncogene products, c-Fos and c-Myc.** Serum contains factors like platelet-derived growth factor (PDGF) that stimulate the growth of quiescent cells. One of the earliest effects of growth factors is to induce expression of c-*fos* and c-*myc,* whose encoded proteins are transcription factors.
[See M. E. Greenberg and E. B. Ziff, 1984, *Nature* **311**:433.]



▲ FIGURE 23-19 **Chromosomal translocation in Burkitt's lymphoma.** As a result of a translocation between chromosomes 8 and 14, the c-*myc* gene is placed adjacent to the gene for part of the antibody heavy chain (C_H), leading to overproduction of the Myc transcription factor in lymphocytes and hence their growth into a lymphoma.

that turn c-*fos* from a normal gene to an oncogene involve genetic deletions of sequences that make the Fos mRNA and protein short-lived. Conversion of the c-*myc* proto-oncogene into an oncogene can occur by several different mechanisms. In cells of the human tumor known as *Burkitt's lymphoma*, the c-*myc* gene is translocated to a site near the heavy-chain antibody genes, which are normally active in antibody-producing white blood cells (Figure 23-19). The c-*myc* translocation is a rare aberration of the normal DNA rearrangements that occur during maturation of antibody-producing cells. The translocated *myc* gene, now regulated by the antibody gene enhancer, is continually expressed, causing the cell to become cancerous. Localized amplification of a segment of DNA containing the *myc* gene, which occurs in several human tumors, also causes inappropriately high production of the otherwise normal Myc protein.

KEY CONCEPTS OF SECTION 23.3

Oncogenic Mutations in Growth-Promoting Proteins

- Mutations or chromosomal translocations that permit RTKs for growth factors to dimerize in the absence of their normal ligands lead to constitutive receptor activity (see Figures 23-14 and 23-15). Such activation ultimately induces changes in gene expression that can transform cells. Overproduction of growth factor receptors can have the same effect and lead to abnormal cell proliferation.
- Certain virus-encoded proteins can bind to and activate host-cell receptors for growth factors, thereby stimulating cell proliferation in the absence of normal signals.
- Most tumor cells produce constitutively active forms of one or more intracellular signal-transduction proteins, causing growth-promoting signaling in the absence of normal growth factors.
- A single point mutation in Ras, a key transducing protein in many signaling pathways, reduces its GTPase activity, thereby maintaining it in an activated state.
- The activity of Src, a cytosolic signal-transducing protein-tyrosine kinase, normally is regulated by reversible phosphorylation and dephosphorylation of a tyrosine residue near the C-terminus (see Figure 23-17). The unregulated activity of Src oncoproteins that lack this tyrosine promotes abnormal proliferation of many cells.
- The Philadelphia chromosome results from a chromosomal translocation that produces the chimeric *bcr-abl* oncogene. The unregulated Abl kinase activity of the Bcr-Abl oncoprotein is responsible for its oncogenic effect. An Abl kinase inhibitor (Gleevec) is effective in treating chronic myelogenous leukemia (CML) and may work against other cancers.
- Inappropriate production of nuclear transcription factors such as Fos, Jun, and Myc can induce transformation. In Burkitt's lymphoma cells, c-*myc* is translocated close to an antibody gene, leading to overproduction of c-Myc (see Figure 23-19).

23.4 Mutations Causing Loss of Growth-Inhibiting and Cell-Cycle Controls

Normal growth and development depends on a finely tuned, highly regulated balance between growth-promoting and growth-inhibiting pathways. Mutations that disrupt this balance can lead to cancer. Most of the mutations discussed in the previous section cause inappropriate activity of growth-promoting pathways. Just as critical are mutations that decrease the activity of growth-inhibiting pathways when they are needed.

For example, transforming growth factor β (TGFβ), despite its name, inhibits proliferation of many cell types, including most epithelial and immune system cells. Binding of TGFβ to its receptor induces activation of cytosolic Smad transcription factors (see Figure 14-2). After translocating to the nucleus, Smads can promote expression of the gene encoding p15, which causes cells to arrest in G_1. TGFβ signaling also promotes expression of genes encoding extracellular matrix proteins and plasminogen activator inhibitor 1 (PAI-1), which reduces the plasmin-catalyzed degradation of the matrix. Loss-of-function mutations in either TGFβ receptors or in Smads thus promote cell proliferation and probably contribute to the invasiveness and metastasis of tumor cells (Figure 23-20). Such mutations have in fact been found in a variety of human cancers, as we describe in Chapter 14.

The complex mechanisms for regulating the eukaryotic cell cycle are prime targets for oncogenic mutations. Both positive- and negative-acting proteins precisely control the

entry of cells into and their progression through the cell cycle, which consists of four main phases: G_1, S, G_2, and mitosis (see Figure 21-2). This regulatory system assures the proper coordination of cellular growth during G_1 and G_2, DNA synthesis during the S phase, and chromosome segregation and cell division during mitosis. In addition, cells that have sustained damage to their DNA normally are arrested before their DNA is replicated. This arrest allows time for the DNA damage to be repaired; alternatively, the arrested cells are directed to commit suicide via programmed cell death. The whole cell-cycle control system functions to prevent cells from becoming cancerous. As might be expected, mutations in this system often lead to abnormal development or contribute to cancer.

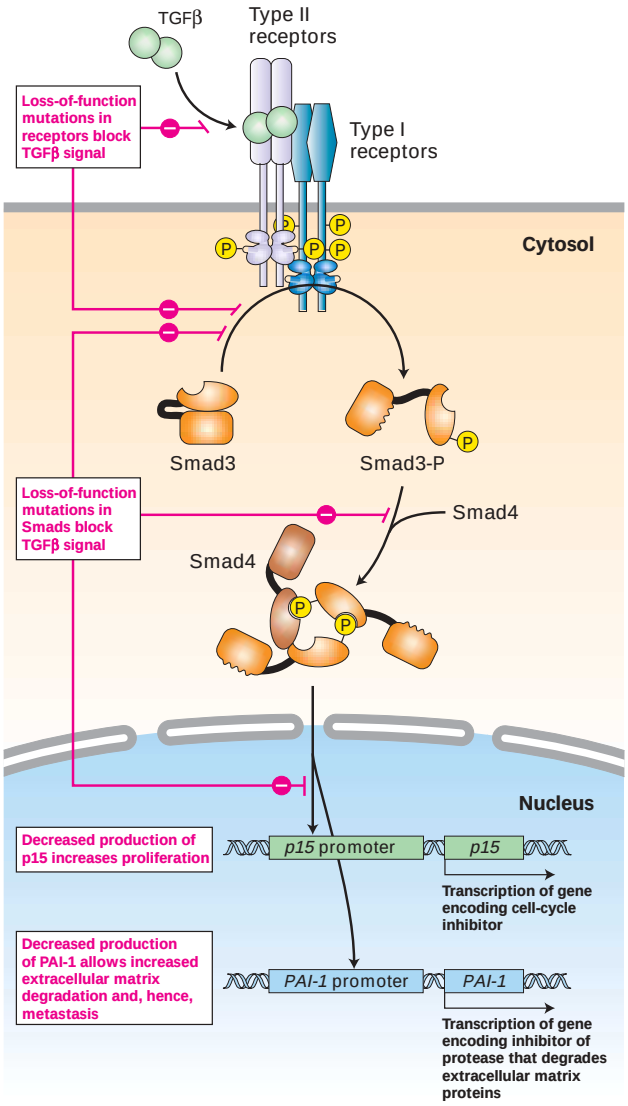
Mutations That Promote Unregulated Passage from G_1 to S Phase Are Oncogenic

Once a cell progresses past a certain point in late G_1, called the **restriction point,** it becomes irreversibly committed to entering the S phase and replicating its DNA (see Figure 21-28). D-type **cyclins, cyclin-dependent kinases (CDKs),** and the Rb protein are all elements of the control system that regulate passage through the restriction point.

The expression of D-type cyclin genes is induced by many extracellular growth factors, or **mitogens.** These cyclins assemble with their partners CDK4 and CDK6 to generate catalytically active cyclin-CDK complexes, whose kinase activity promotes progression past the restriction point. Mitogen withdrawal prior to passage through the restriction point leads to accumulation of p16. Like p15 mentioned above, p16 binds specifically to CDK4 and CDK6, thereby inhibiting their kinase activity and causing G_1 arrest. Under normal circumstances, phosphorylation of Rb protein is initiated midway through G_1 by active cyclin D-CDK4 and cyclin D-CDK6 complexes. Rb phosphorylation is completed by other cyclin-CDK complexes in late G_1, allowing activation of E2F transcription factors, which stimulate transcription of genes encoding proteins required for DNA synthesis. The complete phosphorylation of Rb irreversibly commits the cell to DNA synthesis. Most tumors contain an oncogenic mutation that causes overproduction or loss of one of the components of this pathway such that the cells are propelled into the S phase in the absence of the proper extracellular growth signals (Figure 23-21).

Elevated levels of cyclin D1, for example, are found in many human cancers. In certain tumors of antibody-producing B lymphocytes, for instance, the *cyclin D1* gene is translocated such that its transcription is under control of an antibody-gene enhancer, causing elevated cyclin D1 production throughout the cell cycle, irrespective of extracellular signals. (This phenomenon is analogous to the c-*myc* translocation in Burkitt's lymphoma cells discussed earlier.) That cyclin D1 can function as an oncoprotein was shown by studies with transgenic mice in which the *cyclin D1* gene was placed under control of an enhancer specific for mammary ductal cells. Initially the ductal cells underwent hyperproliferation, and eventually breast tumors developed in these transgenic mice. Amplification of the *cyclin D1* gene and concomitant overproduction of the cyclin D1 protein is common in human breast cancer.

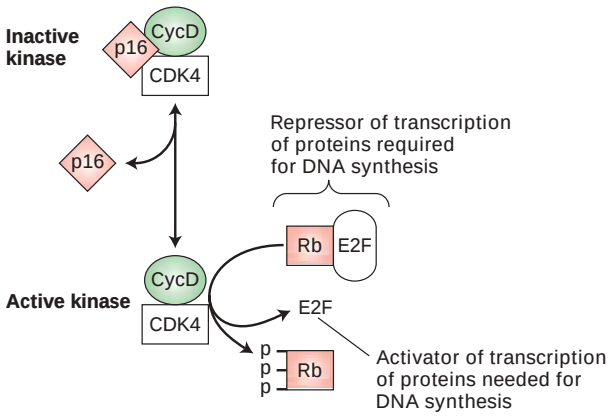
The proteins that function as cyclin-CDK inhibitors play an important role in regulating the cell cycle (Chapter 21). In particular, loss-of-function mutations that prevent p16 from



▲ FIGURE 23-20 **Effect of loss of TGFβ signaling.** Binding of TGFβ, an antigrowth factor, causes activation of Smad transcription factors. In the absence of TGFβ signaling due to either a receptor mutation or a SMAD mutation, cell proliferation and invasion of the surrounding extracellular matrix (ECM) increase. [See X. Hua et al., 1998, *Genes & Develop.* **12**:3084.]

