

# Cancer

# CHAPTER 20

About one in five of us will die of cancer, but that is not why we devote a chapter to this disease. Cancer cells break the most basic rules of cell behavior by which multicellular organisms are built and maintained, and they exploit every kind of opportunity to do so. These transgressions help to reveal what the normal rules are and how they are enforced. As a result, cancer research helps to illuminate the fundamentals of cell biology—especially cell signaling (Chapter 15), the cell cycle and cell growth (Chapter 17), programmed cell death (apoptosis, Chapter 18), and the control of tissue architecture (Chapters 19 and 22). Of course, with a deeper understanding of these normal processes, we also gain a deeper understanding of the disease and better tools to treat it.

In this chapter, we first consider what cancer is and describe the natural history of the disease from a cellular standpoint. We then discuss the molecular changes that make a cell cancerous. And we end the chapter by considering how our enhanced understanding of the molecular basis of cancer is leading to improved methods for its prevention and treatment.

## CANCER AS A MICROEVOLUTIONARY PROCESS

The body of an animal operates as a society or ecosystem, whose individual members are cells that reproduce by cell division and organize themselves into collaborative assemblies called *tissues*. This ecosystem is very peculiar, however, because self-sacrifice—as opposed to survival of the fittest—is the rule. Ultimately, all of the somatic cell lineages in animals are committed to die: they leave no progeny and instead dedicate their existence to the support of the germ cells, which alone have a chance of continued survival (discussed in Chapter 21). There is no mystery in this, for the body is a clone derived from a fertilized egg, and the genome of the somatic cells is the same as that of the germ-cell lineage that gives rise to sperm or eggs. By their self-sacrifice for the sake of the germ cells, the somatic cells help to propagate copies of their own genes.

Thus, unlike free-living cells such as bacteria, which compete to survive, the cells of a multicellular organism are committed to collaboration. To coordinate their behavior, the cells send, receive, and interpret an elaborate set of extracellular signals that serve as *social controls*, directing cells how to act (discussed in Chapter 15). As a result, each cell behaves in a socially responsible manner—resting, growing, dividing, differentiating, or dying—as needed for the good of the organism.

Molecular disturbances that upset this harmony mean trouble for a multicellular society. In a human body with more than  $10^{14}$  cells, billions of cells experience mutations every day, potentially disrupting the social controls. Most dangerously, a mutation may give one cell a selective advantage, allowing it to grow and divide slightly more vigorously and survive more readily than its neighbors and in this way to become a founder of a growing mutant clone. A mutation that promotes such selfish behavior by individual members of the cooperative can jeopardize the future of the whole enterprise. Over time, repeated rounds of mutation, competition, and natural selection operating within the population of somatic cells can cause matters to go from bad to worse. These are the basic ingredients of cancer: it is a disease in which an individual mutant clone of cells begins by

### IN THIS CHAPTER

CANCER AS A  
MICROEVOLUTIONARY  
PROCESS

CANCER-CRITICAL GENES:  
HOW THEY ARE FOUND AND  
WHAT THEY DO

CANCER PREVENTION AND  
TREATMENT: PRESENT AND  
FUTURE

prospering at the expense of its neighbors. In the end—as the clone grows, evolves, and spreads—it can destroy the entire cellular society ([Movie 20.1](#)).

In this section, we discuss the development of cancer as a microevolutionary process that takes place within the course of a human life-span in a subpopulation of cells in the body. But the process depends on the same principles of mutation and natural selection that have driven the evolution of living organisms on Earth for billions of years.

### Cancer Cells Bypass Normal Proliferation Controls and Colonize Other Tissues

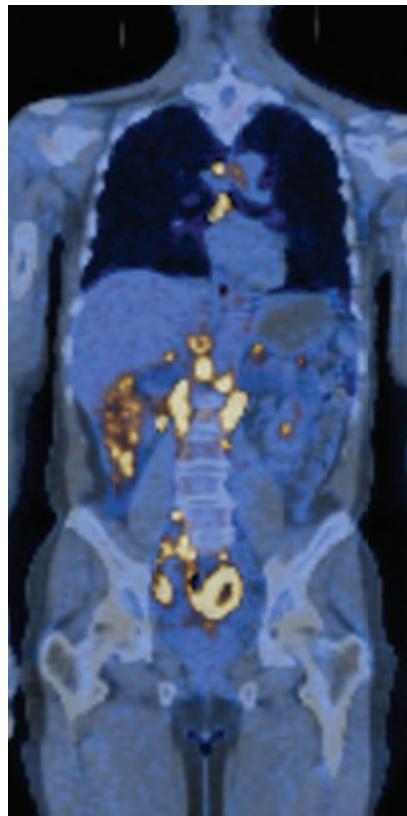
Cancer cells are defined by two heritable properties: (1) they reproduce in defiance of the normal restraints on cell growth and division, and (2) they invade and colonize territories normally reserved for other cells. It is the combination of these properties that makes cancers particularly dangerous. An abnormal cell that grows (increases in mass) and proliferates (divides) out of control will give rise to a tumor, or *neoplasm*—literally, a new growth. As long as the neoplastic cells have not yet become invasive, however, the tumor is said to be **benign**. For most types of such neoplasms, removing or destroying the mass locally usually achieves a complete cure. A tumor is considered a true cancer if it is **malignant**; that is, when its cells have acquired the ability to invade surrounding tissue. Invasiveness is an essential characteristic of cancer cells. It allows them to break loose, enter blood or lymphatic vessels, and form secondary tumors called **metastases** at other sites in the body ([Figure 20–1](#)). In general, the more widely a cancer spreads, the harder it becomes to eradicate. It is generally metastases that kill the cancer patient.

Cancers are traditionally classified according to the tissue and cell type from which they arise. **Carcinomas** are cancers arising from epithelial cells, and they are by far the most common cancers in humans. They account for about 80% of cases, perhaps because most of the cell proliferation in adults occurs in epithelia. In addition, epithelial tissues are the most likely to be exposed to the various forms of physical and chemical damage that favor the development of cancer. **Sarcomas** arise from connective tissue or muscle cells. Cancers that do not fit in either of these two broad categories include the various **leukemias** and **lymphomas**, derived from white blood cells and their precursors (hemopoietic cells), as well as cancers derived from cells of the nervous system. [Figure 20–2](#) shows the types of cancers that are common in the United States, together with their incidence and death rates. Each broad category has many subdivisions according to the specific cell type, the location in the body, and the microscopic appearance of the tumor.

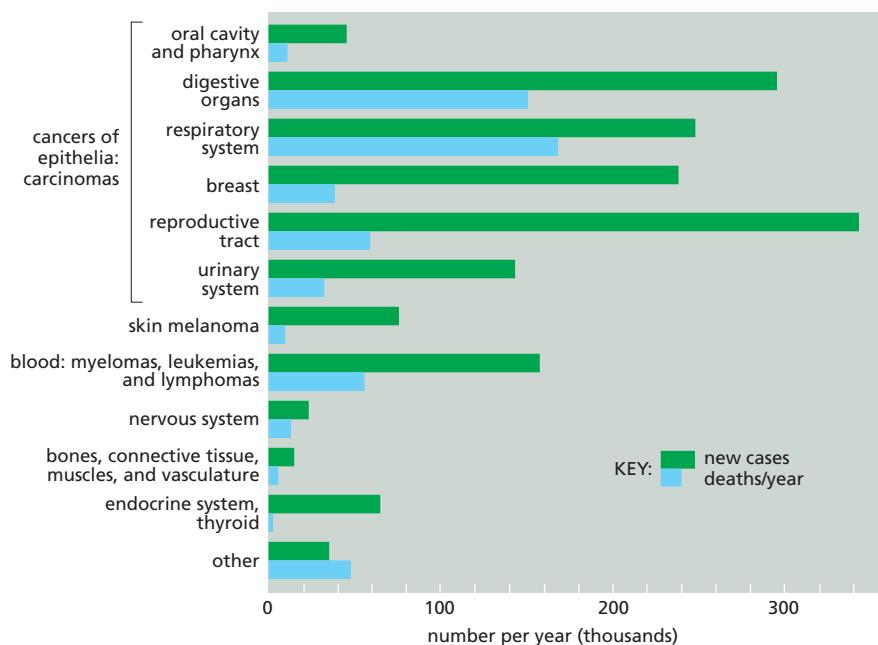
In parallel with the set of names for malignant tumors, there is a related set of names for benign tumors: an *adenoma*, for example, is a benign epithelial tumor with a glandular organization; the corresponding type of malignant tumor is an *adenocarcinoma* ([Figure 20–3](#)). Similarly, a *chondroma* and a *chondrosarcoma* are, respectively, benign and malignant tumors of cartilage.

Most cancers have characteristics that reflect their origin. Thus, for example, the cells of a *basal-cell carcinoma*, derived from a keratinocyte stem cell in the skin, generally continue to synthesize cytokeratin intermediate filaments, whereas the cells of a *melanoma*, derived from a pigment cell in the skin, will often (but not always) continue to make pigment granules. Cancers originating from different cell types are, in general, very different diseases. Basal-cell carcinomas of the skin, for example, are only locally invasive and rarely metastasize, whereas melanomas can become much more malignant and often form metastases. Basal-cell carcinomas are readily cured by surgery or local irradiation, whereas malignant melanomas, once they have metastasized widely, are usually fatal.

Later, we shall see that there is also a different way to classify cancers, one that cuts across the traditional classification by site of origin: we can classify them in terms of the mutations that make the tumor cells cancerous. The final section of the chapter will show how this information can be crucial to the design and choice of treatments.



**Figure 20–1 Metastasis.** Malignant tumors typically give rise to metastases, making the cancer hard to eradicate. Shown in this fusion image is a whole-body scan of a patient with metastatic non-Hodgkin's lymphoma (NHL). The background image of the body's tissues was obtained by CT (computed x-ray tomography) scanning. Overlaid on this image, a PET (positron emission tomography) scan reveals the tumor tissue (yellow), detected by its unusually high uptake of radioactively labeled fluorodeoxyglucose (FDG). High FDG uptake occurs in cells with unusually active glucose uptake and metabolism, which is a characteristic of cancer cells (see [Figure 20–12](#)). The yellow spots in the abdominal region reveal multiple metastases. (Courtesy of S. Gambhir.)



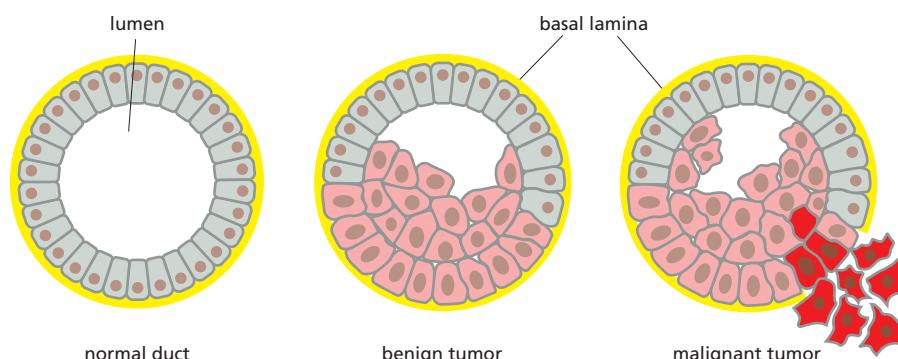
**Figure 20–2** Cancer incidence and mortality in the United States. The total number of new cases diagnosed in 2012 in the United States was 1,665,540, and total cancer deaths were 585,720. Note that deaths reflect cases diagnosed at many different times and that somewhat less than half of the people who develop cancer die of it. In the world as a whole, the five most common cancers are those of the lung, stomach, breast, colon/rectum, and uterine cervix (included in the figure under the heading of reproductive tract), and the total number of new cancer cases recorded per year is just over 6 million. Skin cancers other than melanomas are not included in these figures, since almost all are cured easily and many are unrecorded.

The data for the United Kingdom are similar. However, incidences are different in some other parts of the world, reflecting widespread exposures to different infectious agents and environmental toxins. (Data from American Cancer Society, Cancer Facts and Figures, 2014.)

### Most Cancers Derive from a Single Abnormal Cell

Even when a cancer has metastasized, we can usually trace its origins to a single **primary tumor**, arising in a specific organ. The primary tumor is thought to derive by cell division from a single cell that initially experienced some heritable change. Subsequently, additional changes accumulate in some of the descendants of this cell, allowing them to outgrow, out-divide, and often outlive their neighbors. By the time it is first detected, a typical human cancer will have been developing for many years and will already contain a billion cancer cells or more (Figure 20–4). Tumors will usually also contain a variety of other cell types; for example, fibroblasts will be present in the supporting connective tissue associated with a carcinoma, in addition to inflammatory and vascular endothelial cells. How can we be sure that the cancer cells are the clonal descendants of a single abnormal cell?

One way of proving clonal origin is through molecular analysis of the chromosomes in tumor cells. In almost all patients with *chronic myelogenous leukemia (CML)*, for example, we can distinguish the leukemic white blood cells from the patient's normal cells by a specific chromosomal abnormality: the so-called *Philadelphia chromosome*, created by a translocation between the long arms of chromosomes 9 and 22 (Figure 20–5). When the DNA at the site of translocation is cloned and sequenced, it is found that the site of breakage and rejoining of the translocated fragments is identical in all the leukemic cells in any given patient, but that this site differs slightly (by a few hundred or thousand base pairs) from one patient to another. This is the expected result if, and only if, the cancer in each patient arises from a unique accident occurring in a single cell. We will see later



**Figure 20–3** Benign versus malignant tumors. A benign glandular tumor (pink cells; an adenoma) remains inside the basal lamina (yellow) that marks the boundary of the normal structure (a duct, in this example). In contrast, a malignant glandular tumor (red cells; an adenocarcinoma) can develop from a benign tumor cell, and it destroys the integrity of the tissue, as shown. There are many different forms that such tumors may take.

**Figure 20–4** The growth of a typical human tumor, such as a tumor of the breast. The diameter of the tumor is plotted on a logarithmic scale. Years may elapse before the tumor becomes noticeable. The doubling time of a typical breast tumor, for example, is about 100 days. However, particularly virulent tumors may grow much more rapidly.

how this particular translocation promotes the development of CML by creating a novel hybrid gene encoding a protein that promotes cell proliferation.

Many other lines of evidence, from a variety of cancers, point to the same conclusion: most cancers originate from a single aberrant cell.

### Cancer Cells Contain Somatic Mutations

If a single abnormal cell is to give rise to a tumor, it must pass on its abnormality to its progeny: the aberration has to be heritable. Thus, the development of a clone of cancer cells depends on genetic changes. The tumor cells contain **somatic mutations**: they have one or more shared detectable abnormalities in their DNA sequence that distinguish them from the normal cells surrounding the tumor, as in the example of CML just described. (The mutations are called *somatic* because they occur in the soma, or body cells, not in the germ line). Cancers are also driven by *epigenetic changes*—persistent, heritable changes in gene expression that result from modifications of chromatin structure without alteration of the cell's DNA sequence. But somatic mutations that alter DNA sequence appear to be a fundamental and universal feature, and cancer is in this sense a genetic disease.

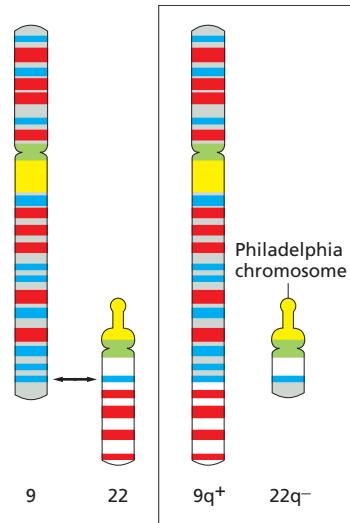
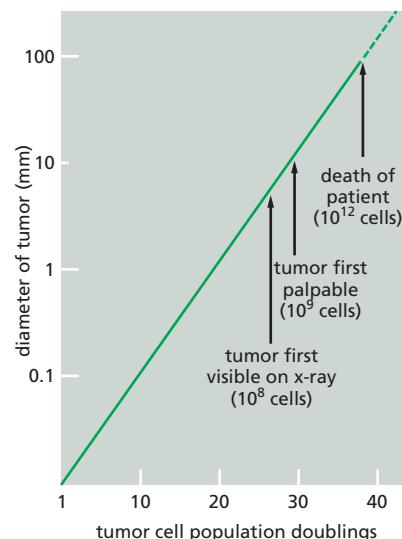
Factors that cause genetic changes tend to provoke the development of cancer. Thus, **carcinogenesis** (the generation of cancer) can be linked to **mutagenesis** (the production of a change in the DNA sequence). This correlation is particularly clear for two classes of external agents: (1) *chemical carcinogens* (which typically cause simple local changes in the nucleotide sequence), and (2) *radiation* such as x-rays (which typically cause chromosome breaks and translocations) or ultraviolet (UV) light (which causes specific DNA base alterations).

As would be expected, people who have inherited a genetic defect in one of several DNA repair mechanisms, causing their cells to accumulate mutations at an elevated rate, run a heightened risk of cancer. Those with the disease *xeroderma pigmentosum*, for example, have defects in the system that repairs DNA damage induced by UV light, and they have a greatly increased incidence of skin cancers.

### A Single Mutation Is Not Enough to Change a Normal Cell into a Cancer Cell

An estimated  $10^{16}$  cell divisions occur in a normal human body in the course of a typical lifetime; in a mouse, with its smaller number of cells and its shorter lifespan, the number is about  $10^{12}$ . Even in an environment that is free of mutagens, mutations would occur spontaneously at an estimated rate of about  $10^{-6}$  mutations per gene per cell division—a value set by fundamental limitations on the accuracy of DNA replication and repair (see pp. 237–238). Thus, in a typical lifetime, every single gene is likely to have undergone mutation on about  $10^{10}$  separate occasions in a human, or on about  $10^6$  occasions in a mouse. Among the resulting mutant cells, we might expect a large number that have sustained deleterious mutations in genes that regulate cell growth and division, causing the cells to disobey the normal restrictions on cell proliferation. From this point of view, the problem of cancer seems to be not why it occurs, but why it occurs so infrequently.

Clearly, if a mutation in a single gene were enough to convert a typical healthy cell into a cancer cell, we would not be viable organisms. Many lines of evidence indicate that the development of a cancer typically requires that a substantial number of independent, rare genetic and epigenetic accidents occur in the lineage that emanates from a single cell. One such indication comes from epidemiological studies of the incidence of cancer as a function of age (Figure 20–6). If a

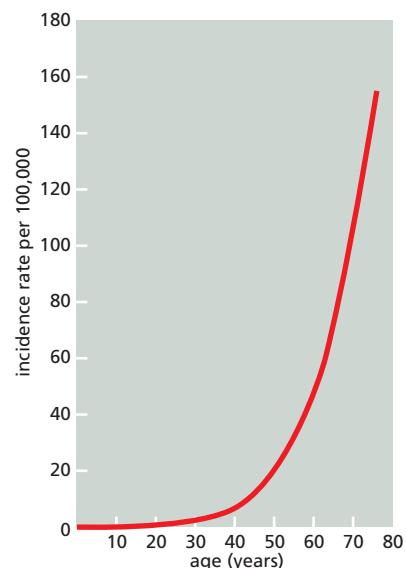


**Figure 20–5** The translocation between chromosomes 9 and 22 responsible for chronic myelogenous leukemia. The normal structures of chromosomes 9 and 22 are shown at the left. When a translocation occurs between them at the indicated site, the result is the abnormal pair at the right. The smaller of the two resulting abnormal chromosomes ( $22q^-$ ) is called the Philadelphia chromosome, after the city where the abnormality was first recorded.

**Figure 20–6 Cancer incidence as a function of age.** The number of newly diagnosed cases of colon cancer in women in England and Wales in 1 year is plotted as a function of age at diagnosis, relative to the total number of individuals in each age group. The incidence of cancer rises steeply as a function of age. If only a single mutation were required to trigger the cancer and this mutation had an equal chance of occurring at any time, the incidence of this cancer would be the same at all ages. Analyses of this type suggest that the development of a solid tumor instead requires five to eight independent accidents (“hits”) that occur randomly over time. This calculation assumes that the mutation rate remains constant as a cancer evolves, where in fact it often increases (see p. 1097). (Data from C. Muir et al., *Cancer Incidence in Five Continents*, Vol. V. Lyon: International Agency for Research on Cancer, 1987.)

single mutation were responsible for cancer, occurring with a fixed probability per year, the chance of developing cancer in any given year of life should be independent of age. In fact, for most types of cancer, the incidence rises steeply with age—as would be expected if cancer is caused by a progressive, random accumulation of a set of mutations in a single lineage of cells.

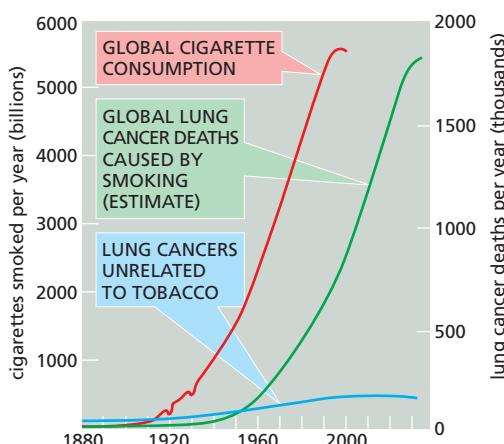
As discussed later, these indirect arguments have now been confirmed by systematically sequencing the genomes of the tumor cells from individual cancer patients and cataloging the mutations that they contain.



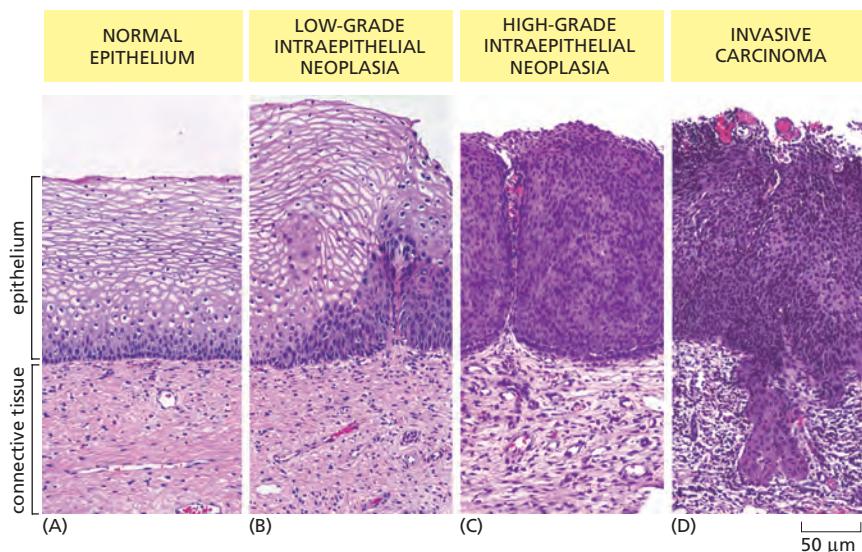
### Cancers Develop Gradually from Increasingly Aberrant Cells

For those cancers known to have a specific external cause, the disease does not usually become apparent until long after exposure to the causal agent. The incidence of lung cancer, for example, does not begin to rise steeply until after decades of heavy smoking (Figure 20–7). Similarly, the incidence of leukemias in Hiroshima and Nagasaki did not show a marked rise until about 5 years after the explosion of the atomic bombs, and industrial workers exposed for a limited period to chemical carcinogens do not usually develop the cancers characteristic of their occupation until 10, 20, or even more years after the exposure. During this long incubation period, the prospective cancer cells undergo a succession of changes, and the same presumably applies to cancers where the initial genetic lesion has no such obvious external cause.

The concept that the development of a cancer requires a gradual accumulation of mutations in a number of different genes helps to explain the well-known phenomenon of **tumor progression**, whereby an initial mild disorder of cell behavior evolves gradually into a full-blown cancer. Chronic myelogenous leukemia again provides a clear example. It begins as a disorder characterized by a nonlethal overproduction of white blood cells and continues in this form for several years before changing into a much more rapidly progressing illness that usually ends in death within a few months. In the early chronic phase, the leukemic cells are distinguished mainly by the chromosomal translocation (the Philadelphia chromosome) mentioned previously, although there may well be other, less visible



**Figure 20–7 Smoking and the onset of lung cancer.** A major increase in cigarette smoking (red line) has caused a dramatic rise in lung cancer deaths (green line), with a lag time of about 35 years. Because global cigarette smoking peaked in 1990, global lung cancer deaths are expected to decline after a similar lag. (Data from R.N. Proctor, *Nat. Rev. Cancer* 1:82–86, 2001).



**Figure 20–8** Stages of progression in the development of cancer of the epithelium of the uterine cervix. Pathologists use standardized terminology to classify the types of disorders they see, so as to guide the choice of treatment. (A) In a stratified squamous epithelium, dividing cells are confined to the basal layer. (B) In this low-grade intraepithelial neoplasia (right half of image), dividing cells can be found throughout the lower third of the epithelium; the superficial cells are still flattened and show signs of differentiation, but this is incomplete. (C) In high-grade intraepithelial neoplasia, cells in all the epithelial layers are proliferating and exhibit defective differentiation. (D) True malignancy begins when the cells move through or destroy the basal lamina that underlies the basal layer of epithelium and invade the underlying connective tissue. (Photographs courtesy of Andrew J. Connolly.)

genetic or epigenetic changes. In the subsequent acute phase, cells that show not only the translocation but also several other chromosomal abnormalities overrun the hemopoietic (blood-forming) system. It appears that cells from the initial mutant clone have undergone further mutations that make them proliferate even more vigorously, so that they come to outnumber both the normal blood cells and their ancestors with the primary chromosomal translocation.

Carcinomas and other solid tumors evolve in a similar way (Figure 20–8). Although many such cancers in humans are not diagnosed until a relatively late stage, in some cases it is possible to observe the earlier steps and, as we shall see later, to relate them to specific genetic changes.

### Tumor Progression Involves Successive Rounds of Random Inherited Change Followed by Natural Selection

From all the evidence, therefore, it seems that cancers arise by a process in which an initial population of slightly abnormal cells—descendants of a single abnormal ancestor—evolve from bad to worse through successive cycles of random inherited change followed by natural selection. Correspondingly, tumors grow in fits and starts, as additional advantageous inherited changes arise and the cells bearing them flourish. Tumor progression involves a large element of chance and usually takes many years, which may be why the majority of us will die of causes other than cancer.

At each stage of progression, some individual cell acquires an additional mutation or epigenetic change that gives it a selective advantage over its neighbors, making it better able to thrive in its environment—an environment that, inside a tumor, may be harsh, with low levels of oxygen, scarce nutrients, and the natural barriers to growth presented by the surrounding normal tissues. The larger the number of tumor cells, the higher the chance that at least one of them will undergo a change that favors it over its neighbors. Thus, as the tumor grows, progression accelerates. The offspring of the best-adapted cells continue to divide, eventually producing the dominant clones in the developing lesion (Figure 20–9).

Just as in the evolution of plants and animals, a kind of speciation often occurs: the original cancer cell lineage can diversify to give many genetically different vigorous subclones of cells. These may coexist in the same mass of tumor tissue; or they may migrate and colonize separate environments suited to their individual quirks, where they settle, thrive, and progress as independently evolving metastases. As new mutations arise within each tumor mass, different subclones may gain an advantage and come to predominate, only to be overtaken by others or outgrown by their own sub-subclones. The increasing genetic diversity as a cancer progresses is one of the chief factors that make cures difficult.

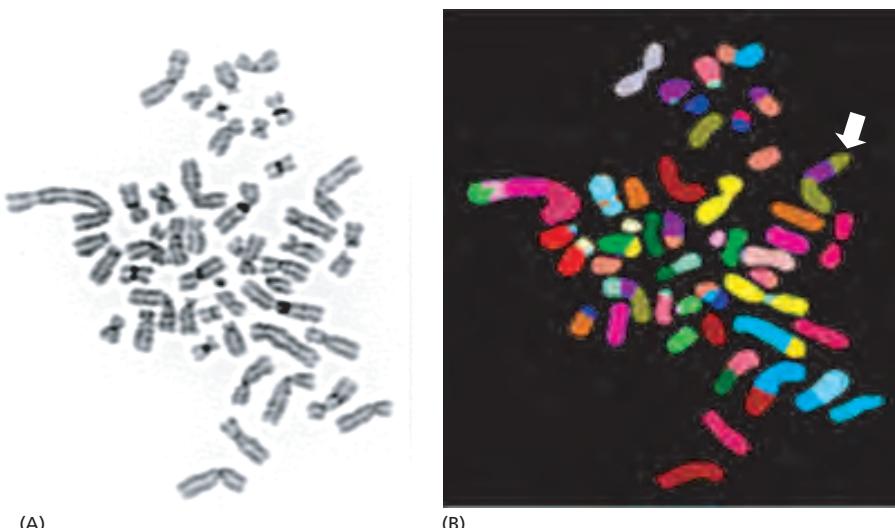
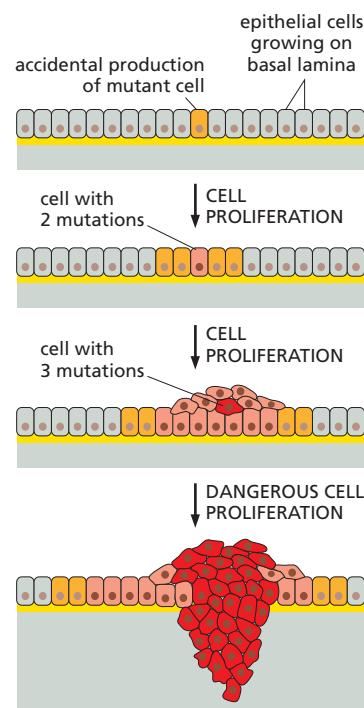
**Figure 20–9 Clonal evolution.** In this schematic diagram, a tumor develops through repeated rounds of mutation and proliferation, giving rise eventually to a clone of fully malignant cancer cells. At each step, a single cell undergoes a mutation that either enhances cell proliferation or decreases cell death, so that its progeny become the dominant clone in the tumor. Proliferation of each clone hastens the occurrence of the next step of tumor progression by increasing the size of the cell population that is at risk of undergoing an additional mutation. The final step depicted here is invasion through the basement membrane, an initial step in metastasis. In reality, there are more than the three steps shown here, and a combination of genetic and epigenetic changes are involved. Not shown here is the fact that, over time, a variety of competing subclones will often arise in a tumor. As we will discuss later, this heterogeneity complicates cancer therapies (see Figure 20–30).

### Human Cancer Cells Are Genetically Unstable

Most human cancer cells accumulate genetic changes at an abnormally rapid rate and are said to be **genetically unstable**. The extent of this instability and its molecular origins differ from cancer to cancer and from patient to patient, as we shall discuss in a later section. The basic phenomenon was evident even before modern molecular analyses. For example, the cells of many cancers show grossly abnormal sets of chromosomes, with duplications, deletions, and translocations that are visible at mitosis (**Figure 20–10**). When the cells are maintained in culture, these patterns of chromosomal disruption can often be seen to evolve rapidly and in a seemingly haphazard way. And for many years, pathologists have used an abnormal appearance of the cell nucleus to identify and classify cancer cells in tumor biopsies; in particular, cancer cells can contain an unusually large amount of heterochromatin—a condensed form of interphase chromatin that silences genes (see pp. 194–195). This suggested that epigenetic changes of chromatin structure can also contribute to the cancer cell phenotype, as recently confirmed by molecular analysis.

The genetic instability observed in cancer cells can arise from defects in the ability to repair DNA damage or to correct replication errors of various kinds. These alterations lead to changes in DNA sequence and produce rearrangements such as DNA translocations and duplications. Also common are defects in chromosome segregation during mitosis, which provide another possible source of chromosome instability and changes in karyotype.

From an evolutionary perspective, none of this should be a surprise: anything that increases the probability of random changes in gene function heritable from one cell generation to the next—and that is not too deleterious—is likely to speed the evolution of a clone of cells toward malignancy, thereby causing this property to be selected for during tumor progression.



**Figure 20–10 Chromosomes from a breast tumor displaying abnormalities in structure and number.** Chromosomes were prepared from a breast tumor cell in metaphase, spread on a glass slide, and stained with (A) a general DNA stain or (B) a combination of fluorescently labeled DNA molecules that color each normal human chromosome differently (see Figure 4–10). The staining (displayed in false color) shows multiple translocations, including a doubly translocated chromosome (white arrow) that is made up of two pieces of chromosome 8 (green-brown) and a piece of chromosome 17 (purple). The karyotype also contains 48 chromosomes, instead of the normal 46. (Courtesy of Joanne Davidson and Paul Edwards.)

## Cancer Cells Display an Altered Control of Growth

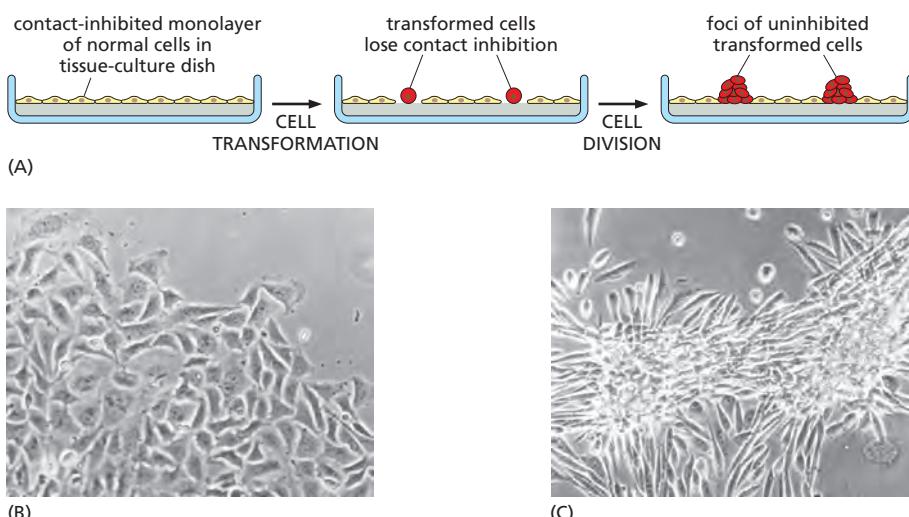
Mutability and large cell population numbers create the opportunities for mutations to occur, but the driving force for development of a cancer has to come from some sort of selective advantage possessed by the mutant cells. Most obviously, a mutation or epigenetic change can confer such an advantage by increasing the rate at which a clone of cells proliferates or by enabling it to continue proliferating when normal cells would stop. Cancer cells that can be grown in culture, or cultured cells artificially engineered to contain the types of mutations encountered in cancers, typically show a **transformed** phenotype. They are abnormal in their shape, their motility, their responses to growth factors in the culture medium, and, most characteristically, in the way they react to contact with the substratum and with one another. Normal cells will not divide unless they are attached to the substratum; transformed cells will often divide even if held in suspension. Normal cells become inhibited from moving and dividing when the culture reaches confluence (where the cells are touching one another); transformed cells continue moving and dividing even after confluence, and so pile up in layer upon layer in the culture dish (Figure 20-11). In addition, transformed cells no longer require all of the positive signals from their surroundings that normal cells require.

Their behavior in culture gives a hint of the ways in which cancer cells may misbehave in their natural environment, embedded in a tissue. But cancer cells in the body show other peculiarities that mark them out from normal cells, beyond those just described.

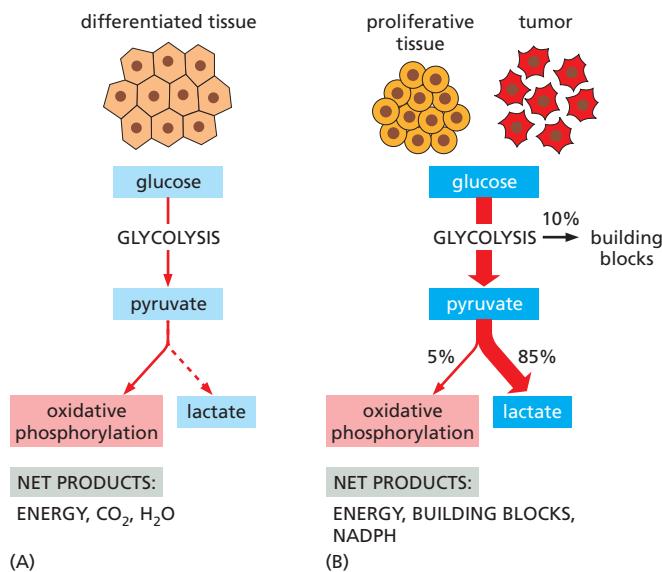
## Cancer Cells Have an Altered Sugar Metabolism

Given sufficient oxygen, normal adult tissue cells will generally fully oxidize almost all the carbon in the glucose they take up to  $\text{CO}_2$ , which is lost from the body as a waste product. A growing tumor needs nutrients in abundance to provide the building blocks to make new macromolecules. Correspondingly, most tumors have a metabolism more similar to that of a growing embryo than to that of normal adult tissue. Tumor cells consume glucose avidly, importing it from the blood at a rate that can be as much as 100 times higher than neighboring normal cells. Moreover, only a small fraction of this imported glucose is used for production of ATP by oxidative phosphorylation. Instead, a great deal of lactate is produced, and many of the remaining carbon atoms derived from glucose are diverted for use as raw materials for synthesis of the proteins, nucleic acids, and lipids required for tumor growth (Figure 20-12).

This tendency of tumor cells to de-emphasize oxidative phosphorylation even when oxygen is plentiful, while at the same time taking up large quantities of glucose, can be shown to promote cancer cell growth and is called the *Warburg*



**Figure 20-11** Loss of contact inhibition by cancer cells in cell culture. Most normal cells stop proliferating once they have carpeted the dish with a single layer of cells: proliferation seems to depend on contact with the dish, and to be inhibited by contacts with other cells—a phenomenon known as “contact inhibition.” Cancer cells, in contrast, usually disregard these restraints and continue to grow, so that they pile up on top of one another, as shown (Movie 20.2). (A) Schematic drawing. (B and C) Light micrographs of normal (B) and transformed (C) fibroblasts. (B and C, courtesy of Lan Bo Chen.)



**Figure 20–12** The Warburg effect in tumor cells reflects a dramatic change in glucose uptake and sugar metabolism. (A) Cells that are not proliferating will normally oxidize nearly all of the glucose that they import from the blood to produce ATP through the oxidative phosphorylation that takes place in their mitochondria. Only when deprived of oxygen will these cells generate most of their ATP from glycolysis, converting the pyruvate produced to lactate in order to regenerate the  $\text{NAD}^+$  that they need to keep glycolysis going (see Figure 2–47). (B) Tumor cells, by contrast, will generally produce abundant lactate even in the presence of oxygen. This results from a greatly increased rate of glycolysis that is fed by a very large increase in the rate of glucose import. In this way, tumor cells resemble the rapidly proliferating cells in embryos (and during tissue repair), which likewise require for biosynthesis a large supply of the small-molecule building blocks that can be produced from imported glucose (see also Figure 20–26).

**effect**—so named because Otto Warburg first noticed the phenomenon in the early twentieth century. It is this abnormally high glucose uptake that allows tumors to be selectively imaged in whole-body scans (see Figure 20–1), thereby providing a way to monitor cancer progression and responses to treatment.