MEDIA CONNECTIONS
Focus Animation: TGFβ Signaling Pathway

MEDIA CONNECTIONS
Overview Animation: Cell-Cycle Control



▲ FIGURE 23-21 **Restriction point control.** Unphosphorylated Rb protein binds transcription factors collectively called E2F and thereby prevents E2F-mediated transcriptional activation of many genes whose products are required for DNA synthesis (e.g., DNA polymerase). The kinase activity of cyclin D-CDK4 phosphorylates Rb, thereby activating E2F; this kinase activity is inhibited by p16. Overproduction of cyclin D, a positive regulator, or loss of the negative regulators p16 and Rb, commonly occurs in human cancers.

inhibiting cyclin D-CDK4/6 kinase activity are common in several human cancers. As Figure 23-21 makes clear, loss of p16 mimics overproduction of cyclin D1, leading to Rb hyperphosphorylation and release of active E2F transcription factor. Thus p16 normally acts as a tumor suppressor. Although the *p16* tumor-suppressor gene is deleted in some human cancers, in others the *p16* sequence is normal. In these latter cancers (e.g., lung cancer), the *p16* gene is inactivated by hypermethylation of its promoter region, which prevents transcription. What promotes this change in the methylation of *p16* is not known, but it prevents production of this important cell-cycle control protein.

We've seen already that inactivating mutations in both *RB* alleles lead to childhood retinoblastoma, a relatively rare type of cancer. However, loss of *RB* gene function also is found in more common cancers that arise later in life (e.g., carcinomas of lung, breast, and bladder). These tissues, unlike retinal tissue, most likely produce other proteins whose function is redundant with that of Rb, and thus loss of Rb is not so critical. Several proteins are known that are related in structure and probably function to Rb. In addition to inactivating mutations, Rb function can be eliminated by the binding of an inhibitory protein, designated E7, that is encoded by human papillomavirus (HPV), another nasty viral trick to create virus-producing tissue.

Tumors with inactivating mutations in Rb generally produce normal levels of cyclin D1 and functional p16 protein. In contrast, tumor cells that overproduce cyclin D1 or have lost p16 function generally retain wild-type Rb. Thus loss of only one component of this regulatory system for controlling passage through the restriction point is all that is necessary to subvert normal growth control and set the stage for cancer.

Loss-of-Function Mutations Affecting Chromatin-Remodeling Proteins Contribute to Tumors

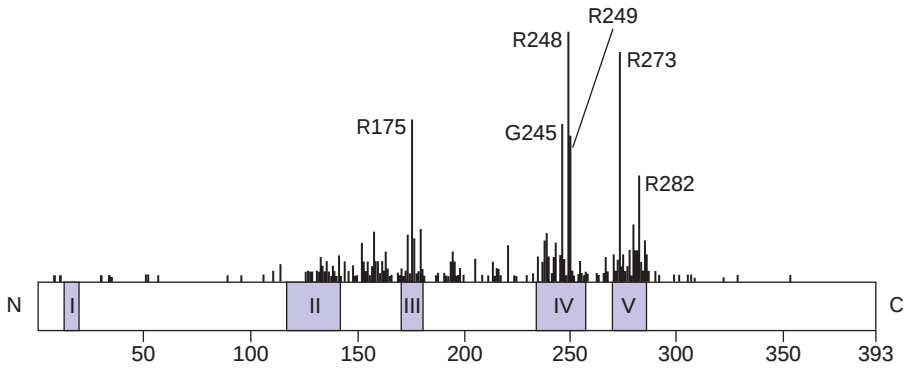
Mutations can undermine growth control by inactivating tumor-suppressor genes, but these genes can also be silenced by repressive chromatin structures. In recent years the importance of chromatin-remodeling machines, such as the Swi/Snf complex, in transcriptional control has become increasingly clear. These large and diverse multiprotein complexes have at their core an ATP-dependent helicase and often control acetylation of histones. By causing changes in the positions or structures of nucleosomes, Swi/Snf complexes make genes accessible or inaccessible to DNA-binding proteins that control transcription (Chapter 11). If a gene is normally activated or repressed by Swi/Snf-mediated chromatin changes, mutations in the genes encoding the Swi or Snf proteins will cause changes in expression of the target gene.

Our knowledge of the target genes regulated by Swi/Snf and other such complexes is incomplete, but the targets evidently include some growth-regulating genes. For example, studies with transgenic mice suggest that Swi/Snf plays a role in repressing the *E2F* genes, thereby inhibiting progression through the cell cycle. The relationship between the genes that encode Swi/Snf proteins and the *E2F* gene was discovered in genetic experiments with flies. Transgenic flies were constructed to overexpress *E2F*, which resulted in mild growth defects. A search for mutations that increase the effect of the *E2F* overexpression in these flies identified three components of the Swi/Snf complex. That loss of function of these genes increases the proliferative effects of E2F indicates that Swi/Snf normally counteracts the function of the E2F transcription factor. Thus loss of Swi/Snf function, just like loss of Rb function, can lead to overgrowth and perhaps cancer. Indeed, in mice, Rb protein recruits Swi/Snf proteins to repress transcription of the *E2F* gene.

With chromatin-remodeling complexes involved in so many cases of transcriptional control, it is expected that Swi/Snf and similar complexes will be linked to many cancers. In humans, for example, mutations in *Brg1*, which encodes the Swi/Snf catalytic subunit, have been found in prostate, lung, and breast tumors. Components of the Swi/Snf complex also have been found to associate with BRCA1, a nuclear protein that helps suppress human breast cancer. BRCA-1 is involved in the repair of double-strand DNA breaks (discussed in the final section of this chapter) and in transcriptional control, so the Swi/Snf complex may assist BRCA-1 in these functions.

Loss of p53 Abolishes the DNA-Damage Checkpoint

A critical feature of cell-cycle control is the G_1 checkpoint, which prevents cells with damaged DNA from entering the

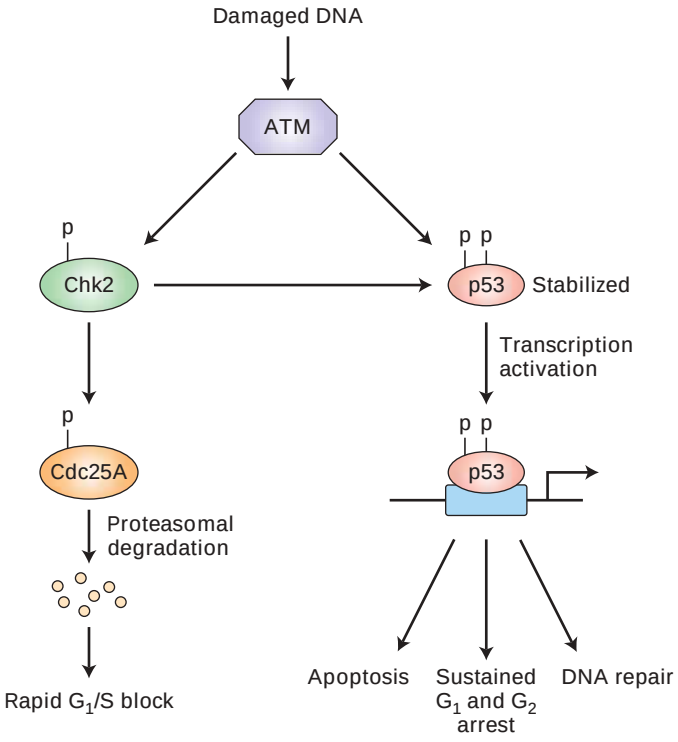


▲ EXPERIMENTAL FIGURE 23-22 **Mutations in human tumors that inactivate the function of p53 protein are highly concentrated in a few residues.** Colored boxes represent sequences in the *p53* gene that are highly conserved in evolution. Vertical lines represent the frequency at which point mutations are found at each residue in various human tumors. These mutations are clustered in conserved regions II–V. The locations in the protein sequence of the most frequently occurring point mutations are labeled. In these labels, R = arginine; G = glycine. [Adapted from C. C. Harris, 1993, *Science* **262**:1980, and L. Ko and C. Prives, 1996, *Genes & Develop.* **10:**1054.]

S phase (see Figure 21-32, step 4a). The **p53 protein** is a sensor essential for the checkpoint control that arrests cells with damaged DNA in G_1. Although p53 has several functions, its ability to activate transcription of certain genes is most relevant to its tumor-suppressing function. Virtually all p53 mutations abolish its ability to bind to specific DNA sequences and activate gene expression. Mutations in the *p53* tumor-suppressor gene occur in more than 50 percent of human cancers (Figure 23-22).

Cells with functional p53 become arrested in G_1 when exposed to DNA-damaging irradiation, whereas cells lacking functional p53 do not. Unlike other cell-cycle proteins, p53 is present at very low levels in normal cells because it is extremely unstable and rapidly degraded. Mice lacking p53 are viable and healthy, except for a predisposition to develop multiple types of tumors. Expression of the *p53* gene is heightened only in stressful situations, such as ultraviolet or γ irradiation, heat, and low oxygen. DNA damage by γ irradiation or by other stresses somehow leads to the activation of ATM, a serine kinase that phosphorylates and thereby stabilizes p53, leading to a marked increase in its concentration (Figure 23-23). The stabilized p53 activates expression of the gene encoding $p21^{CIP}$, which binds to and inhibits mammalian G_1 cyclin-CDK complexes. As a result, cells with damaged DNA are arrested in G_1, allowing time for DNA repair by mechanisms discussed later. If repair is successful, the levels of p53 and $p21^{CIP}$ will fall, and the cells then can progress into the S phase.

When the p53 G_1 checkpoint control does not operate properly, damaged DNA can replicate, perpetuating mutations and DNA rearrangements that are passed on to daughter cells, contributing to the likelihood of transformation into metastatic cells. In addition, $p21^{CIP}$ and two other proteins induced by p53 inhibit the cyclin B-CDK1 complex required for entry into mitosis, thus causing cells to arrest in G_2 (see Figure 21-32, step 4d). p53 also represses expression



▲ FIGURE 23-23 **G_1 arrest in response to DNA damage.** The kinase activity of ATM is activated in response to DNA damage due to various stresses (e.g., UV irradiation, heat). Activated ATM then triggers two pathways leading to arrest in G_1. Phosphorylation of p53 stabilizes it, permitting p53-activated expression of genes encoding proteins that (1) cause arrest in G_1 and in some cases G_2, (2) promote apoptosis, or (3) participate in DNA repair. In the other pathway phosphorylated Chk2 in turn phosphorylates Cdc25A, thereby marking it for degradation and blocking its role in CDK2 activation. See the text for a discussion.

of the genes encoding cyclin B and topoisomerase II, which also are required for the $G_2 \rightarrow$ mitosis transition. Thus if DNA is damaged following its replication, p53-induced G_2 arrest will prevent its transmission to daughter cells.