### Cancer Cells Have an Abnormal Ability to Survive Stress and DNA Damage

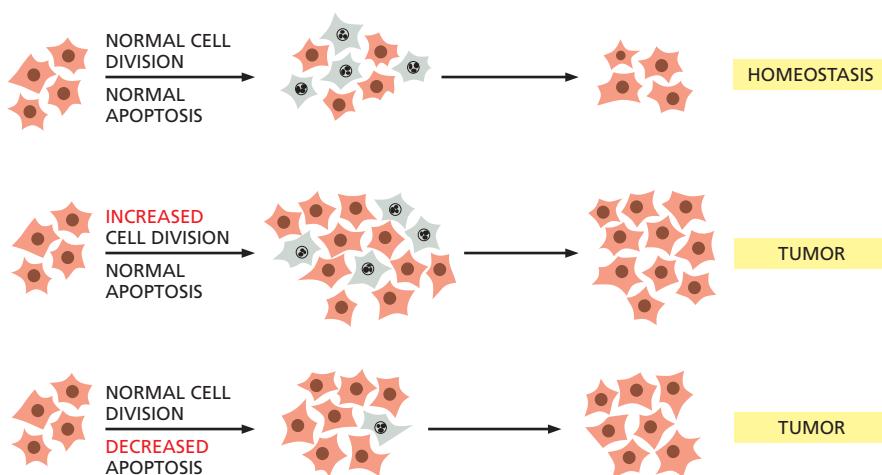
In a large multicellular organism, there are powerful safety mechanisms that guard against the trouble that can be caused by damaged and deranged cells. For example, internal disorder gives rise to danger signals in the faulty cell, activating protective devices that can eventually lead to apoptosis (see Chapter 18). To survive, cancer cells require additional mutations to elude or break through these defenses against cellular misbehavior.

Cancer cells are found to contain mutations that drive the cell into an abnormal state, where metabolic processes may be unbalanced and essential cell components may be produced in ill-matched proportions. States of this type, where the cell's homeostatic mechanisms are inadequate to cope with an imposed disturbance, are loosely referred to as states of *cell stress*. As one example, chromosome breakage and other forms of DNA damage are commonly observed during the development of cancer, reflecting the genetic instability that cancer cells display. Thus, to survive and divide without limit, a prospective cancer cell must accumulate mutations that disable the normal safety mechanisms that would otherwise induce a cell that is stressed, in this or in other ways, to commit suicide. In fact, one of the most important properties of many types of cancer cells is that they fail to undergo apoptosis when a normal cell would do so (Figure 20–13).

While cancer cells tend to avoid apoptosis, this does not mean that they rarely die. On the contrary, in the interior of a large solid tumor, cell death often occurs on a massive scale: living conditions are difficult, with severe competition among the cancer cells for oxygen and nutrients. Many die, but typically much more by necrosis than by apoptosis (Figure 20–14). The tumor grows because the cell birth rate outpaces the cell death rate, but often by only a small margin. For this reason, the time that a tumor takes to double in size can be far longer than the cell-cycle time of the tumor cells.

### Human Cancer Cells Escape a Built-in Limit to Cell Proliferation

Many normal human cells have a built-in limit to the number of times they can divide when stimulated to proliferate in culture: they permanently stop dividing



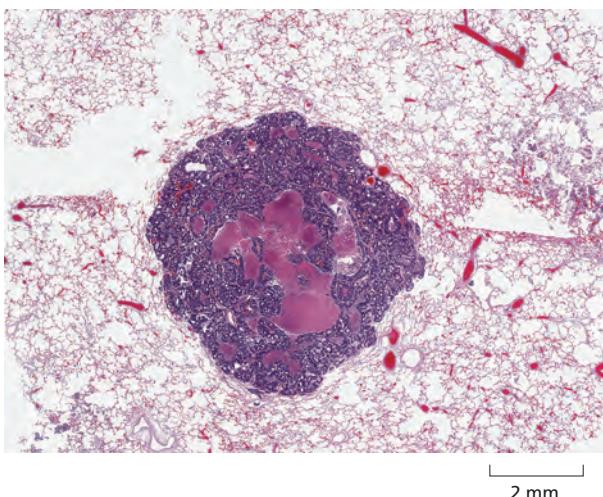
**Figure 20–13** Both increased cell division and decreased apoptosis can contribute to tumorigenesis. In normal tissues, apoptosis balances cell division to maintain homeostasis (see Movie 18.1). During the development of cancer, either an increase in cell division or an inhibition of apoptosis can lead to the increased cell numbers important for tumorigenesis. The cells fated to undergo apoptosis are gray in this diagram. Both an increase in cell division and a decrease in apoptosis normally contribute to tumor growth.

after a certain number of population doublings (25–50 for human fibroblasts, for example). This cell-division-counting mechanism is termed **replicative cell senescence**, and it generally depends on the progressive shortening of the telomeres at the ends of chromosomes, a process that eventually changes their structure (discussed in Chapter 17). As discussed in Chapter 5, the replication of telomere DNA during S phase depends on the enzyme *telomerase*, which maintains a special telomeric DNA sequence that promotes the formation of protein cap structures to protect chromosome ends. Because many proliferating human cells (stem cells being an exception) are deficient in telomerase, their telomeres shorten with every division, and their protective caps deteriorate, creating a DNA damage signal. Eventually, the altered chromosome ends can trigger a permanent cell-cycle arrest, causing a normal cell to die.

Human cancer cells avoid replicative cell senescence in one of two ways. They can maintain the activity of telomerase as they proliferate, so that their telomeres do not shorten or become uncapped, or they can evolve an alternate mechanism based on homologous recombination (called ALT) for elongating their chromosome ends. Regardless of the strategy used, the result is that the cancer cells continue to proliferate under conditions when normal cells would stop.

### The Tumor Microenvironment Influences Cancer Development

While the cancer cells in a tumor are the bearers of dangerous mutations and are often grossly abnormal, the other cells in the tumor—especially those of the supporting connective tissue, or **stroma**—are far from passive bystanders. The



**Figure 20–14** Cross-section of a colon adenocarcinoma that has metastasized to the lung. This tissue slice shows well-differentiated colorectal cancer cells forming cohesive glands in the lung. The metastasis has central pink areas of necrosis where dying cancer cells have outgrown their blood supply. Such anoxic regions are common in the interior of large tumors. (Courtesy of Andrew J. Connolly.)

development of a tumor relies on a two-way communication between the tumor cells and the tumor stroma, just as the normal development of epithelial organs relies on communication between epithelial cells and mesenchymal cells (discussed in Chapter 22).

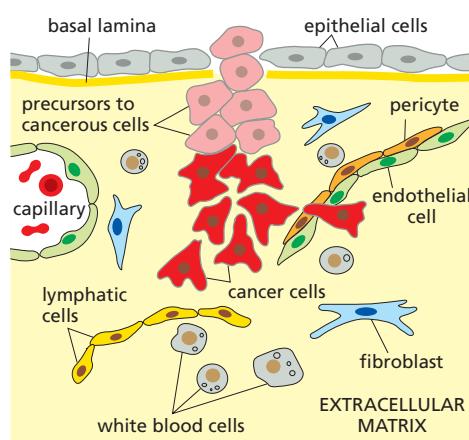
The stroma provides a framework for the tumor. It is composed of normal connective tissue containing fibroblasts and inflammatory white blood cells, as well as the endothelial cells that form blood and lymphatic vessels with their attendant pericytes and smooth muscle cells (Figure 20–15). As a carcinoma progresses, the cancer cells induce changes in the stroma by secreting signal proteins that alter the behavior of the stromal cells, as well as proteolytic enzymes that modify the extracellular matrix. The stromal cells in turn act back on the tumor cells, secreting signal proteins that stimulate cancer cell growth and division as well as proteases that further remodel the extracellular matrix. In these ways, the tumor and its stroma evolve together, like weeds and the ecosystem that they invade, and the tumor becomes dependent on its particular stromal cells. Experiments using mice indicate that the growth of some transplanted carcinomas depends on the tumor-associated fibroblasts and normal fibroblasts will not do. Such environmental requirements help to protect us from cancer, as we discuss next in considering the critical phenomenon called metastasis.

### Cancer Cells Must Survive and Proliferate in a Foreign Environment

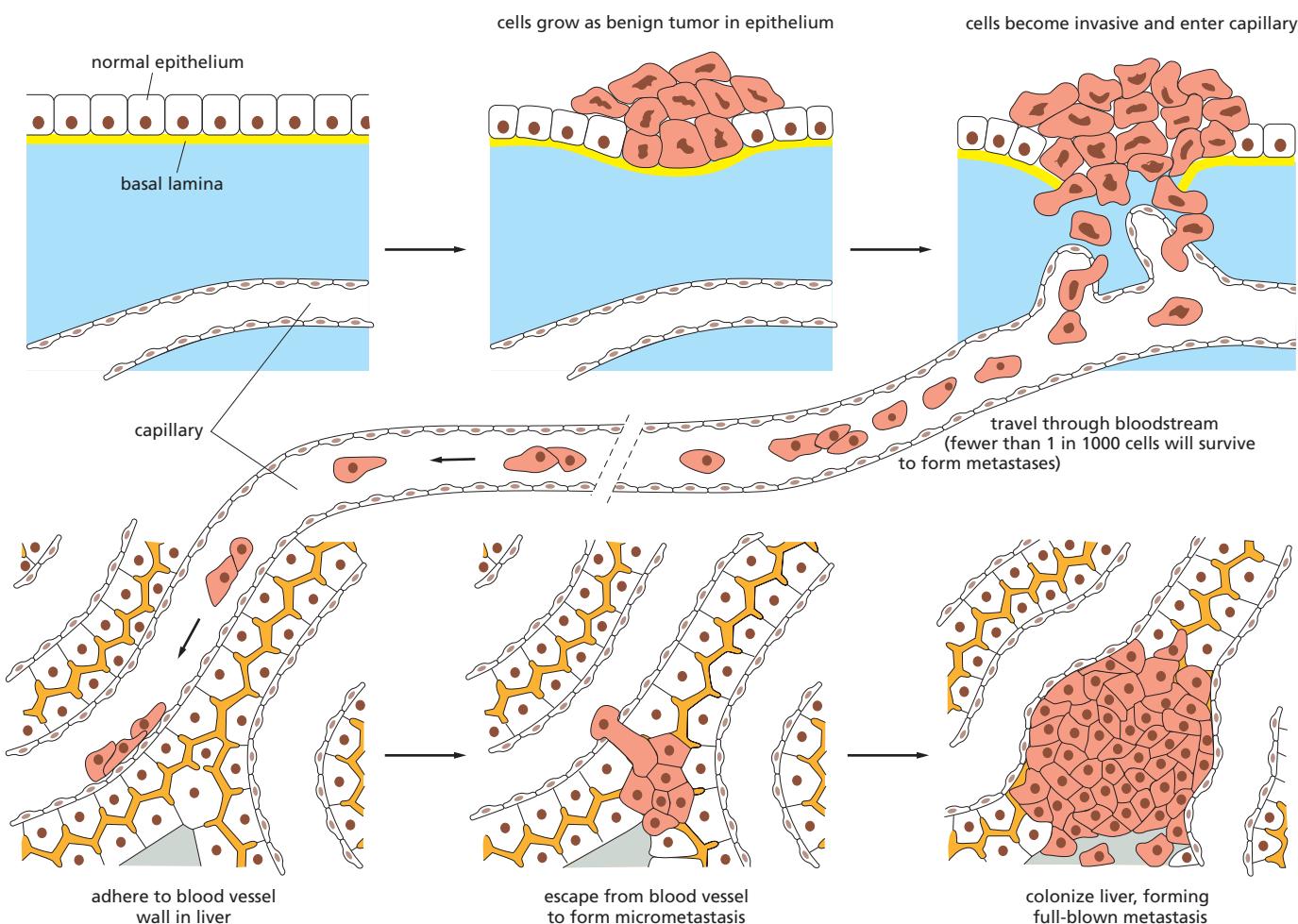
Cancer cells generally need to spread and multiply at new sites in the body in order to kill us, through a process called metastasis. This is the most deadly—and least understood—aspect of cancer, being responsible for 90% of cancer-associated deaths. By spreading through the body, a cancer becomes almost impossible to eradicate by either surgery or local irradiation. **Metastasis** is itself a multi-step process: the cancer cells first have to invade local tissues and vessels, move through the circulation, leave the vessels, and then establish new cellular colonies at distant sites (Figure 20–16). Each of these events is complex, and most of the molecular mechanisms involved are not yet clear.

For a cancer cell to become dangerous, it must break free of constraints that keep normal cells in their proper places and prevent them from invading neighboring tissues. Invasiveness is thus one of the defining properties of malignant tumors, which show a disorganized pattern of growth and ragged borders, with extensions into the surrounding tissue (see, for example, Figure 20–8). Although the underlying molecular changes are not well understood, invasiveness almost certainly requires a disruption of the adhesive mechanisms that normally keep cells tethered to their proper neighbors and to the extracellular matrix. For carcinomas, this change resembles the *epithelial-mesenchymal transition (EMT)* that occurs in some epithelial tissues during normal development (see p. 1042).

The next step in metastasis—the establishment of colonies in distant organs—begins with entry into the circulation: the invasive cancer cells must penetrate the



**Figure 20–15** The tumor microenvironment plays a role in tumorigenesis. Tumors consist of many cell types, including cancer cells, endothelial cells, pericytes (vascular smooth muscle cells), fibroblasts, and inflammatory white blood cells. Communication among these and other cell types plays an important part in tumor development. Note, however, that only the cancer cells are thought to be genetically abnormal in a tumor.



**Figure 20–16 Steps in the process of metastasis.** This example illustrates the spread of a tumor from an organ such as the bladder to the liver. Tumor cells may enter the bloodstream directly by crossing the wall of a blood vessel, as diagrammed here, or, more commonly perhaps, by crossing the wall of a lymphatic vessel that ultimately discharges its contents (lymph) into the bloodstream. Tumor cells that have entered a lymphatic vessel often become trapped in lymph nodes along the way, giving rise to lymph-node metastases.

Studies in animals show that typically far fewer than one in every thousand malignant tumor cells that enter the bloodstream will colonize a new tissue so as to produce a detectable tumor at a new site.

wall of a blood or lymphatic vessel. Lymphatic vessels, being larger and having more flimsy walls than blood vessels, allow cancer cells to enter in small clumps; such clumps may then become trapped in lymph nodes, giving rise to lymph-node metastases. The cancer cells that enter blood vessels, in contrast, seem to do so singly. With modern techniques for sorting cells according to their surface properties, it has become possible in some cases to detect these *circulating tumor cells* (CTCs) in samples of blood from cancer patients, even though they are only a minute fraction of the total blood-cell population. These cells, in principle at least, provide a useful sample of the tumor-cell population for genetic analysis.

Of the cancer cells that enter the lymphatics or bloodstream, only a tiny proportion succeed in making their exit, settling in new sites, and surviving and proliferating there as founders of metastases. Experiments show that fewer than one in thousands, perhaps one in millions, manage this feat. The final step of colonization seems to be the most difficult: like the Vikings who landed on the inhospitable shores of Greenland, the migrant cells may fail to survive in the alien environment; or they may only thrive there for a short while to found a little colony—a *micrometastasis*—that then dies out ([Movie 20.3](#)).

Many cancers are discovered before they have managed to found metastatic colonies and can be cured by destruction of the primary tumor. But on occasion,

an undetected micrometastasis will remain dormant for many years, only to reveal its presence by erupting into growth to form a large secondary tumor long after the primary tumor has been removed.

### Many Properties Typically Contribute to Cancerous Growth

Clearly, to produce a cancer, a cell must acquire a range of aberrant properties—a collection of subversive new skills—as it evolves. Different cancers require different combinations of these properties. Nevertheless, cancers all share some common features. By definition, they all ignore or misinterpret normal social controls so as to proliferate and spread where normal cells would not. These defining properties are commonly combined with other features that help the miscreants to arise and thrive. A list of the key attributes of cancer cells in general would include the following, all of which we have just discussed:

1. They grow (biosynthesize) when they should not, aided by a metabolism shifted from oxidative phosphorylation toward aerobic glycolysis.
2. They go through the cell-division cycle when they should not.
3. They escape from their home tissues (that is, they are invasive) and survive and proliferate in foreign sites (that is, they metastasize).
4. They have abnormal stress responses, enabling them to survive and continue dividing in conditions of stress that would arrest or kill normal cells, and they are less prone than normal cells to commit suicide by apoptosis.
5. They are genetically and epigenetically unstable.
6. They escape replicative cell senescence, either by producing telomerase or by acquiring another way of stabilizing their telomeres.

In the next section of the chapter, we examine the mutations and molecular mechanisms that underlie these and other properties of cancer cells.

### Summary

*Cancer cells, by definition, grow and proliferate in defiance of normal controls (that is, they are neoplastic) and are able to invade surrounding tissues and colonize distant organs (that is, they are malignant). By giving rise to secondary tumors, or metastases, they become difficult to eradicate by surgery or local irradiation. Cancers are thought to originate from a single cell that has experienced an initial mutation, but the progeny of this cell must undergo many further changes, requiring additional mutations and epigenetic events, to become cancerous. Tumor progression usually takes many years and reflects the operation of a Darwinian-like process of evolution, in which somatic cells undergo mutation and epigenetic changes accompanied by natural selection.*

*Cancer cells acquire a variety of special properties as they evolve, multiply, and spread. Their mutant genomes enable them to grow and divide in defiance of the signals that normally keep cell proliferation under tight control. As part of the evolutionary process of tumor progression, cancer cells acquire a collection of additional abnormalities, including defects in the controls that permanently stop cell division or induce apoptosis in response to cell stress or DNA damage, and in the mechanisms that normally keep cells from straying from their proper place. All of these changes increase the ability of cancer cells to survive, grow, and divide in their original tissue and then to metastasize, founding new colonies in foreign environments. The evolution of a tumor also depends on other cells present in the tumor microenvironment, collectively called stromal cells, that the cancer attracts and manipulates.*

*Since many changes are needed to confer this collection of asocial behaviors, it is not surprising that most cancer cells are genetically and/or epigenetically unstable. This instability is thought to be selected for in the clones of aberrant cells that are able to produce tumors, because it greatly accelerates the accumulation of the further genetic and epigenetic changes that are required for tumor progression.*

## CANCER-CRITICAL GENES: HOW THEY ARE FOUND AND WHAT THEY DO

As we have seen, cancer depends on the accumulation of inherited changes in somatic cells. To understand it at a molecular level we need to identify the mutations and epigenetic changes involved and to discover how they give rise to cancerous cell behavior. Finding the relevant cells is often easy; they are favored by natural selection and call attention to themselves by giving rise to tumors. But how do we identify those genes with the cancer-promoting changes among all the other genes in the cancerous cells? A typical cancer depends on a whole set of mutations and epigenetic changes—usually a somewhat different set in each individual patient. In addition, a given cancer cell will also contain a large number of somatic mutations that are accidental by-products—so-called *passengers* rather than *drivers*—of its genetic instability, and it can be difficult to distinguish these meaningless changes from those changes that have a causative role in the disease. Despite these difficulties, many of the genes that are repeatedly altered in human cancers have been identified over the past 40 years. We will call such genes, for want of a better term, **cancer-critical genes**, meaning all genes whose alteration contributes to the causation or evolution of cancer by driving tumorigenesis.

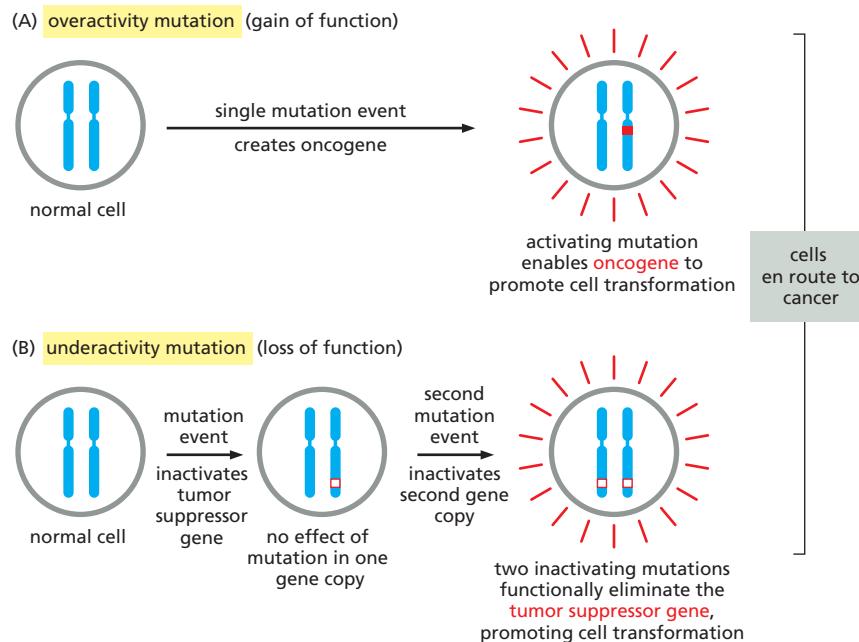
In this section, we shall first discuss how cancer-critical genes are identified. We shall then examine their functions and the parts they play in conferring on cancer cells the properties outlined in the first part of the chapter. We shall end the section by discussing colon cancer as an extended example, showing how a succession of changes in cancer-critical genes enables a tumor to evolve from one pattern of bad behavior to another that is worse.

### The Identification of Gain-of-Function and Loss-of-Function Cancer Mutations Has Traditionally Required Different Methods

Cancer-critical genes are grouped into two broad classes, according to whether the cancer risk arises from too much activity of the gene product or too little. Genes of the first class, in which a gain-of-function mutation can drive a cell toward cancer, are called **proto-oncogenes**; their mutant, overactive or overexpressed forms are called **oncogenes**. Genes of the second class, in which a loss-of-function mutation can contribute to cancer, are called **tumor suppressor genes**. In either case, the mutation may lead toward cancer directly (by causing cells to proliferate when they should not) or indirectly—for example, by causing genetic or epigenetic instability and so hastening the occurrence of other inherited changes that directly stimulate tumor growth. Those genes whose alteration results in genomic instability represent a subclass of cancer-critical genes that are sometimes called *genome maintenance genes*.

As we shall see, mutations in oncogenes and tumor suppressor genes can have similar effects in promoting the development of cancer; overproduction of a signal for cell proliferation, for example, can result from either kind of mutation. Thus, from the point of view of a cancer cell, oncogenes and tumor suppressor genes—and the mutations that affect them—are flip sides of the same coin. The techniques that led to the discovery of these two categories of genes, however, are quite different.

The mutation of a single copy of a proto-oncogene that converts it to an oncogene has a dominant, growth-promoting effect on a cell (Figure 20–17A). Thus, we can identify the oncogene by its effect when it is *added*—by DNA transfection, for example, or through infection with a viral vector—to the genome of a suitable type of tester cell or experimental animal. In the case of the tumor suppressor gene, on the other hand, the cancer-causing alleles produced by the change are generally recessive: often (but not always) both copies of the normal gene must be removed or inactivated in the diploid somatic cell before an effect is seen (Figure 20–17B). This calls for a different experimental approach, one focusing on discovering what is *missing* in the cancer cell.



**Figure 20–17** Cancer-critical mutations fall into two readily distinguishable categories, dominant and recessive. In this diagram, activating mutations are represented by solid red boxes, inactivating mutations by hollow red boxes. (A) Oncogenes act in a dominant manner: a gain-of-function mutation in a single copy of the cancer-critical gene can drive a cell toward cancer. (B) Mutations in tumor suppressor genes, on the other hand, generally act in a recessive manner: the function of both alleles of the cancer-critical gene must be lost to drive a cell toward cancer. Although in this diagram the second allele of the tumor suppressor gene is inactivated by mutation, it is often inactivated instead by loss of the second chromosome. Not shown is the fact that mutation of some tumor suppressor genes can have an effect even when only one of the two gene copies is damaged.

We begin by discussing some examples of each class of cancer-critical genes to illustrate basic principles. These examples are chosen also for their historical importance: the experiments that led to their discovery—at different times and by different methods—marked turning points in the understanding of cancer.

### Retroviruses Can Act as Vectors for Oncogenes That Alter Cell Behavior

The search for the genetic causes of human cancer took a devious route, beginning with clues that came from the study of **tumor viruses**. Although viruses are involved only in a minority of human cancers, a set of viruses that infect animals provided critical early tools for studying cancer.

One of the first animal viruses to be implicated in cancer was discovered over 100 years ago in chickens, when an infectious agent that causes connective-tissue tumors, or sarcomas, was characterized as a virus—the *Rous sarcoma virus*. Like all the other *RNA tumor viruses* discovered since, it is a **retrovirus**. When it infects a cell, its RNA genome is copied into DNA by reverse transcription, and the DNA is inserted into the host genome, where it can persist and be inherited by subsequent generations of cells. Something in the DNA inserted by the Rous sarcoma virus made the host cells cancerous, but what was it? The answer was a surprise. It turned out to be a piece of DNA that was unnecessary for the virus's own survival or reproduction; instead, it was a passenger, a gene called *v-Src*, that the virus had picked up on its travels. *v-Src* was unmistakably similar, but not identical, to a gene—*c-Src*—that was discovered in the normal vertebrate genome. *c-Src* had evidently been caught up accidentally by the retrovirus from the genome of a previously infected host cell, and it had undergone mutation in the process to become an oncogene (*v-Src*).

This Nobel Prize-winning finding was followed by a flood of discoveries of other viral oncogenes carried by retroviruses that cause cancer in nonhuman animals. Each such oncogene turned out to have a counterpart proto-oncogene in the normal vertebrate genome. As was the case for *Src*, these other oncogenes generally differed from their normal counterparts, either in structure or in level of expression. But how did this relate to typical human cancers, most of which are not infectious and in which retroviruses play no part?

## Different Searches for Oncogenes Converged on the Same Gene—*Ras*

In an attempt to answer the above question, other researchers searched directly for oncogenes in the genomes of human cancer cells. They did this by searching for DNA fragments from cancer cells that could provoke uncontrolled proliferation when introduced into noncancerous cell lines. As tester cells for the assay, cell lines derived from mouse fibroblasts were used. These cells had been previously selected for their ability to proliferate indefinitely in culture, and they are thought to already contain alterations that take them part of the way toward malignancy. For this reason, the addition of a single oncogene can sometimes be enough to produce a dramatic effect.

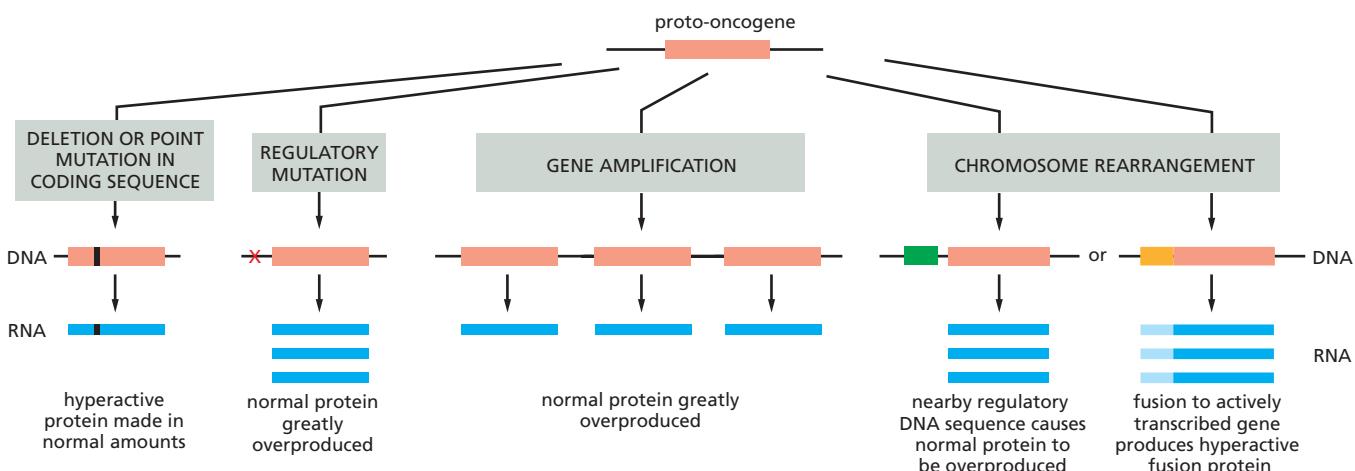
When DNA was extracted from the human tumor cells, broken into fragments, and introduced into the cultured cells, occasional colonies of abnormally proliferating cells began to appear in the culture dish. These cells showed a transformed phenotype, outgrowing the untransformed cells in the culture and piling up in layer upon layer (see Figure 20–11). Each colony was a clone originating from a single cell that had incorporated a DNA fragment that drove cancerous behavior. This fragment, which carried markers of its human origin, could be isolated from the transformed cultured mouse cells. And once isolated and sequenced, it could be recognized: it contained a human version of a gene already known from study of a retrovirus that caused tumors in rats—an oncogene called *v-Ras*.

The newly discovered oncogene was clearly derived by mutation from a normal human gene, one of a small family of proto-oncogenes called *Ras*. This discovery in the early 1980s of the same oncogene in human tumor cells and in an animal tumor virus was electrifying. The implication that cancers are caused by mutations in a limited number of cancer-critical genes transformed our understanding of the molecular biology of cancer.

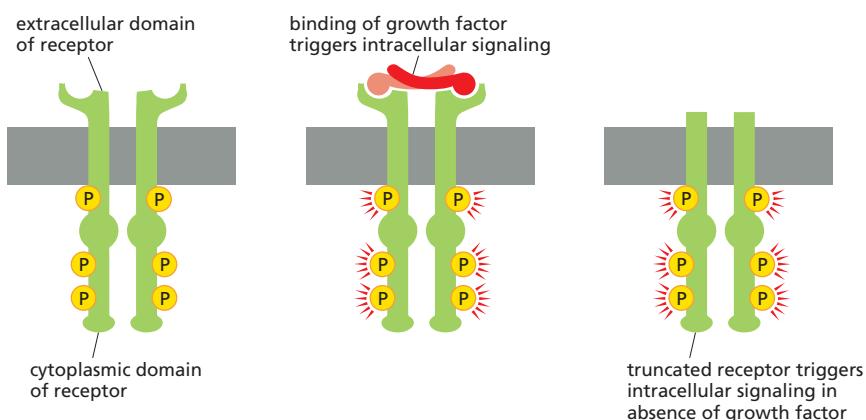
As discussed in Chapter 15, normal Ras proteins are monomeric GTPases that help transmit signals from cell-surface receptors to the cell interior (see Movie 15.7). The *Ras* oncogenes isolated from human tumors contain point mutations that create a hyperactive Ras protein that cannot shut itself off by hydrolyzing its bound GTP to GDP. Because this makes the protein hyperactive, its effect is dominant—that is, only one of the cell’s two gene copies needs to change to have an effect. One or another of the three human *Ras* family members is mutated in perhaps 30% of all human cancers. *Ras* genes are thus among the most important of all cancer-critical genes.

## Genes Mutated in Cancer Can Be Made Overactive in Many Ways

**Figure 20–18** summarizes the types of accidents that can convert a proto-oncogene into an oncogene. (1) A small change in DNA sequence such as a point



**Figure 20–18** The types of accidents that can convert a proto-oncogene into an oncogene.



**Figure 20–19** Mutation of the epidermal growth factor (EGF) receptor can make it active even in the absence of EGF, and consequently oncogenic. Only one of the possible types of activating mutations is illustrated here.

mutation or deletion may produce a hyperactive protein when it occurs within a protein-coding sequence, or lead to protein overproduction when it occurs within a regulatory region for that gene. (2) Gene amplification events, such as those that can be caused by errors in DNA replication, may produce extra gene copies; this can lead to overproduction of the protein. (3) A chromosomal rearrangement—involved in the breakage and rejoining of the DNA helix—may either change the protein-coding region, resulting in a hyperactive fusion protein, or alter the control regions for a gene so that a normal protein is overproduced.

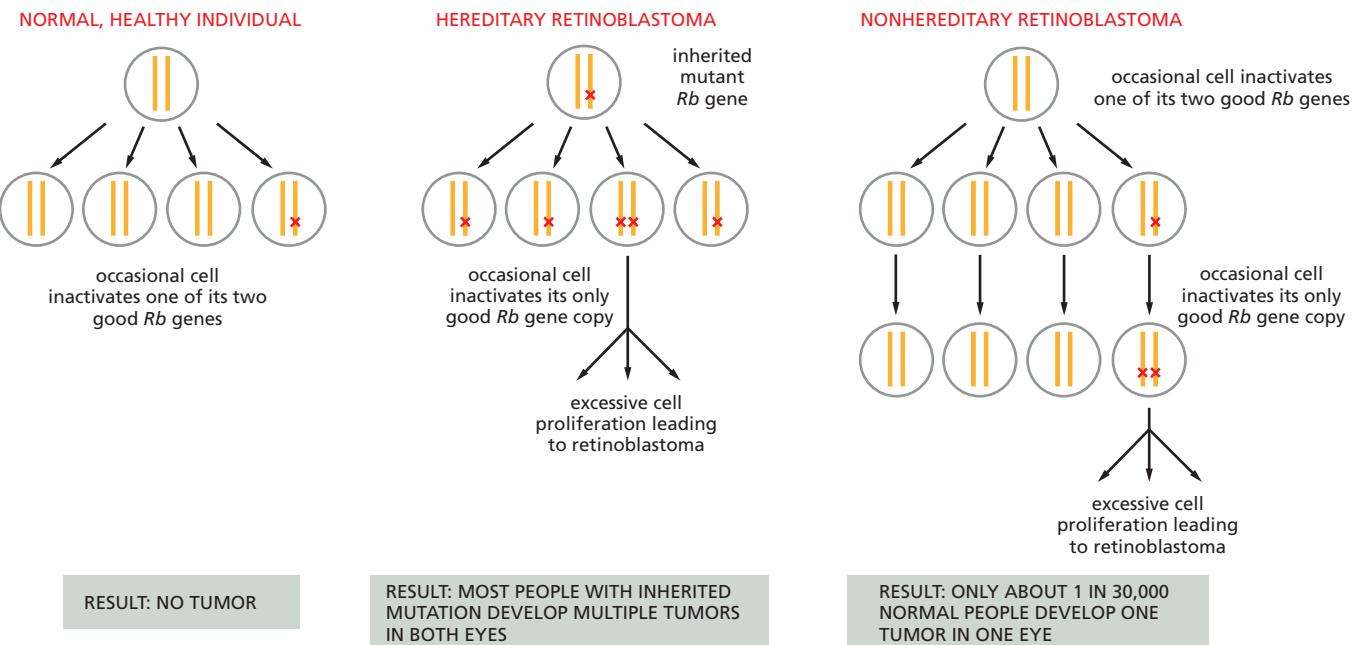
As one example, the receptor for the extracellular signal protein *epidermal growth factor* (EGF) can be activated by a deletion that removes part of its extracellular domain, causing it to be active even in the absence of EGF (Figure 20–19). It thus produces an inappropriate stimulatory signal, like a faulty doorbell that rings even when nobody is pressing the button. Mutations of this type are frequently found in the most common type of human brain tumor, called glioblastoma.

As another example, the *Myc protein*, which acts in the nucleus to stimulate cell growth and division (see Chapter 17), generally contributes to cancer by being overproduced in its normal form. In some cases, the gene is amplified—that is, errors of DNA replication lead to the creation of large numbers of gene copies in a single cell. Or a point mutation can stabilize the protein, which normally turns over very rapidly. More commonly, the overproduction appears to be due to a change in a regulatory element that acts on the gene. For example, a chromosomal translocation can inappropriately bring powerful gene regulatory sequences next to the *Myc* protein-coding sequence, so as to produce unusually large amounts of *Myc* mRNA. Thus, in Burkitt's lymphoma, a translocation brings the *Myc* gene under the control of sequences that normally drive the expression of antibody genes in B lymphocytes. As a result, the mutant B cells tend to proliferate excessively and form a tumor. Different specific chromosome translocations are common in other cancers.

### Studies of Rare Hereditary Cancer Syndromes First Identified Tumor Suppressor Genes

Identifying a gene that has been inactivated in the genome of a cancer cell requires a different strategy from finding a gene that has become hyperactive: one cannot, for example, use a cell transformation assay to identify something that simply is not there. The key insight that led to the discovery of the first tumor suppressor gene came from studies of a rare type of human cancer, **retinoblastoma**, which arises from cells in the retina of the eye that are converted to a cancerous state by an unusually small number of mutations. As often happens in biology, the discovery arose from examination of a special case, but it turned out to reveal a gene of widespread importance.

Retinoblastoma occurs in childhood, and tumors develop from neural precursor cells in the immature retina. About one child in 20,000 is afflicted. One form of the disease is hereditary, and the other is not. In the hereditary form,



multiple tumors usually arise independently, affecting both eyes; in the nonhereditary form, only one eye is affected, and by only one tumor. A few individuals with retinoblastoma have a visibly abnormal karyotype, with a deletion of a specific band on chromosome 13 that, if inherited, predisposes an individual to the disease. Deletions of this same region are also encountered in tumor cells from some patients with the nonhereditary disease, which suggested that the cancer was caused by loss of a critical gene in that location.

Using the location of this chromosomal deletion, it was possible to clone and sequence the **Rb gene**. It was then discovered that those who suffer from the hereditary form of the disease have a deletion or loss-of-function mutation present in one copy of the *Rb* gene in every somatic cell. These cells are predisposed to becoming cancerous, but do not do so if they retain one good copy of the gene. The retinal cells that are cancerous are defective in both copies of *Rb* because of a somatic event that has eliminated the function of the previously good copy.

In patients with the nonhereditary form of the disease, by contrast, the noncancerous cells show no defect in either copy of *Rb*, while the cancerous cells have become defective in both copies. These nonhereditary retinoblastomas are very rare because they require two independent events that inactivate the same gene on two chromosomes in a single retinal cell lineage (Figure 20–20).

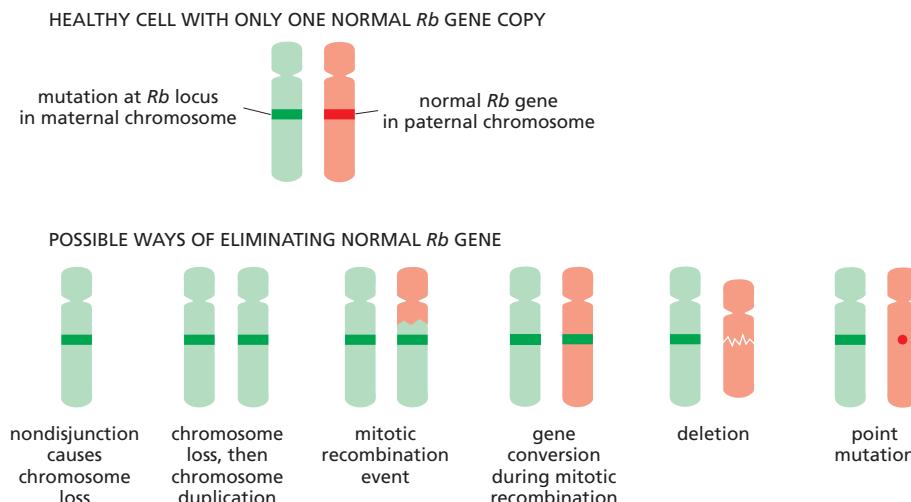
The *Rb* gene is also missing in several common types of sporadic cancer, including carcinomas of lung, breast, and bladder. These more common cancers arise by a more complex series of genetic changes than does retinoblastoma, and they make their appearance much later in life. But in all of them, it seems, loss of *Rb* function is frequently a major step in the progression toward malignancy.