The active form of p53 is a tetramer of four identical subunits. A missense point mutation in one of the two *p53* alleles in a cell can abrogate almost all p53 activity because virtually all the oligomers will contain at least one defective subunit, and such oligomers cannot function as a transcription factor. Oncogenic *p53* mutations thus act as **dominant negatives,** with mutations in a single allele causing a loss of function. As we learned in Chapter 9, dominant-negative mutations can occur in proteins whose active forms are multimeric or whose function depends on interactions with other proteins. In contrast, loss-of-function mutations in other tumor-suppressor genes (e.g., *RB*) are recessive because the encoded proteins function as monomers and mutation of a single allele has little functional consequence.

Under stressful conditions, the ATM kinase also phosphorylates and thus activates Chk2, a protein kinase that phosphorylates the protein phosphatase Cdc25A, marking it for ubiquitin-mediated destruction. This phosphatase removes the inhibitory phosphate on CDK2, a prerequisite for cells to enter the S phase. Decreased levels of Cdc25A thus block progression into and through the S phase (see Figures 23-23 and 21-32, step 4b). Loss-of-function mutations in the *ATM* or *Chk2* genes have much the same effect as *p53* mutations.

The activity of p53 normally is kept low by a protein called *Mdm2*. When Mdm2 is bound to p53, it inhibits the transcription-activating ability of p53 and catalyzes the addition of ubiquitin molecules, thus targeting p53 for proteasomal degradation. Phosphorylation of p53 by ATM displaces bound Mdm2 from p53, thereby stabilizing it. Because the *Mdm2* gene is itself transcriptionally activated by p53, Mdm2 functions in an autoregulatory feedback loop with p53, perhaps normally preventing excess p53 function. The *Mdm2* gene is amplified in many sarcomas and other human tumors that contain a normal *p53* gene. Even though functional p53 is produced by such tumor cells, the elevated Mdm2 levels reduce the p53 concentration enough to abolish the p53-induced G_1 arrest in response to irradiation.

The activity of p53 also is inhibited by a human papillomavirus (HPV) protein called E6. Thus HPV encodes three proteins that contribute to its ability to induce stable transformation and mitosis in a variety of cultured cells. Two of these—E6 and E7—bind to and inhibit the p53 and Rb tumor suppressors, respectively. Acting together, E6 and E7 are sufficient to induce transformation in the absence of mutations in cell regulatory proteins. The HPV E5 protein, which causes sustained activation of the PDGF receptor, enhances proliferation of the transformed cells.

The activity of p53 is not limited to inducing cell-cycle arrest. In addition, this multipurpose tumor suppressor stimulates production of pro-apoptotic proteins and DNA-repair enzymes (see Figure 23-23).

Apoptotic Genes Can Function as Proto-Oncogenes or Tumor-Suppressor Genes

During normal development many cells are designated for **programmed cell death,** also known as apoptosis (see Chapter 22). Many abnormalities, including errors in mitosis, DNA damage, and an abnormal excess of cells not needed for development of a working organ, also can trigger apoptosis. In some cases, cell death appears to be the default situation, with signals required to ensure cell survival. Cells can receive instructions to live and instructions to die, and a complex regulatory system integrates the various kinds of information.

If cells do not die when they should and instead keep proliferating, a tumor may form. For example, chronic lymphoblastic leukemia (CLL) occurs because cells survive when they should be dying. The cells accumulate slowly, and most are not actively dividing, but they do not die. CLL cells have chromosomal translocations that activate a gene called *bcl-2*, which we now know to be a critical blocker of apoptosis (see Figure 22-31). The resultant inappropriate overproduction of Bcl-2 protein prevents normal apoptosis and allows survival of these tumor cells. CLL tumors are therefore attributable to a failure of cell death. Another dozen or so proto-oncogenes that are normally involved in negatively regulating apoptosis have been mutated to become oncogenes. Overproduction of their encoded proteins prevents apoptosis even when it is needed to stop cancer cells from growing.

Conversely, genes whose protein products stimulate apoptosis behave as tumor suppressors. An example is the *PTEN* gene discussed in Chapter 14. The phosphatase encoded by this gene dephosphorylates phosphatidylinositol 3,4,5-trisphosphate, a second messenger that functions in activation of protein kinase B (see Figure 14-27). Cells lacking PTEN phosphatase have elevated levels of phosphatidylinositol 3,4,5-trisphosphate and active protein kinase B, which promotes cell survival and prevents apoptosis by several pathways. Thus PTEN acts as a pro-apoptotic tumor suppressor by decreasing the anti-apoptotic effect of protein kinase B.

The most common pro-apoptotic tumor-suppressor gene implicated in human cancers is *p53*. Among the genes activated by p53 are several encoding pro-apoptotic proteins such as Bax (see Figure 22-32). When most cells suffer extensive DNA damage, the p53-induced expression of pro-apoptotic proteins leads to their quick demise (see Figure 23-23). While this may seem like a drastic response to DNA damage, it prevents proliferation of cells that are likely to accumulate multiple mutations.

When p53 function is lost, apoptosis cannot be induced and the accumulation of mutations required for cancer to develop becomes more likely. Tumors marked by loss of *p53* or another gene needed for apoptosis are difficult to treat with chemical or radiation therapy, since the resulting DNA damage is not translated into programmed cell death. ▮

Failure of Cell-Cycle Checkpoints Can Also Lead to Aneuploidy in Tumor Cells

It has long been known that chromosomal abnormalities abound in tumor cells. We have already encountered several examples of oncogenes that are formed by translocation, amplification, or both (e.g., c-*myc*, *bcr-abl*, *bcl-2*, and *cyclin D1*). Another chromosomal abnormality characteristic of nearly all tumor cells is **aneuploidy,** the presence of an aberrant number of chromosomes—generally too many.

Cells with abnormal numbers of chromosomes form when certain cell-cycle checkpoints are nonfunctional. As discussed in Chapter 21, the unreplicated-DNA checkpoint normally prevents entry into mitosis unless all chromosomes have completely replicated their DNA; the spindle-assembly checkpoint prevents entry into anaphase unless all the replicated chromosomes attach properly to the metaphase mitotic apparatus; and the chromosome-segregation checkpoint prevents exit from mitosis and cytokinesis if the chromosomes segregate improperly (see Figure 21-32, steps 1–3). As advances are made in identifying the proteins that detect these abnormalities and mediate cell-cycle arrest, the molecular basis for the functional defects leading to aneuploidy in tumor cells will become clearer.

KEY CONCEPTS OF SECTION 23.4

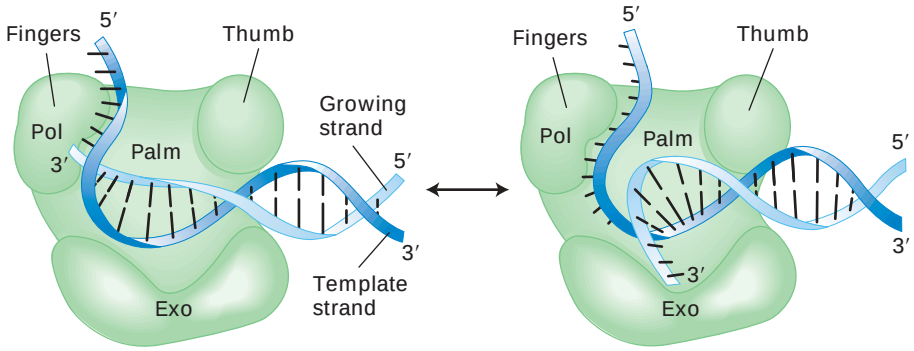
Mutations Causing Loss of Growth-Inhibiting and Cell-Cycle Controls

- Loss of signaling by TGFβ, a negative growth regulator, promotes cell proliferation and development of malignancy (see Figure 23-20).
- Overexpression of the proto-oncogene encoding cyclin D1 or loss of the tumor-suppressor genes encoding p16 and Rb can cause inappropriate, unregulated passage through the restriction point in late G_1. Such abnormalities are common in human tumors.
- p53 is a multipurpose tumor suppressor that promotes arrest in G_1 and G_2, apoptosis, and DNA repair in response to damaged DNA (see Figure 23-23). Loss-of-function mutations in the *p53* gene occur in more than 50 percent of human cancers.
- Overproduction of Mdm2, a protein that normally inhibits the activity of p53, occurs in several cancers (e.g., sarcomas) that express normal p53 protein.
- Human papillomavirus (HPV) encodes three oncogenic proteins: E6 (inhibits p53), E7 (inhibits Rb), and E5 (activates PDGF receptor).
- Mutations affecting the Swi/Snf chromatin-remodeling complex, which participates in transcriptional control, are associated with a variety of tumors. In some cases, interaction of the Swi/Snf complex with a nuclear tumor-suppressor protein may have a repressing effect on gene expression.
- Overproduction of anti-apoptotic proteins (e.g., Bcl-2) can lead to inappropriate cell survival and is associated with chronic lymphoblastic leukemia (CLL) and other cancers. Loss of proteins that promote apoptosis (e.g., p53 transcription factor and PTEN phosphatase) have a similar oncogenic effect.
- Most human tumor cells are aneuploid, containing an abnormal number of chromosomes (usually too many). Failure of cell-cycle checkpoints that normally detect unreplicated DNA, improper spindle assembly, or mis-segregation of chromosomes permits aneuploid cells to arise.

23.5 The Role of Carcinogens and DNA Repair in Cancer

In this final section, we examine how alterations in the genome arise that may lead to cancer and how cells attempt to correct them. DNA damage is unavoidable and arises by spontaneous cleavage of chemical bonds in DNA, by reaction with genotoxic chemicals in the environment or with certain chemical by-products of normal cellular metabolism, and from environmental agents such as ultraviolet and ionizing radiation. Changes in the DNA sequence can also result from copying errors introduced by DNA polymerases during replication and by mistakes made when DNA polymerase attempts to read from a damaged template. If DNA sequence changes, whatever their cause or nature, are left uncorrected, both proliferating and quiescent somatic cells might accumulate so many mutations that they could no longer function properly. In addition, the DNA in germ cells might incur too many mutations for viable offspring to be formed. Thus the prevention of DNA sequence errors in all types of cells is important for survival, and several cellular mechanisms for repairing damaged DNA and correcting sequence errors have evolved.

As our previous discussion has shown, alterations in DNA that lead to decreased production of functional tumor-suppressor proteins or increased, unregulated production or activation of oncoproteins are the underlying cause of most cancers. These oncogenic mutations in key growth and cell-cycle regulatory genes include insertions, deletions, and point mutations, as well as chromosomal amplifications and translocations. Most cancer cells lack one or more DNA-repair systems, which may explain the large number of mutations that they accumulate. Moreover, some repair mechanisms themselves introduce errors in the nucleotide sequence; such error-prone repair also contributes to oncogenesis. The inability of tumor cells to maintain genomic integrity leads to formation of a heterogeneous population of malignant cells. For this reason, chemotherapy directed toward a single gene or even a group of genes is likely to be ineffective in wiping out all malignant cells. This problem adds to the interest in therapies that interfere with the blood supply to tumors or in other ways act upon multiple types of tumor cells.

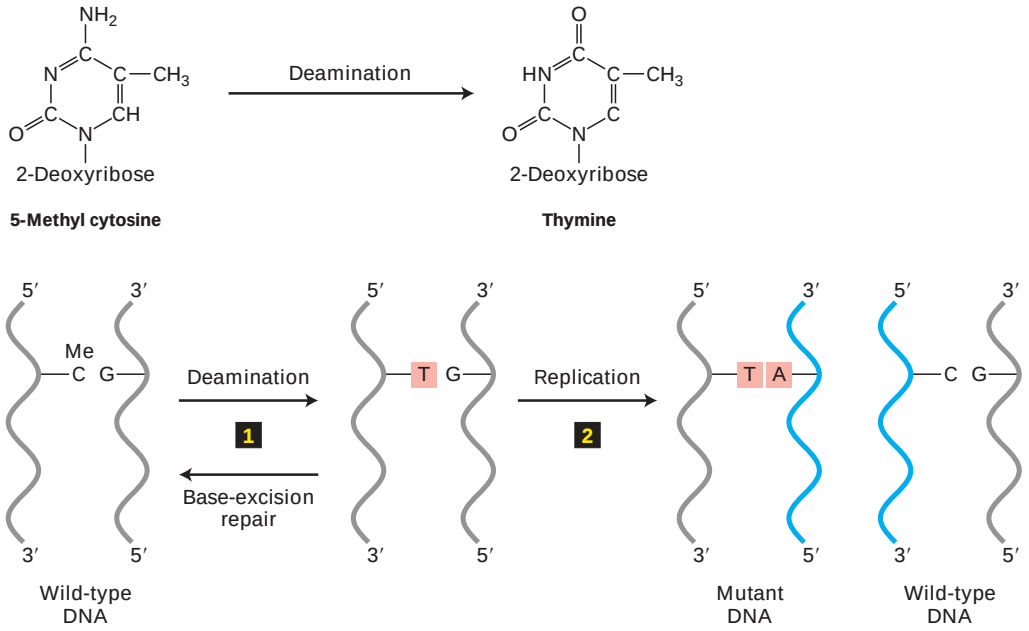


▲ FIGURE 23-24 **Schematic model of the proofreading function of DNA polymerases.** All DNA polymerases have a similar three-dimensional structure, which resembles a half-opened right hand. The "fingers" bind the single-stranded segment of the template strand, and the polymerase catalytic activity (Pol) lies in the junction between the fingers and palm. As long as the correct nucleotides are added to the 3′ end of the growing strand, it remains in the polymerase site. Incorporation of an incorrect base at the 3′ end causes melting of the newly formed end of the duplex. As a result, the polymerase pauses, and the 3′ end of the growing strand is transferred to the 3′ → 5′ exonuclease site (Exo) about 3 nm away, where the mispaired base and probably other bases are removed. Subsequently, the 3′ end flips back into the polymerase site and elongation resumes. [Adapted from C. M. Joyce and T. T. Steitz, 1995, *J. Bacteriol.* **177**:6321, and S. Bell and T. Baker, 1998, *Cell* **92**:295.]

DNA Polymerases Introduce Copying Errors and Also Correct Them

The first line of defense in preventing mutations is DNA polymerase itself. Occasionally, when replicative DNA polymerases progress along the template DNA, an incorrect nucleotide is added to the growing 3′ end of the daughter strand (see Figure 4-34). *E. coli* DNA polymerases, for instance, introduce about 1 incorrect nucleotide per 10^4 polymerized nucleotides. Yet the measured mutation rate in bacterial cells is much lower: about 1 mistake in 10^9 nucleotides incorporated into a growing strand. This remarkable accuracy is largely due to *proofreading* by *E. coli* DNA polymerases.

Proofreading depends on the 3′→5′ exonuclease activity of some DNA polymerases. When an incorrect base is incorporated during DNA synthesis, the polymerase pauses, then transfers the 3′ end of the growing chain to the exonuclease site, where the incorrect mispaired base is removed (Figure 23-24). Then the 3′ end is transferred back to the polymerase site, where this region is copied correctly. All three *E. coli* DNA polymerases have proofreading activity, as do the two DNA polymerases, δ and ε, used for DNA replication in animal cells. It seems likely that proofreading is indispensable for all cells to avoid excessive mutations.



▶ FIGURE 23-25 **Formation of a spontaneous point mutation by deamination of 5-methyl cytosine (C) to form thymine (T).** If the resulting T·G base pair is not restored to the normal C·G base pair by base excision-repair mechanisms (1), it will lead to a permanent change in sequence following DNA replication (i.e., a mutation) (2). After one round of replication, one daughter DNA molecule will have the mutant T·A base pair and the other will have the wild-type C·G base pair.

Chemical Damage to DNA Can Lead to Mutations

DNA is continually subjected to a barrage of damaging chemical reactions; estimates of the number of DNA damage events in a single human cell range from 10^4 to 10^6 per day! Even if DNA were not exposed to damaging chemicals, DNA is inherently unstable. For example, the bond connecting a purine base to deoxyribose is prone to hydrolysis, leaving a sugar without an attached base. Thus coding information is lost, and this can lead to a mutation during DNA replication. Normal cellular reactions, including the movement of electrons along the electron-transport chain in mitochondria and lipid oxidation in peroxisomes, produce several chemicals that react with and damage DNA, including hydroxyl radicals and superoxide (O_2^-). These too can cause mutations including those that lead to cancers.

Many spontaneous mutations are **point mutations,** which involve a change in a single base pair in the DNA sequence. One of the most frequent point mutations comes from *deamination* of a cytosine (C) base, which converts it into a uracil (U) base. In addition, the common modified base 5-methyl cytosine forms thymine when it is deaminated. If these alterations are not corrected before the DNA is replicated, the cell will use the strand containing U or T as template to form a U·A or T·A base pair, thus creating a permanent change to the DNA sequence (Figure 23-25).

Some Carcinogens Have Been Linked to Specific Cancers

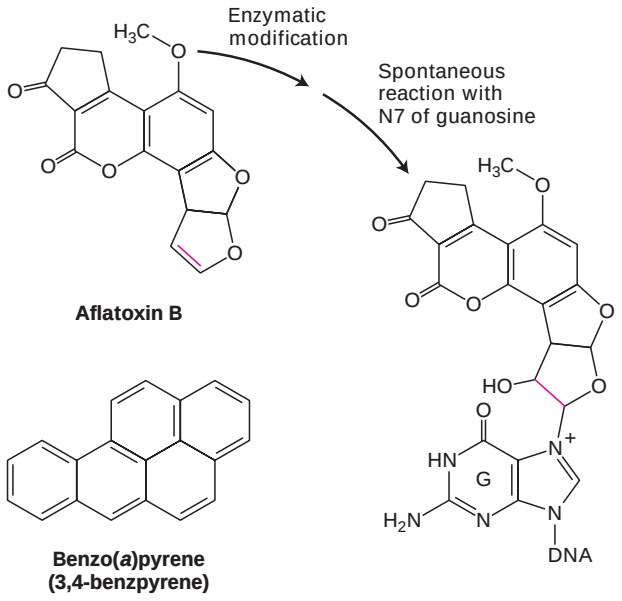
Environmental chemicals were originally associated with cancer through experimental studies in animals. The classic experiment is to repeatedly paint a test substance on the back of a mouse and look for development of both local and systemic tumors in the animal. Likewise, the ability of ionizing radiation to cause human cancer, especially leukemia, was dramatically shown by the increased rates of leukemia among survivors of the atomic bombs dropped in World War II, and more recently by the increase in melanoma (skin cancer) in individuals exposed to too much sunlight (UV radiation).

The ability of chemical and physical carcinogens to induce cancer can be accounted for by the DNA damage that they cause and by the errors introduced into DNA during the cells' efforts to repair this damage. Thus all carcinogens also are **mutagens.** The strongest evidence that carcinogens act as mutagens comes from the observation that cellular DNA altered by exposure of cells to carcinogens can change cultured cells, such as 3T3 cells, into fast-growing cancer-like cells (see Figure 23-4). The mutagenic effect of carcinogens is roughly proportional to their ability to transform cells and induce cancer in animals.

Although substances identified as chemical carcinogens have a broad range of structures with no obvious unifying features, they can be classified into two general categories. *Direct-acting carcinogens,* of which there are only a few, are mainly reactive electrophiles (compounds that seek out and react with negatively charged centers in other compounds). By chemically reacting with nitrogen and oxygen atoms in DNA, these compounds can modify bases in DNA so as to distort the normal pattern of base pairing. If these modified nucleotides are not repaired, they allow an incorrect nucleotide to be incorporated during replication. This type of carcinogen includes ethylmethane sulfonate (EMS), dimethyl sulfate (DMS), and nitrogen mustards.

In contrast, *indirect-acting carcinogens* generally are unreactive, water-insoluble compounds that can act as potent cancer inducers only after the introduction of electrophilic centers. In animals, *cytochrome P-450 enzymes* localized to the endoplasmic reticulum of liver cells normally function to add electrophilic centers to nonpolar foreign chemicals, such as certain insecticides and therapeutic drugs, in order to solubilize them so that they can be excreted from the body. But P-450 enzymes also can turn otherwise harmless chemicals into carcinogens.