The *Rb* gene encodes the **Rb protein**, which is a universal regulator of the cell cycle present in almost all cells of the body (see Figure 17–61). It acts as one of the main brakes on progress through the cell-division cycle, and its loss can allow cells to enter the cell cycle inappropriately, as we discuss later.

### Both Genetic and Epigenetic Mechanisms Can Inactivate Tumor Suppressor Genes

For tumor suppressor genes, it is their inactivation that is dangerous. This inactivation can occur in many ways, with different combinations of mishaps serving to eliminate or cripple both gene copies. The first copy may, for example, be lost by a small chromosomal deletion or inactivated by a point mutation. The second copy is commonly eliminated by a less specific and more probable mechanism:

**Figure 20–20** The genetic mechanisms that cause retinoblastoma. In the hereditary form, all cells in the body lack one of the normal two functional copies of the *Rb* tumor suppressor gene, and tumors occur where the remaining copy is lost or inactivated by a somatic event (either mutation or epigenetic silencing). In the nonhereditary form, all cells initially contain two functional copies of the gene, and the tumor arises because both copies are lost or inactivated through the coincidence of two somatic events in a single line of cells.



**Figure 20-21** Six ways of losing the remaining good copy of a tumor suppressor gene through a change in DNA sequences. A cell that is defective in only one of its two copies of a tumor suppressor gene—for example, the *Rb* gene—usually behaves as a normal, healthy cell; the diagrams below show how this cell may lose the function of the other gene copy as well and thereby progress toward cancer. A seventh possibility, frequently encountered with some tumor suppressors, is that the gene may be silenced by an epigenetic change, without alteration of the DNA sequence, as illustrated in Figure 20-22. (After W.K. Cavenee et al., *Nature* 305:779–784, 1983. With permission from Macmillan Publishers Ltd.)

the chromosome carrying the remaining normal copy may be lost from the cell through errors in chromosome segregation; or the normal gene, along with neighboring genetic material, may be replaced by a mutant version through either a *mitotic recombination* event or a *gene conversion* that accompanies it (see p. 286).

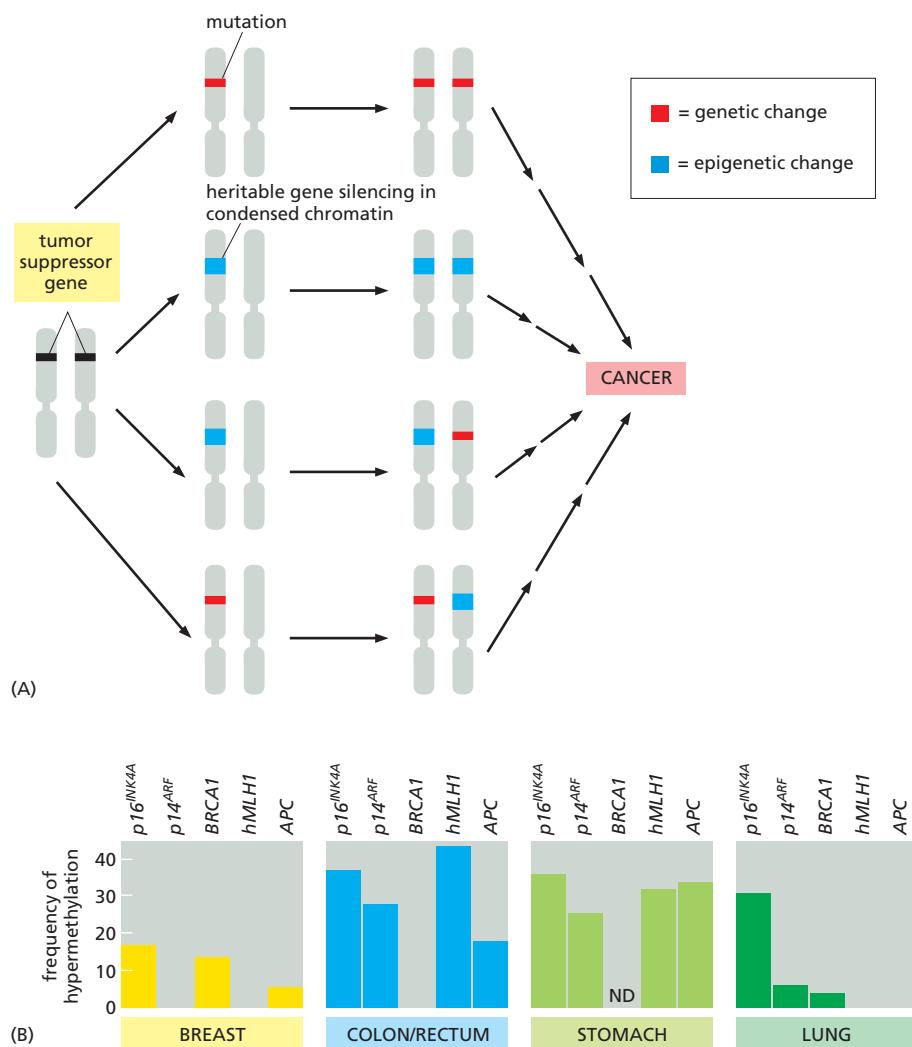
**Figure 20-21** summarizes the range of ways in which the remaining good copy of a tumor suppressor gene can be lost through a DNA sequence change, using the *Rb* gene as an example. It is important to note that, except for the point mutation mechanism illustrated at the far right, these pathways all produce cells that carry only a single type of DNA sequence in the chromosomal region containing their *Rb* genes—a sequence that is identical to the sequence in the original mutant chromosome.

Epigenetic changes provide another important way to permanently inactivate a tumor suppressor gene. Most commonly, the gene may become packaged into heterochromatin and/or the C nucleotides in CG sequences in its promoter may become methylated in a heritable manner (see pp. 404–405). These mechanisms can irreversibly silence the gene in a cell and in all of its progeny. Analysis of methylation patterns in cancer genomes shows that epigenetic gene silencing is a frequent event in tumor progression, and epigenetic mechanisms are now thought to help inactivate several different tumor suppressor genes in most human cancers (**Figure 20-22**).

### Systematic Sequencing of Cancer Cell Genomes Has Transformed Our Understanding of the Disease

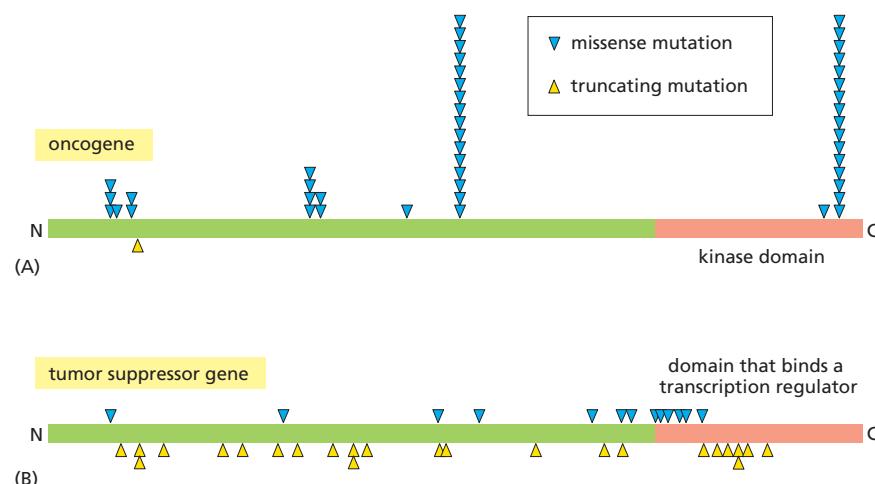
Methods such as those we have described above shone a spotlight on a set of cancer-critical genes that were identified in a piecemeal fashion. Meanwhile, the rest of the cancer cell genome remained in darkness: it was a mystery how many other mutations might lurk there, of what types, in which varieties of cancer, at what frequencies, with what variations from patient to patient, and with what consequences. With the sequencing of the human genome and the dramatic advances in DNA sequencing technology (see Panel 8-1, pp. 478–481), it has become possible to see the whole picture—to view cancer cell genomes in their entirety. This transforms our understanding of the disease.

Cancer cell genomes can be scanned systematically in several different ways. At one extreme—the most costly, but no longer prohibitively so—one can determine a tumor's complete genome sequence. More cheaply, one can focus just on the 21,000 or so genes in the human genome that code for protein (the so-called *exome*), looking for mutations in the cancer cell DNA that alter the amino acid sequence of the product or prevent its synthesis (**Figure 20-23**). There are also efficient techniques to survey the genome for regions that have undergone



**Figure 20-22** The pathways leading to loss of tumor suppressor gene function in cancer involve both genetic and epigenetic changes. (A) As indicated, the changes that silence tumor suppressor genes can occur in any order. Both DNA methylation and the packaging of a gene into condensed chromatin can prevent its expression in a way that is inherited when a cell divides (see Figure 4-44). (B) The frequency of gene silencing by hypermethylation observed in four different types of cancer. The five genes listed at the top can all function as tumor suppressor genes; *BRCA1* and *hMLH1* affect genome stability and are in the subclass known as genome maintenance genes. ND, no data. (Adapted from M. Esteller et al., *Cancer Res.* 61:3225–3229, 2001.)

deletion or duplication, without the need for complete sequence information. The genome can be scanned for epigenetic changes. And finally, alterations in levels of gene expression can be systematically determined by analysis of mRNAs (see Figure 7-3). These approaches generally involve comparing cancer cells with normal controls—ideally, noncancerous cells originating in the same tissue and from the same patient.



**Figure 20-23** The distinct types of DNA sequence changes found in oncogenes compared to tumor suppressor genes. In this diagram, mutations that change an amino acid are denoted by blue arrowheads, whereas mutations that truncate the polypeptide chain are marked by yellow arrowheads. (A) As in this example, oncogene mutations can be detected by the fact that the same nucleotide change is repeatedly found among the missense mutations in a gene. (B) For tumor suppressor genes, by contrast, missense mutations that abort protein synthesis by creating stop codons predominate. (Adapted from B. Vogelstein et al., *Science* 339:1546–1558, 2013.)

## Many Cancers Have an Extraordinarily Disrupted Genome

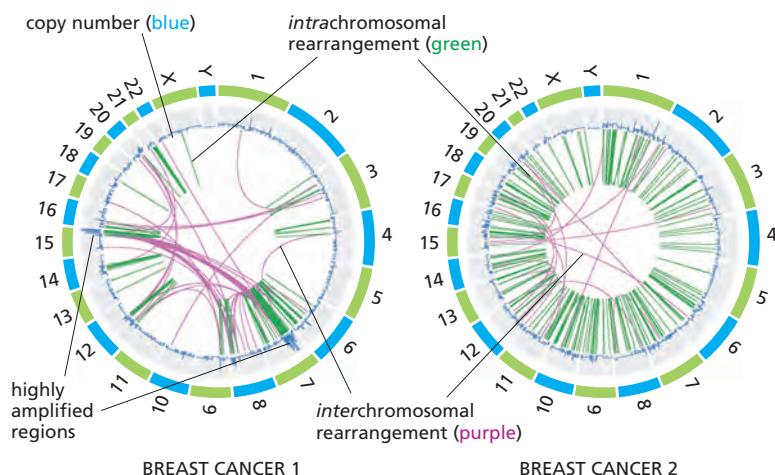
Cancer genome analysis reveals, first of all, the scale of gross genetic disruption in cancer cells. This varies greatly from one type of cancer and one cancer patient to another, both in severity and in character. In some cases, the karyotype—the set of chromosomes as they appear at mitosis—is normal or nearly so, but many point mutations are detected in individual genes, suggesting a failure of the repair mechanisms that normally correct local errors in the replication or maintenance of DNA sequences. Often, however, the karyotype is severely disordered, with many chromosome breaks and rearrangements. In some breast cancers, for example, genome sequencing reveals an astonishing scene of genetic chaos (Figure 20–24), with hundreds of chromosome breaks and translocations, resulting in many deletions, duplications, and amplifications of parts of the genome. In such cells, the normal machinery for avoidance or repair of DNA double-strand breaks is evidently somehow defective, destabilizing the genome by giving rise to broken chromosomes whose fragments then rejoin in random combinations. From the pattern of changes, one can infer that this disruptive process has occurred repeatedly during the evolution of the tumor, with a progressive increase of genetic disorder. Breast cancers showing the most extreme chromosome disorder are usually hard to treat and have a gloomy prognosis.

One survey of more than 3000 individual cancer specimens showed that on average 24 separate blocks of genetic material were duplicated in each tumor, amounting to 17% of the normal genome, and 18 blocks were deleted, amounting to 16% of the normal genome. Many of these changes were found repeatedly, suggesting that they contain cancer-critical genes whose loss (tumor suppressor genes) or gain (oncogenes) confers a selective advantage.

Whole-genome analysis also helps to explain some cancers that seem, at first sight, to be exceptions to the general rules. An example is retinoblastoma, with its early onset during childhood. If cancers in general require an accumulation of many genetic changes and are thus diseases of old age, what makes retinoblastoma different? Whole-genome sequencing confirms that in retinoblastoma, the tumor cells contain loss-of-function mutations in the *Rb* gene; but, amazingly, they contain practically no mutations or genome rearrangements that affect any other oncogene or tumor suppressor gene. Instead, they contain many epigenetic modifications, which alter the level of expression of many known cancer-critical genes—as many as 15 in one well-analyzed case.

## Many Mutations in Tumor Cells are Merely Passengers

Cancer cells generally contain many mutations in addition to gross chromosome abnormalities: point mutations can be scattered over the genome as a whole at a rate of about one per million nucleotide pairs, in addition to the abnormalities



**Figure 20–24** The chromosomal rearrangements in breast cancer cells. The results of an extensive DNA sequencing analysis performed on two different primary tumors are displayed as “Circos plots.” In each plot, the reference DNA sequences of the 22 autosomes and single sex chromosome (X) of a normal human female (3.2 billion nucleotide pairs) are aligned end-to-end to form a circle. Colored lines within the circle are then used to indicate the chromosome alterations found in the particular primary tumor. As indicated, purple lines connect sites at which two different chromosomes have become joined to create an interchromosomal rearrangement, while green lines connect the sites of rearrangements found within a single chromosome. The intrachromosomal rearrangements can be seen to predominate, and most join neighboring sections of DNA that were originally located within 2 million nucleotide pairs of each other. The increases in copy number, shown in blue, reveal the amplified DNA sequences (see the highly amplified regions indicated). (Adapted from P.J. Stephens et al., *Nature* 462:1005–1010, 2009.)

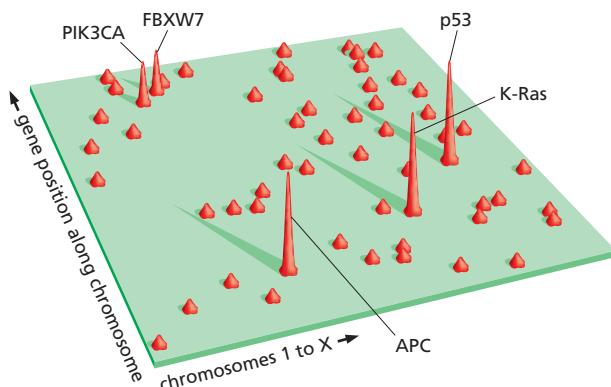
attributed to chromosome breakage and rejoining. Systematic surveys of the protein-coding genes in common solid tumors—such as those of the breast, colon, brain, or pancreas—have revealed that an average of 33 to 66 genes have undergone somatic mutation affecting the sequence of their protein product. Mutations in noncoding regions of the genome are much more numerous, as one would expect from the much larger fraction of the genome that noncoding DNA represents. But they are considerably more difficult to interpret.

The high frequency of mutations testifies to the genetic instability of many cancer cells, but it leaves us with a difficult problem. How can we discover which of the mutations are **drivers** of cancer—that is, causal factors in the development of the disease—and which are merely **passengers**—mutations that happen to have occurred in the same cell as the driver mutations, thanks to genetic instability, but are irrelevant to the development of the disease? A simple criterion is based on frequency of occurrence. Driver mutations affecting a gene that plays a part in the disease will be seen repeatedly, in many different patients. In contrast, passenger mutations, occurring at more-or-less random locations in the genome and conferring no selective advantage on the cancer cell, are unlikely to be found in the same genes in different patients.

**Figure 20–25** shows the results of an analysis of this sort for a large sample of colorectal cancers. The different sites in the genome are laid out on a two-dimensional array, with chromosome serial number along one axis and position within each chromosome along the other. The frequency with which mutations are encountered is shown by height above this plane, creating a mutation “landscape” with mountains (sites where mutations are found in a large proportion of the tumors in the sample), hills (where mutations are found less frequently but still more often than would be expected for a random scattering over the genome), and hillocks (sites of occasional mutations, occurring at a frequency no higher than would be expected for mutations scattered at random in each individual tumor). The mountains and the hills are strong candidates to be the sites of driver mutations—in other words, sites of cancer-critical genes; the hillocks are likely to correspond to passengers. Indeed, many of the mountains and hills turn out to be sites of known oncogenes or tumor suppressor genes, whereas the hillocks mostly correspond to genes that have no known or probable role in causation of cancer. Of course, some hillocks may correspond to genes that are mutated in only a few rare patients but are nevertheless cancer-critical for them.

### About One Percent of the Genes in the Human Genome Are Cancer-Critical

From studies such as the one just described, it is estimated that the number of driver mutations for an individual case of cancer (the sum of meaningful epigenetic and genetic changes in both coding sequences and regulatory regions) is typically on the order of 10, explaining why cancer progression generally involves an increase in genetic and/or epigenetic instability that enhances the rate of such changes.



**Figure 20–25** The mutation landscape in colorectal cancer. In this two-dimensional representation of the human genome, the green surface depicts the 22 human autosomes plus the X sex chromosome as being laid out side-by-side in numerical order from left to right, with the DNA sequence of each chromosome running from back to front. The mountains represent the locations of genes mutated with high frequency in different, independent tumors. As indicated, these are suspected driver mutations in the adenomatous polyposis coli (APC), K-Ras, p53, phosphoinositide 3-kinase (PIK3CA), and ubiquitin ligase (FBXW7) proteins. (Adapted from L.D. Wood et al., *Science* 318:1108–1113, 2007.)

By compiling the data for different types of cancer, each with its own range of identified driver mutations, we can develop a comprehensive catalog of genes that are strongly suspected to be cancer-critical. Current estimates put the total number of such genes at about 300, about 1% of the genes in the human genome. These cancer-critical genes are amazingly diverse. Their products include secreted signal proteins, transmembrane receptors, GTP-binding proteins, protein kinases, transcription regulators, chromatin modifiers, DNA repair enzymes, cell-cell adhesion molecules, cell-cycle controllers, apoptosis regulators, scaffold proteins, metabolic enzymes, components of the RNA splicing machinery, and more besides. All these are susceptible to mutations that can contribute, in one way or another, in one tissue or another, to the evolution of cells with the cancerous properties that we listed earlier on page 1103.

Clearly, the molecular changes that cause cancer are complex. As we now explain, however, the complexity is not quite as daunting as it may initially seem.

### Disruptions in a Handful of Key Pathways Are Common to Many Cancers

Some genes, like *Rb* and *Ras*, are mutated in many cases of cancer and in cancers of many different types. The involvement of genes such as *Rb* and *Ras* in cancer is no surprise, now that we understand their normal functions: they control fundamental processes of cell division and growth. But even these common culprits feature in considerably less than half of individual cases. What is happening to the control of these processes in the many cases of cancer where, for example, *Rb* is intact or *Ras* is not mutated? What part do mutations in the hundreds of other cancer-critical genes play in the development of the disease? With our increasing knowledge of the normal functions of the genes in the human genome, it is becoming easier to see patterns in the catalogued driver mutations and to give some simplifying answers to these questions.

*Glioblastoma*—the commonest type of human brain tumor—provides a good example. Analysis of the genomes of tumor cells from 91 patients identified a total of at least 79 genes that were mutated in more than one individual. The normal functions of most of these genes were known or could be guessed, allowing them to be assigned to specific biochemical or regulatory pathways. Three functional groupings stood out, accounting for a total of 21 of the recurrently mutated genes. One of these groupings consisted of genes in the *Rb pathway* (that is, *Rb* itself, along with genes that directly regulate *Rb*); this pathway governs initiation of the cell-division cycle. Another consisted of genes in the same regulatory subnetwork as *Ras*—a more loosely defined system of genes referred to as the *RTK/Ras/PI3K pathway*, after three of its core components; this pathway serves to transmit signals for cell growth and cell division from the cell exterior into the heart of the cell. The third grouping consisted of genes in a pathway regulating responses to stress and DNA damage—the *p53 pathway*. We shall have more to say about each of these pathways below.

Out of all tumors, 74% had identifiable mutations in all three pathways. If one were to trace these three pathways further upstream and include all the components, known and unknown, on which they depend, this percentage would almost certainly be even higher. In other words, in almost every case of glioblastoma, there are mutations that disrupt each of three fundamental controls: the control of cell growth, the control of cell division, and the control of responses to stress and DNA damage.

Strikingly, in any given tumor-cell clone, there is a strong tendency for no more than one gene to be mutated in each pathway. Evidently, what matters for tumor evolution is the disruption of the control mechanism, and not the genetic means by which that is achieved. Thus, for example, in a patient whose tumor cells have no mutation in *Rb* itself, there is generally a mutation in some other component of the *Rb* pathway, producing a similar biological effect.

Similar patterns are seen in other types of cancers. A survey of many specimens of the major variety of ovarian cancer, for example, identified 67% of patients as

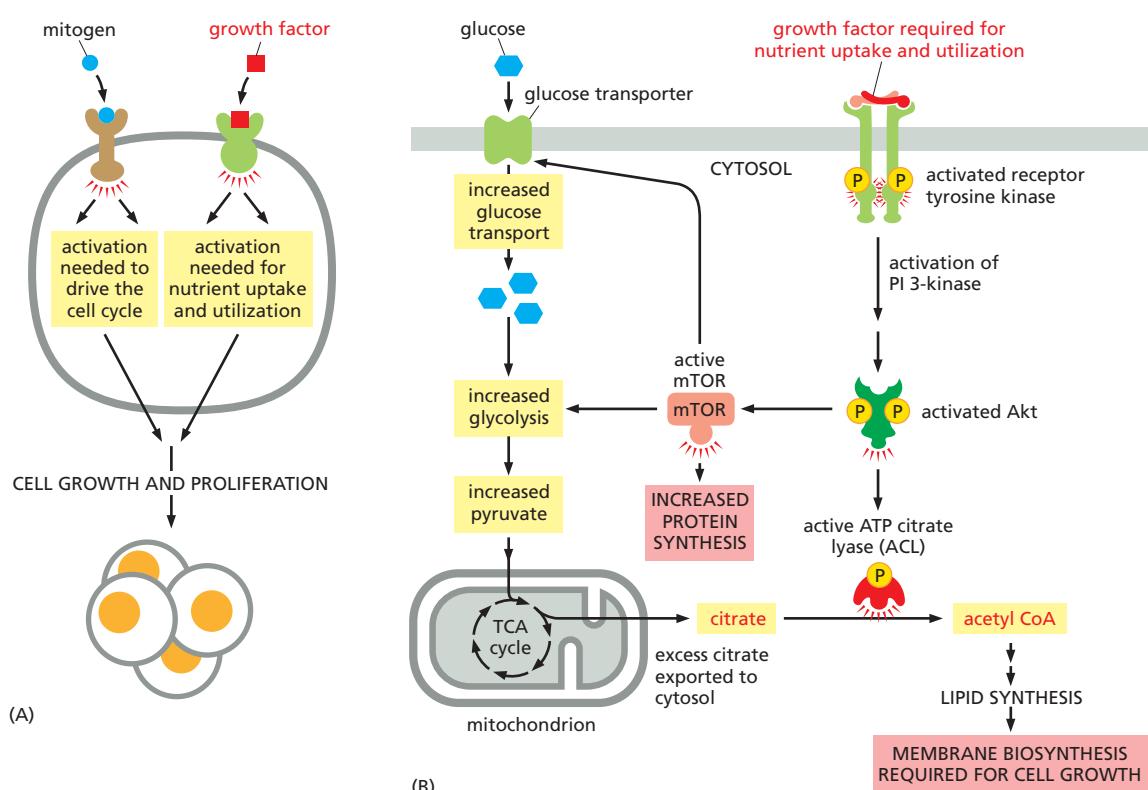
having mutations in the Rb pathway, 45% in the Ras/PI3K pathway (defined more narrowly than in the glioblastoma study), and more than 96% in the p53 pathway. Allowing for additional pathway components not included in the analysis, it seems that most cases of this type of cancer, too, have mutations disrupting the same three controls, leading to misregulated cell growth, misregulated cell proliferation, and abnormal disregard of stress and DNA damage. It seems that these three fundamental controls are subverted in one way or another in virtually every type of cancer.

We have devoted an entire chapter to the cell cycle and growth controls (Chapter 17). Some important details of the other two control pathways are reviewed next.

### Mutations in the PI3K/Akt/mTOR Pathway Drive Cancer Cells to Grow

Cell proliferation is not simply a matter of progression through the cell cycle; it also requires cell growth, which involves complex anabolic processes through which the cell synthesizes all the necessary macromolecules from small-molecule precursors. If a cell divides inappropriately without growing first, it will get smaller at each division and will ultimately die or become too small to divide. Cells appear to require two separate signals to grow and divide (Figure 20–26). Cancer depends, therefore, not only on a loss of restraints on cell-cycle progression, but also on disrupted control of cell growth.

The phosphoinositide 3-kinase (PI 3-kinase)/Akt/mTOR intracellular signaling pathway is critical for cell growth control. As described in Chapter 15, various extracellular signal proteins, including insulin and insulin-like growth factors,



**Figure 20–26** Cells seem to require two types of signals to proliferate. (A) In order to multiply successfully, most normal cells are suspected to require both extracellular signals that drive cell-cycle progression (shown here as blue mitogen) and extracellular signals that drive cell growth (shown here as red growth factor). How mitogens activate the Rb pathway to drive entry into the cell cycle is described in Figure 17–61. (B) Diagram of the signaling system containing Akt that drives cell growth through greatly stimulating glucose uptake and utilization, including a conversion of the excess citric acid produced from sugar intermediates in mitochondria into the acetyl CoA that is needed in the cytosol for lipid synthesis and new membrane production. As indicated, protein synthesis is also increased. This system becomes abnormally activated early in tumor progression. TCA cycle indicates the tricarboxylic acid cycle (citric acid cycle).

normally activate this pathway. In cancer cells, however, the pathway is activated by mutation so that the cell can grow in the absence of such signals. The resulting abnormal activation of the protein kinases Akt and mTOR not only stimulates protein synthesis (see Figure 17–64), but also greatly increases both glucose uptake and the production of the acetyl CoA in the cytosol required for cell lipid synthesis, as outlined in Figure 20–26B.

The abnormal activation of the PI 3-kinase/Akt/mTOR pathway, which normally occurs early in the process of tumor progression, helps to explain the excessive rate of glycolysis that is observed in tumor cells, known as the Warburg effect, as discussed earlier (see Figure 20–12). As expected from our previous discussion, cancers can activate this pathway in many different ways. Thus, for example, a growth factor receptor can become abnormally activated, as in Figure 20–19. Also very common in cancers is the loss of the PTEN phosphatase, an enzyme that normally suppresses the PI 3-kinase/Akt/mTOR pathway by dephosphorylating the PI (3,4,5) P<sub>3</sub> molecules that the PI 3-kinase forms (see pp. 859–861). *PTEN* is thus a common tumor-suppressor gene.

Of course, mutation is not the only way to overactivate the pathway: high levels of insulin in the circulation can have a similar effect. This may explain why the risk of cancer is significantly increased, by a factor of two or more, in people who are obese or have type 2 diabetes. Their insulin levels are abnormally high, driving cancer cell growth without need of mutation in the PI 3-kinase/Akt/mTOR pathway.

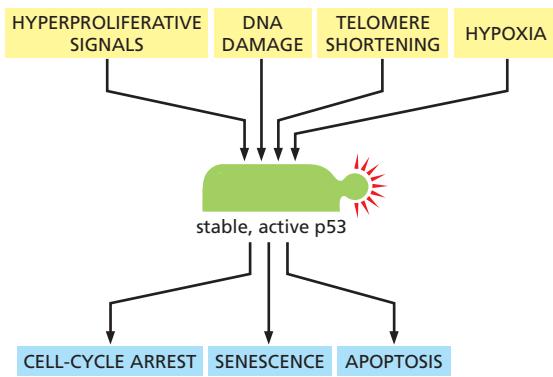
### Mutations in the p53 Pathway Enable Cancer Cells to Survive and Proliferate Despite Stress and DNA Damage

That cancer cells must break the normal rules governing cell growth and cell division is obvious: that is part of the definition of cancer. It is not so obvious why cancer cells should also be abnormal in their response to stress and DNA damage, and yet this too is an almost universal feature. The gene that lies at the center of this response, the *p53* gene, is mutated in about 50% of all cases of cancer—a higher proportion than for any other known cancer-critical gene. When we include with *p53* the other genes that are closely involved in its function, we find that most cases of cancer harbor mutations in the *p53* pathway. Why should this be? To answer, we must first consider the normal function of this pathway.

In contrast to Rb, most cells in the body have very little p53 protein under normal conditions: although the protein is synthesized, it is rapidly degraded. Moreover, p53 is not essential for normal development. Mice in which both copies of the gene have been deleted or inactivated typically appear normal in all respects except one—they universally develop cancer before 10 months of age. These observations suggest that p53 has a function that is required only in special circumstances. In fact, cells raise their concentration of p53 protein in response to a whole range of conditions that have only one obvious thing in common: they are, from the cell's point of view, pathological, putting the cell in danger of death or serious injury. These conditions include DNA damage, putting the cell at risk from a faulty genome; telomere loss or shortening (see p. 1016), also dangerous to the integrity of the genome; hypoxia, depriving the cell of the oxygen it needs to keep its metabolism going; osmotic stress, causing the cell to swell or shrivel; and oxidative stress, generating dangerous levels of highly reactive free radicals.

Yet another form of stress that can activate the p53 pathway arises, it seems, when regulatory signals are so intense or uncoordinated as to drive the cell beyond its normal limits and into a danger zone where its mechanisms of control and coordination break down, as in an engine driven badly or too fast. The p53 concentration rises, for example, when *Myc* is overexpressed to oncogenic levels.

All these circumstances call for desperate action, which may take either of two forms: the cell can block any further progress through the division cycle in order to take time out to repair or recover from the pathological condition; or it can accept that it must die, and do so in a way that minimizes damage to the organism. A good death, from this point of view, is a death by apoptosis. In apoptosis,



**Figure 20–27 Modes of action of the p53 tumor suppressor.** The p53 protein is a cellular stress sensor. In response to hyperproliferative signals, DNA damage, hypoxia, telomere shortening, and various other stresses, the p53 levels in the cell rise. As indicated, this may either arrest cell cycling in a way that allows the cell to adjust and survive, trigger cell suicide by apoptosis, or cause cell “senescence”—an irreversible cell-cycle arrest that stops damaged cells from dividing.

the cell is phagocytosed by its neighbors and its contents are efficiently recycled. A bad death is a death by necrosis. In necrosis, the cell bursts or disintegrates and its contents are spilled into the extracellular space, inducing inflammation.

The p53 pathway, therefore, behaves as a sort of antenna, sensing the presence of a wide range of dangerous conditions, and when any are detected, triggering appropriate action—either a temporary or permanent arrest of cell cycling (senescence), or suicide by apoptosis (Figure 20–27). These responses serve to prevent deranged cells from proliferating. Cancer cells are indeed generally deranged, and their survival and proliferation thus depend on inactivation of the p53 pathway. If the p53 pathway were active in them, they would be halted in their tracks or die (Movie 20.4).

The p53 protein performs its job mainly by acting as a transcription regulator (see Movie 17.8). Indeed, the most common mutations observed in p53 in human tumors are in its DNA-binding domain, where they cripple the ability of p53 to bind to its DNA target sequences. Because p53 binds to DNA as a tetramer, a single mutant subunit within a tetrameric complex can be enough to block its function. Thus, mutations in *p53* can have a dominant negative effect, causing loss of p53 function even when the cell also contains a wild-type version of the gene. For this reason, in contrast with other tumor suppressor genes such as *Rb*, the development of cancer does not always require that both copies of *p53* be knocked out.

As discussed in Chapter 17, the p53 protein exerts its inhibitory effects on the cell cycle, in part at least, by inducing the transcription of *p21*, which encodes a protein that binds to and inhibits the cyclin-dependent kinase (Cdk) complexes required for progression through the cell cycle. By blocking the kinase activity of these Cdk complexes, the p21 protein prevents the cell from progressing through S phase and replicating its DNA.

The mechanism by which p53 induces apoptosis includes stimulation of the expression of many pro-apoptotic genes, and it will be described in Chapter 18.

### Genome Instability Takes Different Forms in Different Cancers

If the p53 pathway is functional, a cell with unrepaired DNA damage will stop dividing or die; it cannot proliferate. Mutations in the p53 pathway are, therefore, generally present in cancer cells showing genome instability—which is to say, the majority. But how does this genome instability originate? Here too, cancer genome studies are illuminating.

In ovarian cancers, for example, chromosome breaks, translocations, and deletions are very common, and these aberrations correlate with a high frequency of mutations and epigenetic silencing in the genes needed for repair of DNA double-strand breaks by homologous recombination, especially *Brca1* and *Brca2* (see pp. 281–282). In a subset of colorectal cancers with DNA mismatch repair defects, on the other hand, one instead finds many point mutations scattered throughout the genome (see pp. 250–251). In both kinds of cancer, the genome is commonly destabilized, but different types of mutations can bring this about.

## Cancers of Specialized Tissues Use Many Different Routes to Target the Common Core Pathways of Cancer

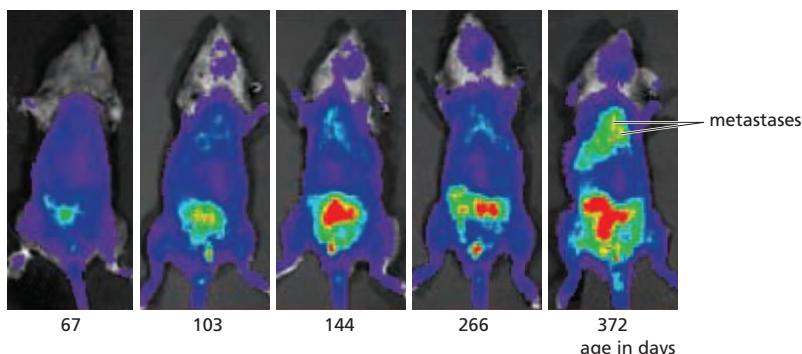
Mutations in core components of the machinery that regulates cell growth, division, and survival, such as Rb, Ras, PTEN, or p53, are not the only way to pervert the control of these processes. Specialized tissues depend on a variety of pathways, as discussed in Chapter 15, to relay environmental signals to the core control machinery, and each pathway lays the cells open to subversion in a different set of ways. Thus, in different cancers, we can find examples of driver mutations in practically all the major signaling pathways through which cells communicate during development and tissue maintenance (discussed in Chapters 21 and 22).

In glioblastoma, for example, most patients have mutations in one or other of a set of cell-surface receptor tyrosine kinases, especially the EGF receptor mentioned earlier (linking into the Ras/PI3K pathway), suggesting that the cells from which the cancer originates are normally controlled by this route. The cells of the prostate gland, on the other hand, respond to the androgen hormone testosterone, and in prostate cancer, components of the androgen receptor signaling pathway (a variety of nuclear hormone receptor signaling; see Chapter 15) are often mutated. In the normal gut lining, Wnt signaling is critical, and Wnt pathway mutations are present in most colorectal cancers. Pancreatic cancers generally have mutations in the transforming growth factor- $\beta$  (TGF $\beta$ ) signaling pathway. Activating mutations in the Notch pathway are present in more than 50% of T cell acute lymphocytic leukemias, and so on.

Cells are generally regulated by several different types of external signals that must act in combination, representing a “fail-safe” control mechanism that protects the organism as a whole from cancer. These signals are different in different tissues. As expected, therefore, the corresponding cancers often have mutations in several signaling pathways concurrently. This is true of the examples we have just listed, which commonly have mutations in other signaling pathways in addition to the ones that we have singled out.

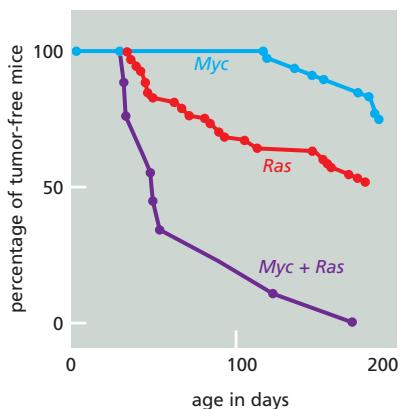
## Studies Using Mice Help to Define the Functions of Cancer-Critical Genes

The ultimate test of a gene’s role in cancer has to come from investigations in the intact, mature organism. The most favored organism for such studies, apart from humans themselves, is the mouse. To explore the function of a candidate oncogene or tumor suppressor gene, one can make a transgenic mouse that overexpresses it or a knockout mouse that lacks it. Using the techniques described in Chapter 8, one can engineer mice in which the misexpression or deletion of the gene is restricted to a specific set of cells, or in which expression of the gene can be switched on at will at a chosen point in time, or both, to see whether and how tumors develop. Moreover, to follow the growth of tumors from day to day in the living organism, the cells of interest can be genetically marked and made visible by expression of a fluorescent or luminescent reporter (Figure 20–28). In these ways, one can begin to clarify the part that each cancer-critical gene plays in cancer initiation or progression.



**Figure 20–28** Monitoring tumor growth and metastasis in a mouse with a luminescent reporter. A mouse was genetically engineered in a way that allows both copies of its *PTEN* tumor suppressor gene to be inactivated in the prostate gland, simultaneously with the prostate-specific activation of a gene engineered to produce the enzyme luciferase (derived from fireflies). After an injection of luciferin (the substrate molecule for luciferase) into the mouse’s bloodstream, the cells in the prostate emit light and can be detected by their bioluminescence in a live mouse, as seen in the 67-day-old animal at the left. Cells lacking the *PTEN* phosphatase enzyme contain elevated amounts of the Akt activator, PI(3,4,5)P<sub>3</sub>, and this causes the prostate cells to proliferate abnormally, progressing over time to form a cancer. In this way, the process of metastasis could be followed in the same animal over the course of a year. The light intensity in these experiments is proportional to the number of prostate-cell descendants, increasing from light blue to green, to yellow, to red in this representation. (Adapted from C.-P. Liao et al., *Cancer Res.* 67:7525–7533, 2007.)