Although chemical carcinogens are believed to be risk factors for many human cancers, a direct linkage to specific cancers has been established only in a few cases, the most important being lung cancer. Epidemiological studies first indicated that cigarette smoking was the major cause of lung cancer, but why this was so was unclear until the discovery that about 60 percent of human lung cancers contain inactivating mutations in the *p53* gene. The chemical *benzo(a)pyrene* (Figure 23-26), found in cigarette smoke, undergoes metabolic activation in the liver to a potent mutagen that mainly causes conversion of guanine (G) to thymine (T) bases, a transversion mutation. When applied to cultured bronchial epithelial cells,



▲ FIGURE 23-26 **Two chemical carcinogens that cause mutations in *p53*.** Like all indirect-acting carcinogens, benzo(*a*)pyrene and aflatoxin must undergo enzyme-catalyzed modification before they can react with DNA. In aflatoxin the colored double bond reacts with an oxygen atom, enabling it to react chemically with the N-7 atom of a guanosine in DNA, forming a large bulky molecule. Both compounds mutate the *p53* tumor-suppressor gene and are known risk factors for human cancer.

activated benzo(*a*)pyrene induces inactivating mutations at codons 175, 248, and 273 of the *p53* gene. These same positions are major mutational hot spots in human lung cancer (see Figure 23-22). Thus, there is a strong correlation between a defined chemical carcinogen in cigarette smoke and human cancer; it is likely that other chemicals in cigarette smoke induce mutations in other genes, as well. Similarly, asbestos exposure is clearly linked to mesothelioma, a type of epithelial cancer.

Lung cancer is not the only major human cancer for which a clear-cut risk factor has been identified. *Aflatoxin*, a fungal metabolite found in moldy grains, induces liver cancer (see Figure 23-26). After chemical modification by liver enzymes, aflatoxin becomes linked to G residues in DNA and induces G-to-T transversions. Aflatoxin also causes a mutation in the *p53* gene. Exposure to other chemicals has been correlated with minor cancers. Hard evidence concerning dietary and environmental risk factors that would help us avoid other common cancers (e.g., breast, colon, and prostate cancer, leukemias) is generally lacking.

Loss of High-Fidelity DNA Excision-Repair Systems Can Lead to Cancer

In addition to proofreading, cells have other repair systems for preventing mutations due to copying errors and exposure to mutagens. Several DNA **excision-repair systems** that normally operate with a high degree of accuracy have been well studied. These systems were first elucidated through a combination of genetic and biochemical studies in *E. coli*. Homologs of the key bacterial proteins exist in eukaryotes from yeast to humans, indicating that these error-free mechanisms arose early in evolution to protect DNA integrity. Each of these systems functions in a similar manner—a segment of the damaged DNA strand is excised, and the gap is filled by DNA polymerase and ligase using the complementary DNA strand as template.

Loss of these systems correlates with increased risk for cancer. For example, humans who inherit mutations in genes that encode a crucial mismatch-repair or excision-repair protein have an enormously increased probability of developing certain cancers (Table 23-1). Without proper DNA repair, people with xeroderma pigmentosum or hereditary nonpolyposis colorectal cancer have a propensity to accumulate mutations in many other genes, including those that are critical in controlling cell growth and proliferation.

We will now turn to a closer look at some of the mechanisms of DNA repair, ranging from repair of single base mutations to repair of DNA broken across both strands. Some of these effect their repairs with great accuracy; others are less precise.

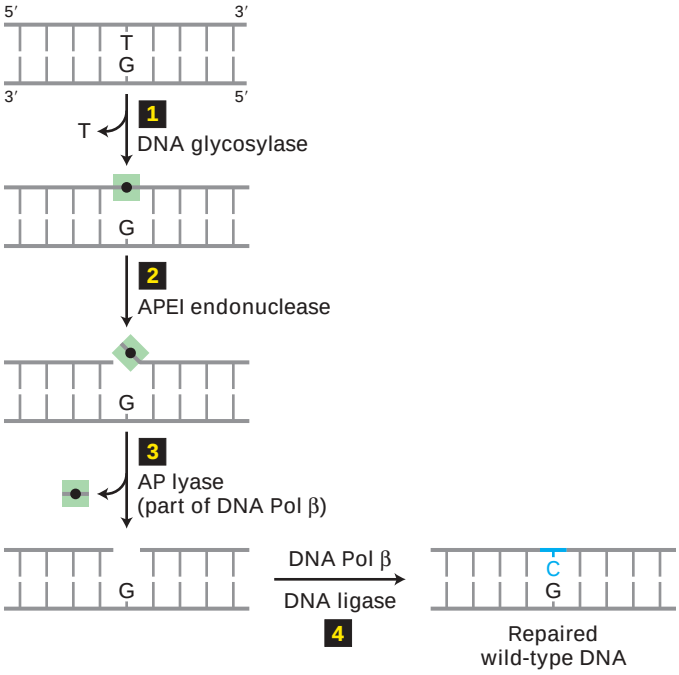
TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411:**366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509:**49.

Base Excision Is Used to Repair Damaged Bases and Single-Base Mispairs

In humans, the most common type of point mutation is a C to T, which is caused by deamination of 5-methyl C to T (see Figure 23-25). The conceptual problem with *base excision repair* is determining which is the normal and which is the mutant DNA strand, and repairing the latter so that it is properly base-paired with the normal strand. But since a G · T mismatch is almost invariably caused by chemical conversion of C to U or 5-methyl C to T, the repair system "knows" to remove the T and replace it with a C. The G · T mismatch is recognized by a DNA glycosylase that flips the thymine base out of the helix and then hydrolyzes the bond that connects it to the sugar-phosphate DNA backbone. Following this initial incision, the segment of the damaged strand containing the baseless deoxyribose is excised by an AP endonuclease that cuts the DNA strand near the abasic site. The resultant single-stranded gap in the damaged strand is filled in by a DNA polymerase and sealed by DNA ligase, restoring the original G · C base pair.



▲ FIGURE 23-27 **Base excision repair of a G·T mismatch**
A DNA glycosylase specific for G · T mismatches, usually formed by deamination of 5-methyl C residues (see Figure 23-25), flips the thymine base out of the helix and then cuts it away from the sugar-phosphate DNA backbone (step 1), leaving just the deoxyribose (black dot). An endonuclease specific for the resultant baseless site then cuts the DNA backbone (step 2), and the deoxyribose phosphate is removed by an endonuclease associated with DNA polymerase β (step 3). The gap is then filled in by DNA Pol β and sealed by DNA ligase (step 4), restoring the original G · C base pair. [After O. Schärer, 2003, *Angewandte Chemie*, in press.]

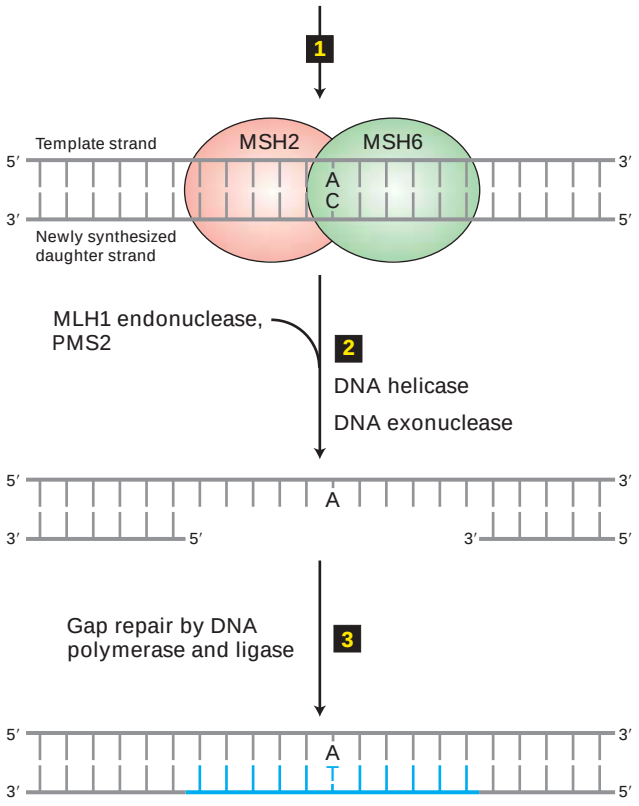
Human cells contain a battery of glycosylases, each of which is specific for a different set of chemically modified DNA bases. For example, one removes 8-oxyguanine, an oxidized form of guanine, allowing its replacement by an undamaged G, and others remove bases modified by alkylating agents. By a process similar to that shown in Figure 23-27, the modified base is cleaved (step 1); the damaged strand is then repaired using the "core" enzymes depicted in steps 2 through 4. A similar mechanism repairs lesions resulting from *depurination*, the loss of a guanine or adenine base from DNA resulting from hydrolysis of the glycosylic bond between deoxyribose and the base. Depurination occurs spontaneously and is fairly common in mammals. The resulting abasic sites, if left unrepaired, generate mutations during DNA replication because they cannot specify the appropriate paired base.

Loss of Mismatch Excision Repair Leads to Colon and Other Cancers

Another process, also conserved from bacteria to man, principally eliminates base-pair mismatches, deletions, and insertions that are accidentally introduced by polymerases during replication. As with base excision repair of a T in a T · G mismatch, the conceptual problem with *mismatch excision repair* is determining which is the normal and which is the mutant DNA strand, and repairing the latter. How this happens in human cells is not known with certainty. It is thought that the proteins that bind to the mismatched segment of DNA distinguish the template and daughter strands; then the mispaired segment of the daughter strand—the one with the replication error—is excised and repaired to become an exact complement of the template strand (Figure 23-28).

Hereditary nonpolyposis colorectal cancer, arising from a common inherited predisposition to cancer, results from an inherited loss-of-function mutation in one allele of either the *MLH1* or the *MSH2* gene; the MSH2 and MLH1 proteins are essential for DNA mismatch repair (see Figure 23-28). Cells with at least one functional copy of each of these genes exhibit normal mismatch repair. However, tumor cells frequently arise from those cells that have experienced a somatic mutation in the second allele and thus have lost the mismatch repair system. Somatic inactivating mutations in these genes are also common in noninherited forms of colon cancer.

One gene frequently mutated in colon cancers because of the absence of mismatch repair encodes the type II receptor for TGFβ (see Figure 23-20 and Chapter 14). The gene encoding this receptor contains a sequence of 10 adenines in a row. Because of "slippage" of DNA polymerase during replication, this sequence often undergoes mutation to a sequence containing 9 or 11 adenines. If the mutation is not fixed by the mismatch repair system, the resultant frameshift in the protein-coding sequence abolishes production of the normal receptor protein. As noted earlier, such inactivating mutations make cells resistant to growth inhibition by



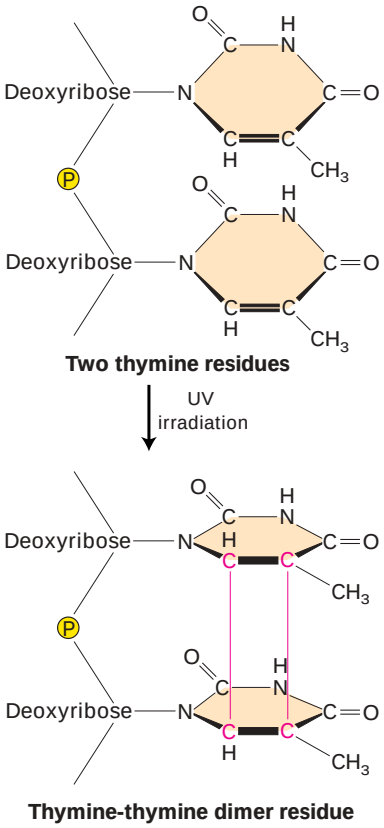
▲ FIGURE 23-28 **Mismatch excision repair of newly replicated DNA in human cells.** A complex of the MSH2 and MSH6 proteins binds to a mispaired segment of DNA in such a way as to distinguish between the template and newly synthesized daughter strands (step 1). This triggers binding of the MLH1 endonuclease, as well as other proteins such as PMS2, which has been implicated in oncogenesis through mismatch-repair mutations, although its specific function is unclear. A DNA helicase unwinds the helix and the daughter strand is cut; an exonuclease then removes several nucleotides, including the mismatched base (step 2). Finally, as with base excision repair, the gap is then filled in by a DNA polymerase (Pol δ, in this case) and sealed by DNA ligase (step 3).

TGFβ, thereby contributing to the unregulated growth characteristic of these tumors. This finding attests to the importance of mismatch repair in correcting genetic damage that might otherwise lead to uncontrolled cell proliferation.

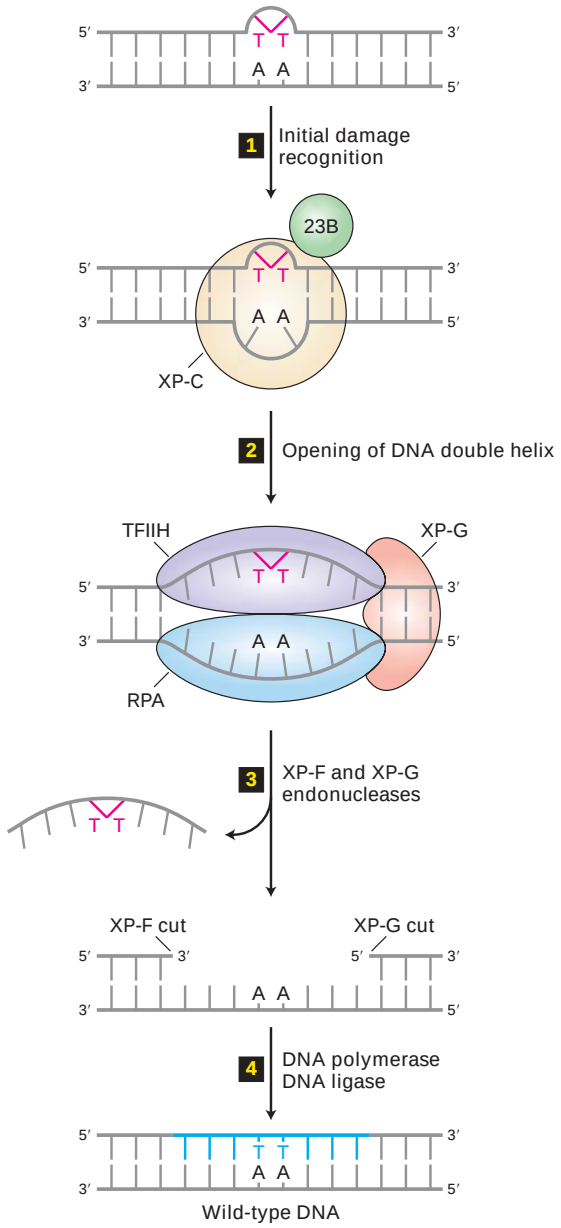
Nucleotide Excision Repair Was Elucidated Through Study of Xeroderma Pigmentosum, a Hereditary Predisposition to Skin Cancers

Cells use *nucleotide excision repair* to fix DNA regions containing chemically modified bases, often called chemical adducts, that distort the normal shape of DNA locally. A key to this type of repair is the ability of certain proteins to slide along the surface of a double-stranded DNA molecule looking for bulges or other irregularities in the shape of the double helix. For example, this mechanism repairs *thymine-thymine dimers*, a common type of damage caused by UV light (Figure 23-29); these dimers interfere with both replication and transcription of DNA. Nucleotide excision repair also can correct DNA regions containing bases altered by covalent attachment of carcinogens such as benzo(*a*)pyrene and aflatoxin (see Figure 23-26), both of which cause G-to-T transversions.

Figure 23-30 illustrates how the nucleotide excision-repair system repairs damaged DNA. Some 30 proteins are involved in this repair process, the first of which were identified through a study of the defects in DNA repair in cultured cells from individuals with xeroderma pigmentosum, a hereditary disease associated with a predisposition to cancer. Individuals with this disease frequently develop the skin cancers called melanomas and squamous cell carcinomas if their skin is exposed to the UV rays in sunlight. Cells of affected patients lack a functional nucleotide excision-repair system system. Mutations in any of at least seven different genes, called *XP-A* through *XP-G*, lead to inactivation of this repair system and cause xeroderma pigmentosum; all produce the same phenotype and have the same consequences. The



▲ FIGURE 23-29 **Formation of thymine-thymine dimers.** The most common type of DNA damage caused by UV irradiation, thymine-thymine dimers can be repaired by an excision-repair mechanism.



▲ FIGURE 23-30 **Nucleotide excision repair in human cells.** A DNA lesion that causes distortion of the double helix, such as a thymine dimer, is initially recognized by a complex of the XP-C (*x*eroderma *p*igmentosum *C* protein) and 23B proteins (step 1). This complex then recruits transcription factor TFIIH, whose helicase subunits, powered by ATP hydrolysis, partially unwind the double helix. XP-G and RPA proteins then bind to the complex and further unwind and stabilize the helix until a bubble of ≈25 bases is formed (step 2). Then XP-G (now acting as an endonuclease) and XP-F, a second endonuclease, cut the damaged strand at points 24–32 bases apart on each side of the lesion (step 3). This releases the DNA fragment with the damaged bases, which is degraded to mononucleotides. Finally the gap is filled by DNA polymerase exactly as in DNA replication (Chapter 4), and the remaining nick is sealed by DNA ligase (step 4). [Adapted from J. Hoeijmakers, 2001, *Nature* **411**:366, and O. Schärer, 2003, *Angewandte Chemie*, in press.]

roles of most of these XP proteins in nucleotide excision repair are now well understood (see Figure 23-30).

Remarkably, five polypeptide subunits of TFIIH, a general transcription factor, are required for nucleotide excision repair in eukaryotic cells, including two with homology to helicases, as shown in Figure 23-30. In transcription, the helicase activity of TFIIH unwinds the DNA helix at the start site, allowing RNA polymerase II to begin (see Figure 11-27). It appears that nature has used a similar protein assembly in two different cellular processes that require helicase activity.

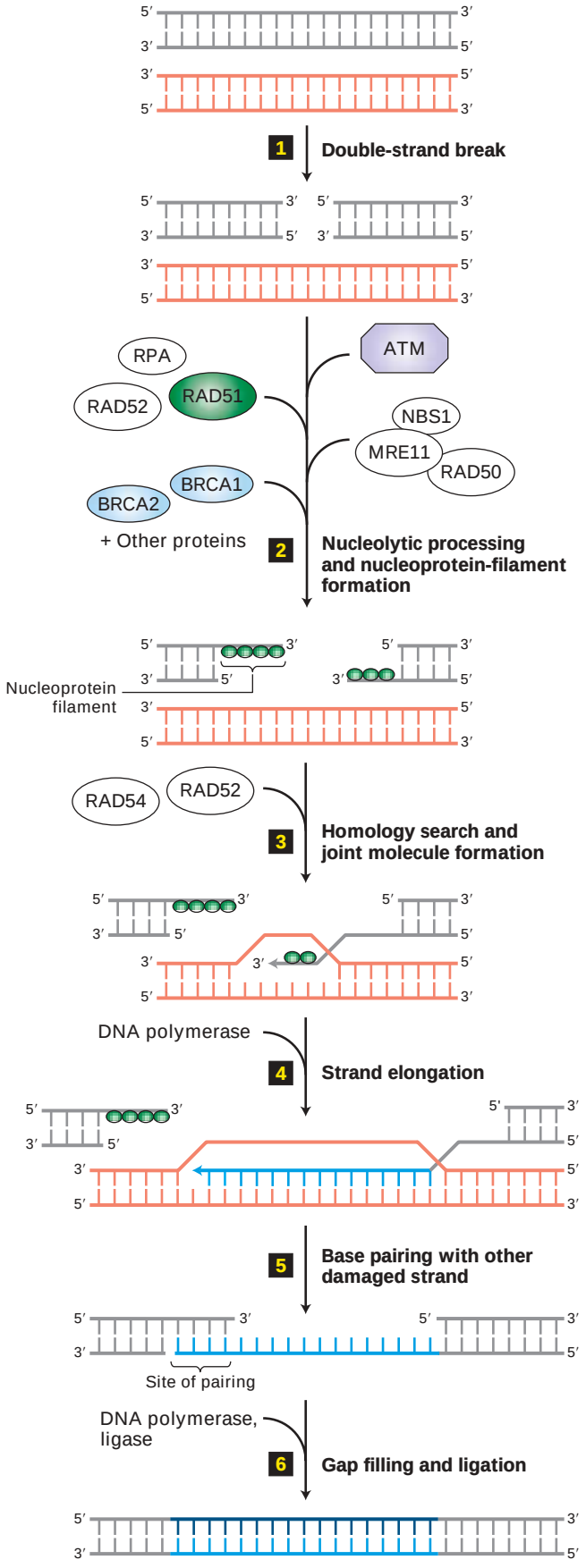
The use of shared subunits in transcription and DNA repair may help explain the observation that DNA damage in higher eukaryotes is repaired at a much faster rate in regions of the genome being actively transcribed than in nontranscribed regions—so-called transcription-coupled repair. Since only a small fraction of the genome is transcribed in any one cell in higher eukaryotes, transcription-coupled repair efficiently directs repair efforts to the most critical regions. In this system, if an RNA polymerase becomes stalled at a lesion on DNA (e.g., a thymine-thymine dimer), a small protein, CSB, is recruited to the RNA polymerase; this triggers opening of the DNA helix at that point, recruitment of TFIIH, and the reactions of steps 2 through 4 depicted in Figure 23-30.