**Figure 20–29 Oncogene collaboration in transgenic mice.** The graphs show the incidence of tumors in three types of transgenic mouse strains, one carrying a *Myc* oncogene, one carrying a *Ras* oncogene, and one carrying both oncogenes. For these experiments, two lines of transgenic mice were first generated. One carries an inserted copy of an oncogene created by fusing the proto-oncogene *Myc* with the mouse mammary tumor virus regulatory DNA (which then drives *Myc* overexpression in the mammary gland). The other line carries an inserted copy of the *Ras* oncogene under control of the same regulatory element. Both strains of mice develop tumors much more frequently than normal, most often in the mammary or salivary glands. Mice that carry both oncogenes together are obtained by crossing the two strains. These hybrids develop tumors at a far higher rate still, much greater than the sum of the rates for the two oncogenes separately. Nevertheless, the tumors arise only after a delay and only from a small proportion of the cells in the tissues where the two genes are expressed. Further accidental changes, in addition to the two oncogenes, are apparently required for the development of cancer. (After E. Sinn et al., *Cell* 49:465–475, 1987. With permission from Elsevier.)



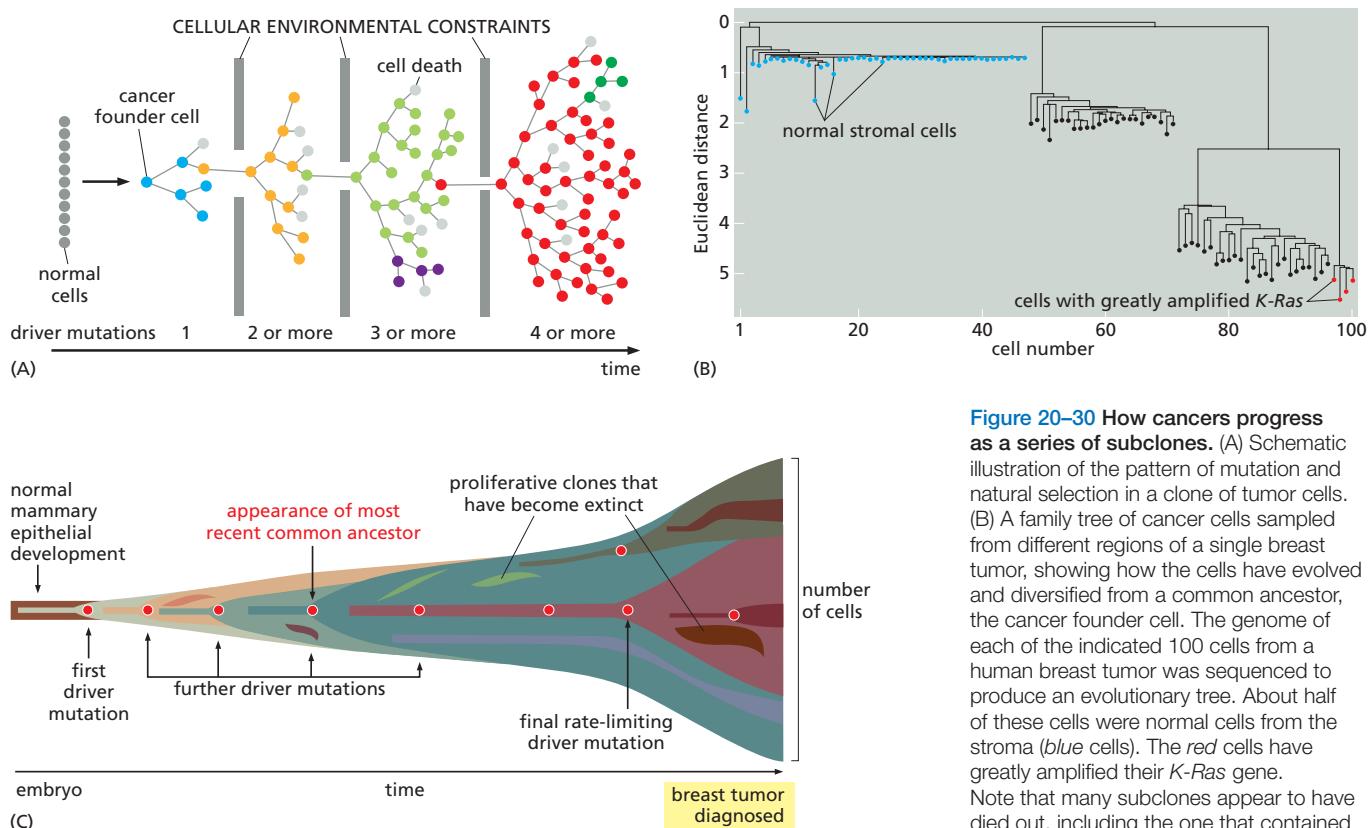
Transgenic mouse studies confirm, for example, that a single oncogene is generally not enough to turn a normal cell into a cancer cell. Thus, in mice engineered to express a *Myc* or *Ras* oncogenic transgene, some of the tissues that express the oncogene may show enhanced cell proliferation, and, over time, occasional cells will undergo further changes to give rise to cancers. Most cells expressing the oncogene, however, do not give rise to cancers. Nevertheless, from the point of view of the whole animal, the inherited oncogene is a serious menace because it creates a high risk that a cancer will arise somewhere in the body. Mice that express both *Myc* and *Ras* oncogenes (bred by mating a transgenic mouse carrying a *Myc* oncogene with one carrying a *Ras* oncogene) develop cancers earlier and at a much higher rate than either parental strain (Figure 20–29); but, again, the cancers originate as scattered, isolated tumors among noncancerous cells. Thus, even cells expressing these two oncogenes must undergo further, randomly generated changes to become cancerous. This strongly suggests that multiple mutations are required for tumorigenesis, as supported by a great deal of other evidence discussed earlier. Experiments using mice with deletions of tumor suppressor genes lead to similar conclusions.

### Cancers Become More and More Heterogeneous as They Progress

From simple histology, looking at stained tissue sections, it is clear that some tumors contain distinct sectors, all clearly cancerous, but differing in appearance because they differ genetically: the cancer cell population is heterogeneous. Evidently, within the initial clone of cancerous cells, additional mutations have arisen and thrived, creating diverse subclones. Today, the ability to analyze cancer genomes lets us look much deeper into the process.

One approach involves taking samples from different regions of a primary tumor and from the metastases that it has spawned. With modern methods, it is even possible to take representative single cells and analyze their genomes. Such studies reveal a classic picture of Darwinian evolution, occurring on a time scale of months or years rather than millions of years, but governed by the same rules of natural selection (Figure 20–30).

One such investigation compared the genomes of 100 individual cells from different regions of a primary tumor of the breast. A large fraction—just over half—of the chosen cells was genetically normal or nearly so: these were connective-tissue cells and other cell types, such as those of the immune system, that were mixed up with the cancer cells. The cancer cells themselves were distinguished by their severely disrupted genomes. The detailed pattern of gene deletions and amplifications in each such cell revealed how closely it was related to the others, and from this data one could draw up a family tree (Figure 20–30B). In this case, three main branches of the tree were seen; that is, the cancer consisted of three major



**Figure 20–30** How cancers progress as a series of subclones. (A) Schematic illustration of the pattern of mutation and natural selection in a clone of tumor cells. (B) A family tree of cancer cells sampled from different regions of a single breast tumor, showing how the cells have evolved and diversified from a common ancestor, the cancer founder cell. The genome of each of the indicated 100 cells from a human breast tumor was sequenced to produce an evolutionary tree. About half of these cells were normal cells from the stroma (blue cells). The red cells have greatly amplified their *K-Ras* gene. Note that many subclones appear to have died out, including the one that contained the founder cells for the three subclones that survive.

(C) A depiction of how driver mutations are thought to cause cancer progression over long periods of time, before producing a large enough clone of proliferating cells to be detected as a tumor. The data indicate that driver mutations occur only rarely in a background of long-lived subclones of cells that continually accumulate passenger mutations without gaining a growth advantage. (A, adapted from M. Greaves, *Semin. Cancer Biol.* 20:65–70, 2010; B, adapted from N. Navin et al., *Nature* 472:90–94, 2011; C, adapted from S. Nik-Zainal et al., *Cell* 149:994–1007, 2012.)

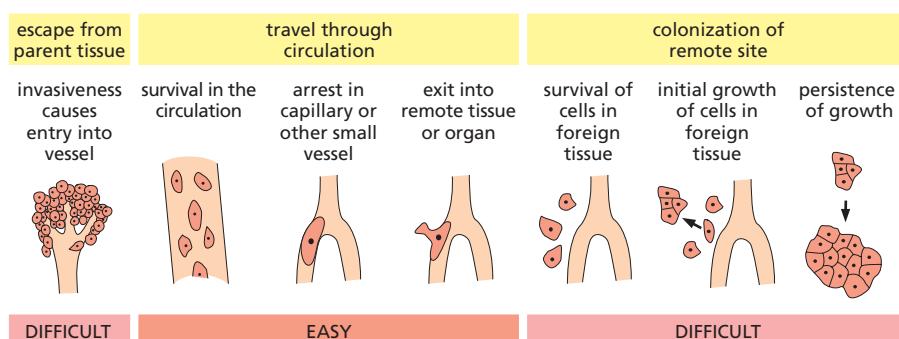
subclones. From the shared abnormalities, one could deduce that their last common ancestor—the presumed founder of the cancer—was already very different from a normal cell, but that the first split between branches occurred early, when the tumor was small. This was followed by a large amount of additional change within each branch. A hint of the future could be seen in the smallest of the three major subclones: its cells were distinguished by a massive amplification of a *Ras* oncogene. Given more time, perhaps they would have out-competed the other cancer cells and taken over the whole tumor.

Similar results have been obtained with other cancers. Clearly, cancer cells are constantly mutating, multiplying, competing, evolving, and diversifying as they exploit new ecological niches and react to the treatments that are used against them (Figure 20–30C). Diversification accelerates as they metastasize and colonize new territories, where they encounter new selection pressures. The longer the evolutionary process continues, the harder it becomes to catch them all in the same net and kill them.

### The Changes in Tumor Cells That Lead to Metastasis Are Still Largely a Mystery

Perhaps the most significant gap in our understanding of cancer concerns invasiveness and metastasis. For a start, it is not clear exactly what new properties a cancer cell must acquire to become metastatic. In some cases, it is possible that invasion and metastasis require no further genetic changes beyond those needed to violate the normal controls on cell growth, cell division, and cell death. On the other hand, it may be that, for some cancers, metastasis requires a large number of additional mutations and epigenetic changes. Clues are coming from comparisons of the genomes of cells of primary tumors with the cells of metastases that they have spawned. The results appear complex and variable from one cancer to another. Nevertheless, some general principles have emerged.

As we discussed earlier, it is helpful to distinguish three phases of tumor progression required for a carcinoma to metastasize (see Figure 20–16). First, the cells



must escape the normal confines of their parent epithelium and begin to invade the tissue immediately beneath. Second, they must travel via the blood or lymph to lodge in distant sites. Third, they must survive there and multiply. It is the first and last steps in this sequence that are the most difficult to accomplish for most cancers (**Figure 20-31**).

The first step, local invasiveness, requires a relaxation of the mechanisms that normally hold epithelial cells together. As mentioned earlier, this step resembles the normal developmental process known as the *epithelial-mesenchymal transition (EMT)*, in which epithelial cells undergo a shift in character, becoming less adhesive and more migratory (discussed in Chapter 19). A key part of the EMT process involves switching off expression of the *E-cadherin* gene. The primary function of the transmembrane *E-cadherin* protein is in cell-cell adhesion, binding epithelial cells together through adherens junctions (see Figure 19-13). In some carcinomas of the stomach and of the breast, *E-cadherin* has been identified as a tumor suppressor gene, and a loss of *E-cadherin* may promote cancer development by facilitating local invasiveness.

The initial entry of tumor cells into the circulation is helped by the presence of a dense supply of blood vessels and sometimes lymphatic vessels, which tumors attract to themselves as they grow larger and become hypoxic in their interior. This process, called *angiogenesis*, is caused by the secretion of angiogenic factors that promote the growth of blood vessels, such as vascular endothelial growth factor (VEGF; see Figure 22-26). An abnormal fragility and leakiness of the new vessels that form may help the cells that have become invasive to enter and then move through the circulation with relative ease.

The remaining steps in metastasis, involving exit from a blood or lymphatic vessel and the effective colonization of remote sites, are much harder to study. To discover which of the later steps in metastasis present cancer cells with the greatest difficulties, one can label the cells with a fluorescent dye or green fluorescent protein (GFP), inject them into the bloodstream of a mouse, and then monitor their fate (**Movie 20.5**). In such experiments, one observes that many cells survive in the circulation, lodge in small vessels, and exit into the surrounding tissue, regardless of whether they come from a tumor that metastasizes or one that does not. Some cells die immediately after they enter foreign tissue; others survive entry into the foreign tissue but fail to proliferate. Still others divide a few times and then stop, forming micrometastases containing ten to several thousand cells. Very few establish full-blown metastases.

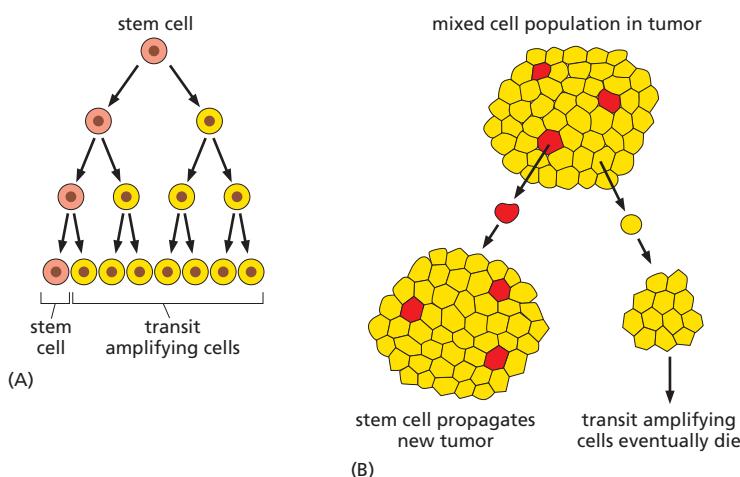
What, if anything, distinguishes the survivors from the failures? A clue may come from the fact that in many types of tumors, the cancer cells show a kind of heterogeneity that resembles the heterogeneity seen among the cells of those normal tissues that renew themselves continually by a stem-cell strategy, as we discuss next.

### A Small Population of Cancer Stem Cells May Maintain Many Tumors

Self-renewing tissues, where cell division continues throughout life, are the breeding ground for the great majority of human cancers. They include the epidermis

**Figure 20-31** The barriers to metastasis.

Studies of labeled tumor cells leaving a tumor site, entering the circulation, and establishing metastases show which steps in the metastatic process, outlined in Figure 20-16, are difficult or “inefficient,” in the sense that they are steps in which large numbers of cells fail and are lost. It is in these difficult steps that cells from highly metastatic tumors are observed to have much greater success than cells from a nonmetastatic source. It seems that the ability to escape from the parent tissue, and an ability to survive and grow in the foreign tissue, are key properties that cells must acquire to become metastatic. (Adapted from A.F. Chambers et al., *Breast Cancer Res.* 2:400–407, 2000. With permission from BioMed Central Ltd.)



**Figure 20–32** Cancer stem cells can be responsible for tumor growth and yet remain only a small part of the tumor-cell population. (A) How stem cells produce transit amplifying cells. (B) How a small proportion of cancer stem cells can maintain a tumor. Suppose, for example, that each daughter of a cancer stem cell has a probability slightly greater than 50% of retaining stem-cell potential and a probability slightly less than 50% of becoming a transit amplifying cell that is committed to a program of cell divisions that stops after 10 division cycles. While the number of cancer stem cells will increase slowly but steadily to give a growing tumor, the non-stem cells that they give rise to will always outnumber the stem cells by a large factor—in this example, by a factor of about 1000. (If the cell-division-cycle and survival times for the two classes of cells are equal.)

(the outer epithelial layer of the skin), the lining of the digestive and reproductive tracts, and the bone marrow, where blood cells are generated (see Chapter 22). In almost all these tissues, renewal depends on the presence of stem cells, which divide to give rise to terminally differentiated cells, which do not divide. This creates a mixture of cells that are genetically identical and closely related by lineage, but are in different states of differentiation. Many tumors seem likewise to consist of cells in varied states of differentiation, with different capacities for cell division and self-renewal.

To see the implications, it is helpful to consider how normal stem-cell systems operate. When a normal stem cell divides, each daughter cell has a choice—it can remain a stem cell, or it can commit to a pathway leading to differentiation. A stem-cell daughter remains in place to generate more cells in the future. A committed daughter typically undergoes some rounds of cell proliferation (as a so-called *transit amplifying cell*) but then stops dividing, terminally differentiates, and eventually is discarded and replaced (it may die by apoptosis, with recycling of its materials, or be shed from the body). On average, the two fates—stem cell or differentiating cell—normally occur with equal probability, so that half the daughters of stem-cell divisions take the one path and half take the other. In a healthy body, feedback controls regulate the process, adjusting this balance of cell-fate choices to correct for any departure from the proper cell population numbers. Thus, the number of stem cells remains approximately constant, and the terminally differentiated cells are continually replaced at a steady rate. Because of the divisions undergone by the transit amplifying cells, the stem cells may be vastly outnumbered by the cells that are committed to terminal differentiation and have lost the capacity for self-renewal. But the stem cells, though few and far between and often relatively slowly dividing, carry the whole responsibility for maintenance of the tissue in the long term.

Some cancers seem to be organized in a similar way: they consist of rare **cancer stem cells** capable of dividing indefinitely, together with much larger numbers of dividing transit amplifying cells that are derived from the cancer stem cells but have a limited capacity for self-renewal (**Figure 20–32**). These non-stem cells appear to constitute the great majority of the cell population in some tumors.

### The Cancer Stem-Cell Phenomenon Adds to the Difficulty of Curing Cancer

Evidence for the cancer stem-cell phenomenon comes chiefly from experiments in which individual cells from a cancer are tested for their ability to give rise to fresh tumors: a standard assay is to implant the cells into an immunodeficient mouse (**Figure 20–33**). It has been known for half a century that there is usually only a small chance—typically much less than 1%—that a tumor cell chosen at random and tested in this way will generate a new tumor. This by itself does not prove that

the tumor cells are heterogeneous: like seeds scattered on difficult ground, each of them may have only a small chance of finding a spot where it can survive and grow. Modern technologies for sorting cells have shown, however, that in some cancers at least, the rate of success in founding new tumors is even lower than it would otherwise be because the cancer cells are heterogeneous in their state of differentiation, and only a small subset of them—the cancer stem cells—have the special properties needed for tumor propagation. For example, in several types of cancer, including breast cancers and leukemias, one can fractionate the tumor cells using monoclonal antibodies that recognize a particular cell-surface marker that is present on the normal stem cells in the tissue of origin of the cancer. The purified cancer cells expressing this marker are found to have a greatly enhanced ability to found new tumors. And the new tumors consist of mixtures of cells that express the marker and cells that do not, all generated from the same founder cell that expressed the marker.

Experiments with breast cancer cells have revealed that, instead of following a rigid program from stem cell to transit amplifying cell to terminally differentiated cell, these cancer cells can randomly switch to and fro—with a certain low transition probability—between different states of differentiation that express different molecular markers. In one state, they behave like stem cells, dividing slowly but capable of founding new tumors; in other states, they behave like transit amplifying cells, dividing rapidly but unable to found new tumors in a standard transplant assay. But a single cell in any of these states—given time in culture, or a congenial environment in the body—will give rise to a mixed population that includes all the other states as well.