Two Systems Repair Double-Strand Breaks in DNA

Ionizing radiation and some anticancer drugs (e.g., bleomycin) cause double-strand breaks in DNA. A cell that has suffered a particular double-strand break usually contains other breaks. These are particularly severe lesions because incorrect rejoining of double strands of DNA can lead to gross chromosomal rearrangements and translocations such as those that produce a hybrid gene or bring a growth-regulatory gene under the control of a different promoter. The B and T cells of the immune system are particularly susceptible to DNA rearrangements caused by double-strand breaks created during rearrangement of their immunoglobulin or T cell receptor genes, explaining the frequent involvement of these gene loci in leukemias and lymphomas.

Two systems have evolved to repair double-strand breaks—*homologous recombination* and *DNA end-joining*. The former is used during and after DNA replication, when the sister chromatid is available for use as a template to repair the damaged DNA strand; homologous recombination is error-free. The alternative mechanism, DNA end-joining, is error-prone, since several nucleotides are invariably lost at the point of repair. This and other error-prone repair systems are thought to mediate much of if not all the carcinogenic effects of chemicals and radiation.

Error-Free Repair by Homologous Recombination Yeasts can repair double-strand breaks induced by γ-irradiation. Isolation and analysis of radiation-sensitive (*RAD*) mutants that are deficient in this homologous recombination repair



◀ FIGURE 23-31 **Repair of double-strand breaks by homologous recombination.** During S phase cells copy each chromosome to create two identical sister chromatids that later segregate into daughter cells. The black and red DNAs represent the homologous sequences on these sister chromatids. Step 1: A double-strand DNA break forms in the chromatids. Step 2: The double-strand break activates the ATM kinase (see Figure 23-23); this leads to activation of a set of exonucleases that remove nucleotides at the break first from the 3′ and then from the 5′ ends of both broken strands, ultimately creating single-stranded 3′ ends. In a process that is dependent on the BRCA1 and BRCA2 proteins, as well as others, the Rad51 protein (green ovals) polymerizes on single-stranded DNA with a free 3′ end to form a nucleoprotein filament. Step 3: Aided by yet other proteins, one Rad51 nucleoprotein filament searches for the homologous duplex DNA sequence on the sister chromatid, then invades the duplex to form a joint molecule in which the single-stranded 3′ end is base-paired to the complementary strand on the homologous DNA strand. Step 4: The replicative DNA polymerases elongate this 3′ end of the damaged DNA (green strand), templated by the complementary sequences in the undamaged homologous DNA segment. Step 5: Next this repaired 3′ end of the damaged DNA pairs with the single-stranded 3′ end of the other damaged strand. Step 6: Any remaining gaps are filled in by DNA polymerase and ligase (light green), regenerating a wild-type double helix in which an entire segment (dark and light green) has been regenerated from the homologous segment of the sister chromatid. [Adapted from D. van Gant et al., 2001, *Nature Rev. Genet.* **2**:196.]

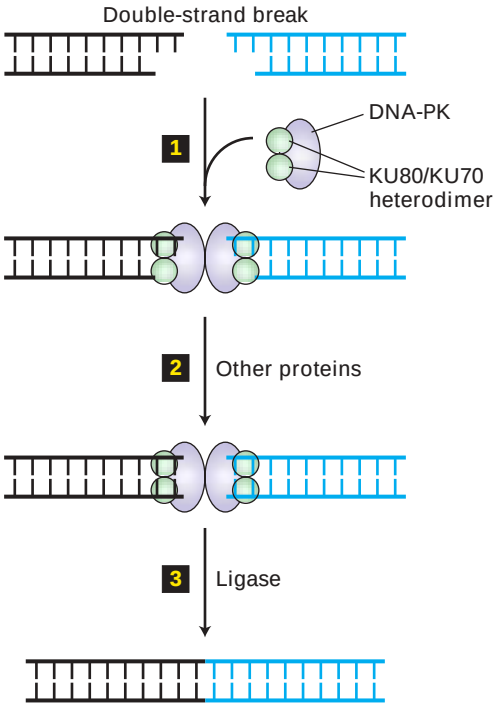
system facilitated study of the process. Virtually all the yeast Rad proteins have homologs in the human genome, and the human and yeast proteins function in an essentially identical fashion (Figure 23-31). At one time homologous recombination was thought to be a minor repair process in human cells. This changed when it was realized that several human cancers are potentiated by inherited mutations in genes essential for homologous recombination repair (see Table 23-1). For example, the vast majority of women with inherited susceptibility to breast cancer have a mutation in one allele of either the BCRA-1 or the BCRA-2 genes that encode proteins participating in this repair process. Loss or inactivation of the second allele inhibits the homologous recombination repair pathway and thus tends to induce cancer in mammary or ovarian epithelial cells.

Repair of a double-strand break by homologous recombination involves reactions between three DNA molecules—the two DNA ends and the intact DNA strands from the sister chromatid (see Figure 23-31). In this process single-stranded DNAs with 3′ ends are formed from the ends of the broken DNAs and then coated with the Rad51 protein. One Rad51 nucleoprotein filament searches for the homologous duplex DNA sequence in the sister chromatid. This 3′ end is then elongated (green in Figure 23-31) by DNA polymerase, templated by the complementary strand on the homologous DNA. When sufficiently long, this single strand

base-pairs with the single-stranded 3′ end of the other broken DNA, and DNA polymerase and DNA ligase fill in the gaps. This process regenerates a wild-type double helix with the correct sequence, and in general no mutations are induced during repair by homologous recombination.

Error-Prone Repair by End-Joining In multicellular organisms, the predominant mechanism for repairing double-strand breaks involves rejoining the nonhomologous ends of two DNA molecules. Even if the joined DNA fragments come from the same chromosome, the repair process results in loss of several base pairs at the joining point (Figure 23-32). Formation of such a possibly mutagenic deletion is one example of how repair of DNA damage can introduce mutations.

Since movement of DNA within the protein-dense nucleus is fairly minimal, the correct ends are generally rejoined together, albeit with loss of base pairs. However, occasionally broken ends from different chromosomes are joined together, leading to translocation of pieces of DNA from one chromosome to another. As we have seen, such translocations may generate chimeric oncogenes or place a proto-oncogene next to, and thus under the inappropriate control of, a promoter from another gene. The devastating effects of double-strand breaks make these the "most unkindest cuts of all," to borrow a phrase from Shakespeare's *Julius Caesar*.



▲ FIGURE 23-32 **Repair of double-strand breaks by end-joining.** In general, nucleotide sequences are butted together that were not apposed in the unbroken DNA. These DNA ends are usually from the same chromosome locus, and when linked together, several base pairs are lost. Occasionally, ends from different chromosomes are accidentally joined together. A complex of two proteins, Ku and DNA-dependent protein kinase, binds to the ends of a double-strand break (1). After formation of a synapse, the ends are further processed by nucleases, resulting in removal of a few bases (2), and the two double-stranded molecules are ligated together (3). As a result, the double-strand break is repaired, but several base pairs at the site of the break are removed. [Adapted from G. Chu, 1997, *J. Biol. Chem.* **272**:24097; M. Lieber et al., 1997, *Curr. Opin. Genet. Devel.* **7**:99; and D. van Gant et al., 2001, *Nature Rev. Genet*. **2**:196.]

Telomerase Expression Contributes to Immortalization of Cancer Cells

Telomeres, the physical ends of linear chromosomes, consist of tandem arrays of a short DNA sequence, TTAGGG in vertebrates. Telomeres provide the solution to the end-replication problem—the inability of DNA polymerases to completely replicate the end of a double-stranded DNA molecule. *Telomerase*, a reverse transcriptase that contains an RNA template, adds TTAGGG repeats to chromosome ends to lengthen or maintain the 5- to 20-kb regions of repeats that decorate the ends of human chromosomes (see Figure 10-34). Germ-line cells and rapidly dividing somatic cells (e.g., stem cells) produce telomerase, but most human somatic cells lack telomerase. As a result, their telomeres shorten with each cell cycle. Complete loss of telomeres leads to end-to-end chromosome fusions and cell death. Extensive shortening of telomeres is detected as a kind of DNA damage, with consequent stabilization and activation of p53 protein, leading to p53-triggered apoptosis.

Most tumor cells, despite their rapid proliferation rate, overcome this fate by expressing telomerase. Many researchers believe that telomerase expression is essential for a tumor cell to become immortal, and specific inhibitors of telomerase have been suggested as cancer therapeutic agents. Introduction of telomerase-producing transgenes into cultured human cells that otherwise lack the enzyme can extend their lifespan by more than 20 doublings while maintaining telomere length. The initial finding that mice homozygous for a deletion of the RNA subunit of telomerase are viable and fertile was surprising. However, after four to six generations defects began to appear in the telomerase-null mice as their very long telomeres (40–60 kb) became significantly shorter. The defects included depletion of tissues that require high rates of cell division, like skin and intestine, and infertility.

When treated with carcinogens, telomerase-null mice develop tumors less readily than normal mice do. For example, papillomas induced by a combination of chemical carcinogens occur 20 times less frequently in mice lacking a functional telomerase than in normal mice. Mice with an *APC* mutation normally develop colon tumors, and these too are reduced if the mice lack telomerase. Some other tumors are less affected by loss of telomerase. These studies demonstrate the relevance of telomerase for unbridled cell division and make the enzyme a possible target for chemotherapy.

KEY CONCEPTS OF SECTION 23.5

The Role of Carcinogens and DNA Repair in Cancer

- Changes in the DNA sequence result from copying errors and the effects of various physical and chemical agents, or carcinogens. All carcinogens are mutagens; that is, they alter one or more nucleotides in DNA.
- Many copying errors that occur during DNA replication are corrected by the proofreading function of DNA polymerases that can recognize incorrect (mispaired) bases at the 3′ end of the growing strand and then remove them by an inherent 3′→5′ exonuclease activity (see Figure 23-24).
- Indirect carcinogens, the most common type, must be activated before they can damage DNA. In animals, metabolic activation occurs via the cytochrome P-450 system, a pathway generally used by cells to rid themselves of noxious foreign chemicals.
- Benzo(*a*)pyrene, a component of cigarette smoke, causes inactivating mutations in the *p53* gene at the same positions that are mutated in many human lung tumors.
- Eukaryotic cells have three excision-repair systems for correcting mispaired bases and for removing UV-induced thymine-thymine dimers or large chemical adducts from DNA. Base excision repair, mismatch repair, and nucleotide excision repair operate with high accuracy and generally do not introduce errors.
- Inherited defects in the nucleotide excision-repair pathway, as in individuals with xeroderma pigmentosum, predispose them to skin cancer. Inherited colon cancer frequently is associated with mutant forms of proteins essential for the mismatch repair pathway.
- Error-free repair of double-strand breaks in DNA is accomplished by homologous recombination using the undamaged sister chromatid as template.
- Defects in repair by homologous recombination are associated with inheritance of one mutant allele of the BRCA-1 or BRCA-2 gene and result in predisposition to breast cancer.
- Repair of double-strand breaks by the end-joining pathway can link segments of DNA from different chromosomes, possibly forming an oncogenic translocation. The repair mechanism also produces a small deletion, even when segments from the same chromosome are joined.
- Inherited defects in other cellular DNA-repair processes found in certain human diseases are associated with an increased susceptibility for certain cancers (see Table 23-1).
- Cancer cells, like germ cells and stem cells but unlike most differentiated cells, produce telomerase, which prevents shortening of chromosomes during DNA replication and may contribute to their immortalization. The absence of telomerase is associated with resistance to generation of certain tumors.

PERSPECTIVES FOR THE FUTURE

The recognition that cancer is fundamentally a genetic disease has opened enormous new opportunities for preventing and treating the disease. Carcinogens can now be assessed for their effects on known steps in cell-cycle control. Genetic defects in the checkpoint controls for detecting damaged DNA and in the systems for repairing it can be readily recognized and used to explore the mechanisms of cancer. The multiple changes that must occur for a cell to grow into a dangerous tumor present multiple opportunities for intervention. Identifying mutated genes associated with cancer points directly to proteins at which drugs can be targeted.

Diagnostic medicine is being transformed by our newfound ability to monitor large numbers of cell characteristics. The traditional methods of assessing possible tumor cells, mainly microscopy of stained cells, will be augmented or replaced by techniques for measuring the expression of tens of thousands of genes, focusing particularly on genes whose activities are identified as powerful indicators of the cell's growth properties and the patient's prognosis. Currently, DNA microarray analysis permits measurement of gene transcription. In the future, techniques for systematically measuring protein production, modification, and localization, all important measures of cell states, will give us even more refined portraits of cells. Tumors now viewed as identical or very similar will instead be recognized as distinctly different and given appropriately different treatments. Earlier detection of tumors, based on better monitoring of cell properties, should allow more successful treatment. A focus on that particularly destructive process, metastasis, should be successful in identifying more of the mechanisms used by cells to migrate, attach, and invade. Manipulation of angiogenesis continues to look hopeful as a means of suffocating tumors.

The molecular cell biology of cancer provides avenues for new therapies, but prevention remains crucial and preferable to therapy. Avoidance of obvious carcinogens, in particular cigarette smoke, can significantly reduce the incidence of lung cancer and perhaps other kinds as well. Beyond minimizing exposure to carcinogens such as smoke or sunlight, certain specific approaches are now feasible. New knowledge of the involvement of human papillomavirus 16 in most cases of cervical cancer holds promise for developing a cancer vaccine that will prevent viral action. Antibodies against cell surface markers that distinguish cancer cells are a source of great hope, especially after successes with the clinical use of monoclonal antibodies against human EGF receptor 2 (Her2), a protein involved in some cases of human breast cancer. Further steps must involve medicine and science. Understanding the cell biology of cancer is a critical first step toward prevention and cure, but the next steps are hard. The success with Gleevec (STI-571) against leukemia is exceptional; many cancers remain difficult to treat and cause enormous suffering. Since *cancer* is a term for a group of highly diverse diseases, interventions that are successful for one type may not be useful for others. Despite these daunting realities,

we are beginning to reap the benefits of decades of research exploring the molecular biology of the cell. We hope that many of the readers of this book will help to overcome the obstacles that remain.

KEY TERMS

aflatoxin *964*
aneuploidy *961*
benign *936*
Burkitt's lymphoma *956*
carcinogens *935*
carcinomas *938*
deamination *963*
DNA end-joining *967*
excision-repair systems *964*
leukemias *938*
loss of heterozygosity (LOH) *947*
malignant *936*
metastasis *937*
mutagens *963*
nondisjunction *947*
oncogene *940*
p53 protein *959*
Philadelphia chromosome *954*
proto-oncogene *935*
sarcomas *938*
slow-acting retroviruses *946*
thymine-thymine dimers *966*
transducing retroviruses *946*
transformation *939*
tumor-suppressor gene *935*

REVIEW THE CONCEPTS

1. What characteristics distinguish benign from malignant tumors? With respect to gene mutations, what distinguishes benign colon polyps from malignant colon carcinoma?

2. Ninety percent of cancer deaths are caused by metastatic rather than primary tumors. Define *metastasis.* Explain the rationale for the following new cancer treatments: (a) batimastat, an inhibitor of matrix metalloproteinases and of the plasminogen activator receptor, (b) antibodies that block the function of integrins, integral membrane proteins that mediate attachment of cells to the basal laminae and extracellular matrices of various tissues, and (c) bisphosphonate, which inhibits the function of bone-digesting osteoclasts.

3. Because of oxygen and nutrient requirements, cells in a tissue must reside within 100 μm of a blood vessel. Based on this information, explain why many malignant tumors often possess gain-of-function mutations in one of the following genes: *bFGF, TGFα*, and *VEGF.*

4. What hypothesis explains the observations that incidence of human cancers increases exponentially with age? Give an example of data that confirm the hypothesis.

5. Distinguish between proto-oncogenes and tumor-suppressor genes. To become cancer promoting, do proto-oncogenes and tumor-suppressor genes undergo gain-of-function or loss-of-function mutations? Classify the following genes as proto-oncogenes or tumor-suppressor genes: *p53, ras, Bcl-2, telomerase, jun,* and *p16.*

6. Hereditary retinoblastoma generally affects children in both eyes, while spontaneous retinoblastoma usually occurs during adulthood only in one eye. Explain the genetic basis for the epidemiologic distinction between these two forms of retinoblastoma. Explain the apparent paradox: loss-of-function mutations in tumor-suppressor genes act recessively, yet hereditary retinoblastoma is inherited as an autosomal dominant.

7. Explain the concept of loss of heterozygosity (LOH). Why do most cancer cells exhibit LOH of one or more genes? How does failure of the spindle assembly checkpoint lead to loss of heterozygosity?

8. Many malignant tumors are characterized by the activation of one or more growth factor receptors. What is the catalytic activity associated with transmembrane growth factor receptors such as the EGF receptor? Describe how the following events lead to activation of the relevant growth factor receptor: (a) expression of the viral protein gp55, (b) translocation that replaces the extracellular domain of the Trk receptor with the N-terminal region of tropomyosin, (c) point mutation that converts a valine to glutamine within the transmembrane region of the Her2 receptor.

9. Describe the common signal-transduction event that is perturbed by cancer-promoting mutations in the genes encoding Ras and NF-1. Why are mutations in Ras more commonly found in cancers than mutations in NF-1?

10. What is the structural distinction between the proteins encoded by c-*src* and v-*src*? How does this difference render v-*src* oncogenic?

11. Describe the mutational event that produces the *myc* oncogene in Burkitt's lymphoma. Why does the particular mechanism for generating oncogenic *myc* result in a lymphoma rather than another type of cancer? Describe another mechanism for generating oncogenic *myc.*

12. Pancreatic cancers often possess loss-of-function mutations in the gene that encodes the SMAD4 protein. How does this mutation promote the loss of growth inhibition and highly metastatic phenotype of pancreatic tumors?

13. Loss of p53 function occurs in the majority of human tumors. Name two ways in which loss of p53 function contributes to a malignant phenotype. Explain the mechanism by which the following agents cause loss of p53 function: (a) human papillomavirus and (b) benzo(*a*)pyrene.

14. DNA-repair systems are responsible for maintaining genomic fidelity in normal cells despite the high frequency with which mutational events occur. What type of DNA mutation is generated by (a) UV irradiation and (b) ionizing radiation? Describe the system responsible for repairing each of these types of mutations in mammalian cells. Postulate why a loss of function in one or more DNA-repair systems typifies many cancers.

15. Which human cell types possess telomerase activity? What characteristic of cancer is promoted by expression of telomerase? What concerns does this pose for medical therapies involving stem cells?

ANALYZE THE DATA

In a recent study by Elizabeth Snyderwine and colleagues, cDNA microarray profiling was used to compare the expression profiles for 6900 genes in normal and malignant breast tissues from rats. RNA was extracted from the following tissues:

a. Breast tissue from virgin rats

b. Breast tissue from pregnant rats

c. Breast tissue from lactating rats

d. Breast carcinoma induced by the meat-derived carcinogen PhIP

e. Breast carcinoma induced by the experimental carcinogen DMBA

After the microarray slides were hybridized with labeled cDNAs derived from these 5 populations of RNAs, the data were analyzed by several different comparisons.

In comparison 1, tissues a, b, and c were grouped together as "normal" tissue samples, and d and e were grouped as "carcinoma" samples. Genes that were either induced (more than twofold increase in expression) or repressed (more than twofold decrease in expression) in both carcinoma samples relative to all three normal samples were determined. A partial listing of differentially expressed genes is shown in the accompanying table.

Induced Genes

Cell-Growth and Cell-Cycle-related Genes

Platelet-derived growth factor A chain (PDGF-A)

Cyclin-dependent kinase 4 (Cdk4)

Cyclin D

Signal-Transduction and Transcription-Related Genes

STAT5a

Repressed Genes

Extracellular Matrix Genes

Alpha 1 type V collagen

Fibronectin 1

Desmin

1. What was the purpose of analyzing breast tissue from pregnant and lactating rats?

2. What characteristic of cancer is promoted by the overexpression of PDGF-A, Cdk4, and cyclin D?

3. What characteristic of cancer might be promoted by the repression of extracellular matrix genes?

4. STAT5a is a transcription factor that regulates the expression of cyclin D, Bcl-X_L, and other genes. Why is it possible for these carcinomas that no mutation occurs in the cyclin D gene despite its overexpression? Why are mutations in transcription-factor genes like STAT5a commonly found in cancer cells?

In comparison 2, expression profiles of carcinomas induced by PhIP and DMBA were compared with each other. In this analysis, some distinctions were found, but the number of differentially expressed genes was far less than the number of genes identified when comparing the grouped "normal" samples (a, b, and c) to the grouped "carcinoma" samples (d and e).

5. On the basis of this information, what can be generalized about the molecular profile of breast carcinomas?

6. Would you anticipate greater or fewer differences in gene expression if two distinct types of cancer (e.g., breast carcinoma vs. B-cell lymphoma) induced by the same carcinogen were compared by microarray analysis?

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