The cancer stem-cell phenomenon, whatever its basis, implies that even when the tumor cells are genetically similar, they are phenotypically diverse. A treatment that wipes out those in one state is likely to allow survival of others that remain a danger. Radiotherapy or a cytotoxic drug, for example, may selectively kill off the rapidly dividing cells, reducing the tumor volume to almost nothing, and yet spare a few slowly dividing cells that go on to resurrect the disease. This greatly adds to the difficulty of cancer therapy, and it is part of the reason why treatments that seem at first to succeed often end in relapse and disappointment.

### Colorectal Cancers Evolve Slowly Via a Succession of Visible Changes

At the beginning of this chapter, we saw that most cancers develop gradually from a single aberrant cell, progressing from benign to malignant tumors by the accumulation of a number of independent genetic and epigenetic changes. We have discussed what some of these changes are in molecular terms and seen how they contribute to cancerous behavior. We now examine one of the common human cancers more closely, using it to illustrate and enlarge upon some of the general principles and molecular mechanisms we have introduced. We take **colorectal cancer** as our example.

Colorectal cancers arise from the epithelium lining the colon (the large intestine) and rectum (the terminal segment of the gut). The organization of this tissue is broadly similar to that of the small intestine, discussed in detail in Chapter 22 (pp. 1217–1221). For both the small and large intestine, the epithelium is renewed at an extraordinarily rapid rate, taking about a week to completely replace most of the epithelial sheet. In both regions, the renewal depends on stem cells that lie in deep pockets of the epithelium, called intestinal crypts. The signals that maintain the stem cells and control the normal organization and renewal of the epithelium are beginning to be quite well understood, as explained in Chapter 22. Mutations that disrupt these signals begin the process of tumor progression for most colorectal cancers (**Movie 20.6**).

Colorectal cancers are common, currently causing nearly 60,000 deaths a year in the United States, or about 10% of total deaths from cancer. Like most cancers, they are not usually diagnosed until late in life (90% occur after the age of 55). However, routine examination of normal adults with a colonoscope (a fiber



**Figure 20–33** An immunodeficient mouse, as used in transplantation assays to test human cancer cells for their ability to found new tumors. This nude mouse has a mutation that blocks development of the thymus and, as a side effect, robs it of hair. Because it has practically no T cells, it tolerates grafts of cells even from other species. (Courtesy of Harlan Sprague Dawley.)

optic device for viewing the interior of the colon and rectum) often reveals a small benign tumor, or adenoma, of the gut epithelium in the form of a protruding mass of tissue called a *polyp* (see Figure 22–4). These adenomatous polyps are believed to be the precursors of a large proportion of colorectal cancers. Because the progression of the disease is usually very slow, there is typically a period of about 10 years in which the slowly growing tumor is detectable but has not yet turned malignant. Thus, when people are screened by colonoscopy in their fifties and the polyps are removed through the colonoscope—a quick and easy surgical procedure—the subsequent incidence of colorectal cancer is much lower: according to some studies, less than a quarter of what it would be otherwise.

In microscopic sections of polyps smaller than 1 cm in diameter, the cells and their arrangement in the epithelium usually appear almost normal. The larger the polyp, the more likely it is to contain cells that look abnormally undifferentiated and form abnormally organized structures. Sometimes, two or more distinct areas can be distinguished within a single polyp, with the cells in one area appearing relatively normal and those in the other appearing clearly cancerous, as though they have arisen as a mutant subclone within the original clone of adenomatous cells. At later stages in the disease, some tumor cells become invasive in a small fraction of the polyps, first breaking through the epithelial basal lamina, then spreading through the layer of muscle that surrounds the gut, and finally metastasizing to lymph nodes via lymphatic vessels and to liver, lung, and other organs via blood vessels.

### A Few Key Genetic Lesions Are Common to a Large Fraction of Colorectal Cancers

What are the mutations that accumulate with time to produce this chain of events? Of those genes so far discovered to be involved in colorectal cancer, three stand out as most frequently mutated: the proto-oncogene *K-Ras* (a member of the *Ras* gene family), in about 40% of cases; *p53*, in about 60% of cases; and the tumor suppressor gene *Apc* (discussed below), in more than 80% of cases. Others are involved in smaller numbers of colon cancers, and some of these are listed in **Table 20–1**.

The role of *Apc* first came to light through study of certain families showing a rare type of hereditary predisposition to colorectal cancer, called *familial*

**TABLE 20–1 Some Genetic Abnormalities Detected in Colorectal Cancer Cells**

| Gene  | Class                                | Pathway affected                   | Human colon cancers (%) |
|---|--------------------------------------|------------------------------------|-------------------------|
| <i>K-Ras</i>  | Oncogene                             | Receptor tyrosine kinase signaling | 40                      |
| <i>β-Catenin</i> <sup>1</sup>   | Oncogene                             | Wnt signaling                      | 5–10                    |
| <i>Apc</i> <sup>1</sup>   | Tumor suppressor                     | Wnt signaling                      | >80                     |
| <i>p53</i>  | Tumor suppressor                     | Response to stress and DNA damage  | 60                      |
| <i>TGFβ receptor II</i> <sup>2</sup>  | Tumor suppressor                     | <i>TGFβ</i> signaling              | 10                      |
| <i>Smad4</i> <sup>2</sup>   | Tumor suppressor                     | <i>TGFβ</i> signaling              | 30                      |
| <i>MLH1</i> and other DNA mismatch repair genes (often silenced by DNA methylation) | Tumor suppressor (genetic stability) | DNA mismatch repair                | 15                      |

<sup>1,2</sup>The genes with the same superscript numeral act in the same pathway, and therefore only one of the components is mutated in an individual cancer.

**Figure 20–34 Colon of familial adenomatous polyposis coli patient compared with normal colon.** (A) The normal colon wall is a gently undulating but smooth surface. (B) The polyposis colon is completely covered by hundreds of projecting polyps, each resembling a tiny cauliflower when viewed with the naked eye. (Courtesy of Andrew Wyllie and Mark Arends.)

*adenomatous polyposis coli (FAP)*. In this syndrome, hundreds or thousands of polyps develop along the length of the colon (Figure 20–34). These polyps start to appear in early adult life, and if they are not removed, one or more will almost always progress to become malignant; the average time from the first detection of polyps to the diagnosis of cancer is 12 years. The disease can be traced to a deletion or inactivation of the tumor suppressor gene *Apc*, named after the syndrome. Individuals with FAP have inactivating mutations or deletions of one copy of the *Apc* gene in all their cells and show loss of heterozygosity in tumors, even in the benign polyps. Most patients with colorectal cancer do not have the hereditary condition. Nevertheless, in more than 80% of the cases, their cancer cells (but not their normal cells) have inactivated both copies of the *Apc* gene through mutations acquired during the patient's lifetime. Thus, by a route similar to that which we discussed for retinoblastoma, mutation of the *Apc* gene was identified as one of the central ingredients of colorectal cancer.

The *Apc* protein, as we now know, is an inhibitory component of the *Wnt signaling pathway* (discussed in Chapter 15). It binds to the  $\beta$ -catenin protein, another component of the Wnt pathway, and helps to induce the protein's degradation. By inhibiting  $\beta$ -catenin in this way, *Apc* prevents the  $\beta$ -catenin from migrating to the nucleus, where it would act as a transcriptional regulator to drive cell proliferation and maintain the stem-cell state (see Figure 15–60). Loss of *Apc* results in an excess of free  $\beta$ -catenin and thus leads to an uncontrolled expansion of the stem-cell population. This causes massive increase in the number and size of the intestinal crypts (see Figure 22–4).

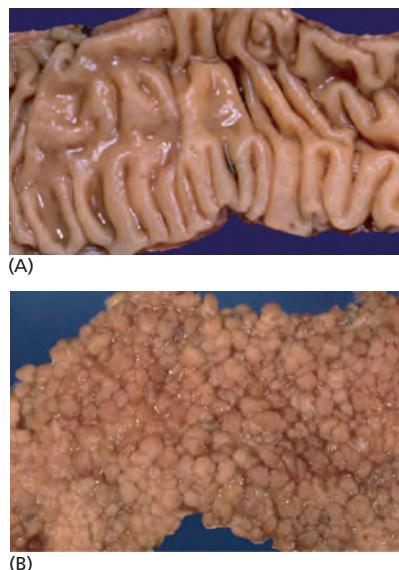
When the  $\beta$ -catenin gene was sequenced in a collection of colorectal tumors, it was discovered that, many of the tumors that did not have *Apc* mutations had activating mutations in  $\beta$ -catenin instead. Thus, it is excessive activity in the Wnt signaling pathway that is critical for the initiation of this cancer, rather than any single oncogene or tumor suppressor gene that the pathway contains.

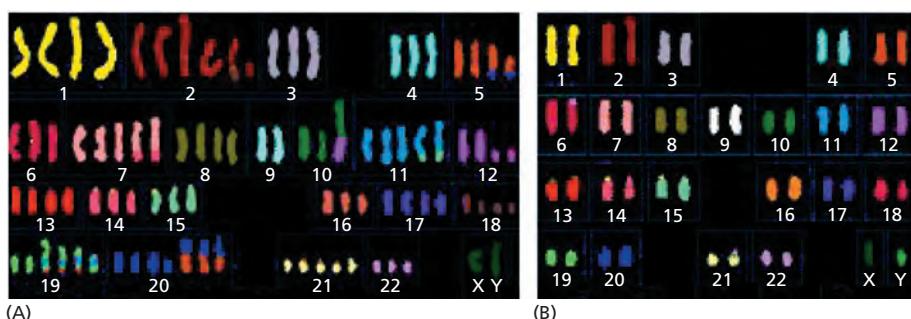
This being so, why is the *Apc* gene in particular so often the most common culprit in colorectal cancer? The *Apc* protein is large and it interacts not only with  $\beta$ -catenin but also with various other cell components, including microtubules. Loss of *Apc* appears to increase the frequency of mitotic spindle defects, leading to chromosome abnormalities when cells divide. This additional, independent cancer-promoting effect could explain why *Apc* mutations feature so prominently in the causation of colorectal cancer.

### Some Colorectal Cancers Have Defects in DNA Mismatch Repair

In addition to the hereditary disease (FAP) associated with *Apc* mutations, there is a second, more common kind of hereditary predisposition to colon carcinoma in which the course of events differs from the one we have described for FAP. In this more common condition, called *hereditary nonpolyposis colorectal cancer (HNPCC)*, the probability of colon cancer is increased without any increase in the number of colorectal polyps (adenomas). Moreover, the cancer cells are unusual, in that they have a normal (or almost normal) karyotype. The majority of colorectal tumors in non-HNPCC patients, in contrast, have gross chromosomal abnormalities, with multiple translocations, deletions, and other aberrations, as well as having many more chromosomes than normal (Figure 20–35).

The mutations that predispose HNPCC individuals to colorectal cancer occur in one of several genes that code for central components of the DNA mismatch repair system. These genes are homologous in structure and function to the *MutL* and *MutS* genes in bacteria and yeast (see Figure 5–19). Only one of the two copies of the involved gene is defective, so the repair system is still able to remove





**Figure 20-35** Chromosome complements (karyotypes) of colon cancers showing different kinds of genetic instability. (A) The karyotype of a typical cancer shows many gross abnormalities in chromosome number and structure. Considerable variation can also exist from cell to cell (not shown). (B) The karyotype of a tumor that has a stable chromosome complement with few chromosomal anomalies; the genetic abnormalities are mostly invisible, having been created by defects in DNA mismatch repair. All of the chromosomes in this figure were stained as in Figure 4-10, the DNA of each human chromosome being marked with a different combination of fluorescent dyes. (Courtesy Wael Abdel-Rahman and Paul Edwards.)

the inevitable DNA replication errors that occur in the patient's cells. However, as discussed previously, these individuals are at risk, because the accidental loss or inactivation of the remaining good gene copy will immediately elevate the spontaneous mutation rate by a hundredfold or more (discussed in Chapter 5). These genetically unstable cells then can presumably speed through the standard processes of mutation and natural selection that allow clones of cells to progress to malignancy.

This particular type of genetic instability produces invisible changes in the chromosomes—most notably changes in individual nucleotides and short expansions and contractions of mono- and dinucleotide repeats such as AAAA... or CACACA.... Once the defect in HNPCC patients was recognized, the epigenetic silencing or mutation of mismatch repair genes was found in about 15% of the colorectal cancers occurring in people with no inherited predisposing mutation.

Thus, the genetic instability found in many colorectal cancers can be acquired in at least two ways. The majority of the cancers display a form of chromosomal instability that leads to visibly altered chromosomes, whereas in the others the instability occurs on a much smaller scale and reflects a defect in DNA mismatch repair. Indeed, many carcinomas show either chromosomal instability or defective mismatch repair—but rarely both. These findings clearly demonstrate that genetic instability is not an accidental by-product of malignant behavior but a contributory cause—and that cancer cells can acquire this instability in multiple ways.

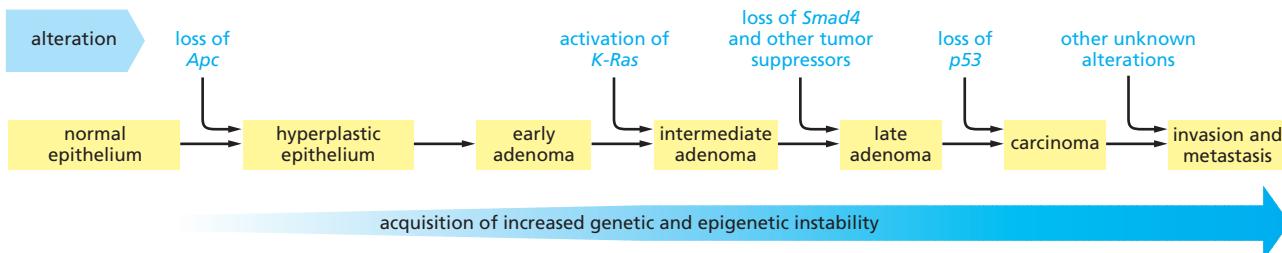
### The Steps of Tumor Progression Can Often Be Correlated with Specific Mutations

In what order do *K-Ras*, *p53*, *Apc*, and the other identified colorectal cancer-critical genes mutate, and what contribution does each of them make to the asocial behavior of the cancer cell? There is no single answer, because colorectal cancer can arise by more than one route: thus, we know that in some cases, the first mutation can be in a DNA mismatch repair gene; in others, it can be in a gene regulating cell proliferation. Moreover, as previously discussed, a general feature such as genetic instability or a tendency to proliferate abnormally can arise in a variety of ways, through mutations in different genes.

Nevertheless, certain sets of mutations are particularly common in colorectal cancer, and they occur in a characteristic order. Thus, in most cases, mutations inactivating the *Apc* gene appear to be the first, or at least a very early step, as they are detected at the same high frequency in small benign polyps as in large malignant tumors. Changes that lead to genetic and epigenetic instability are likely also to arise early in tumor progression, since they are needed to drive the later steps.

Activating mutations in the *K-Ras* gene occur later, as they are rare in small polyps but common in larger ones that show disturbances in cell differentiation and histological pattern.

Inactivating mutations in *p53* are thought to come later still, as they are rare in polyps but common in carcinomas (Figure 20-36). We have seen that loss of *p53* function allows cancer cells to endure stress and to avoid apoptosis and cell-cycle arrest. Additionally, loss of *p53* is related to the heightened activation



of oncogenes such as *Ras*. Experiments in mice show that an initial low level of oncogene activation can give rise to a slowly growing tumor even while *p53* is functional: genes such as *Ras* are, after all, part of the normal machinery of growth control, and moderate activation is not stressful for a cell and does not call the *p53* protein into play. Progression of a tumor from slow to rapid, malignant growth, however, involves activation of oncogenes beyond normal physiological limits to a higher, stressful level. If the *p53* protein is present and functional, this should lead to cell-cycle arrest or death. Only by losing *p53* function can the cancer cells with hyperactive oncogenes survive and progress.

The steps we have just described are only part of the picture. It is important to emphasize that each case of colorectal cancer is different, with its own detailed combination of mutations, and that even for the mutations that are commonly shared, the sequence of occurrence may vary. The same is true for cancers in general.

Advances in molecular biology have recently provided the tools to find out precisely which genes are amplified, deleted, mutated, or misregulated by epigenetic mechanisms in the tumor cells of any given patient. As we discuss in the next section, such information promises to become as important for the diagnosis and treatment of cancer as was the breakthrough of being able to identify microorganisms for the treatment of infectious diseases.

## Summary

*The molecular analysis of cancer cells reveals two classes of cancer-critical genes: oncogenes and tumor suppressor genes. A set of these genes becomes altered by a combination of genetic and epigenetic accidents to drive tumor progression. Many cancer-critical genes code for components of the social control pathways that regulate when cells grow, divide, differentiate, or die. In addition, a subclass of tumor suppressors can be categorized as “genome maintenance genes,” because their normal role is to help maintain genome integrity.*

*The inactivation of the *p53* pathway, which occurs in nearly all human cancers, allows genetically damaged cells to escape apoptosis and continue to proliferate. Inactivation of the *Rb* pathway also occurs in most human cancers, illustrating how fundamental each of these pathways is for protecting us against cancer.*

*The sequencing of cancer cell genomes reveals that—except for the cancers of childhood—many cancers acquire 10 or so driver mutations over the long course of tumor progression, along with a considerably larger number of passenger mutations of no consequence. The same methods reveal how subclones of cells arise and die out as a tumor ages. Tumors thus contain a heterogeneous mixture of cells, some—the so-called cancer stem cells—being much more dangerous than others.*

*We can often correlate the steps of tumor progression with mutations that activate specific oncogenes and inactivate specific tumor suppressor genes, with colon cancer providing a good example. But different combinations of mutations and epigenetic changes are found in different types of cancer, and even in different patients with the same type of cancer, reflecting the random way in which these inherited changes arise. Nevertheless, many of the same changes are encountered repeatedly, suggesting that there are a limited number of ways to breach our defenses against cancer.*

**Figure 20–36** Suggested typical sequence of genetic changes underlying the development of a colorectal carcinoma. This oversimplified diagram provides a general idea of the way mutation and tumor development are related. But many other mutations are generally involved, and different colon cancers can progress through different sequences of mutations (and/or epigenetic changes).

## CANCER PREVENTION AND TREATMENT: PRESENT AND FUTURE

We can apply the growing understanding of the molecular biology of cancer to sharpen our attack on the disease at three levels: prevention, diagnosis, and treatment. Prevention is always better than cure, and indeed many cancers can be prevented, especially by avoiding smoking. Highly sensitive molecular assays promise new opportunities for earlier and more precise diagnosis, with the aim of detecting primary tumors while they are still small and have not yet metastasized. Cancers caught at these early stages can often be nipped in the bud by surgery or radiotherapy, as we saw for colorectal polyps. Nevertheless, full-blown malignant disease will continue to be common for many years to come, and cancer treatments will continue to be needed.

In this section, we first examine the preventable causes of cancer and then consider how advances in our understanding at a molecular level are beginning to transform the treatment of the disease.

### Epidemiology Reveals That Many Cases of Cancer Are Preventable

A certain irreducible background incidence of cancer is to be expected regardless of circumstances. As discussed in Chapter 5, mutations can never be absolutely avoided because they are an inescapable consequence of fundamental limitations on the accuracy of DNA replication and repair. If a person could live long enough, it is inevitable that at least one of his or her cells would eventually accumulate a set of mutations sufficient for cancer to develop.

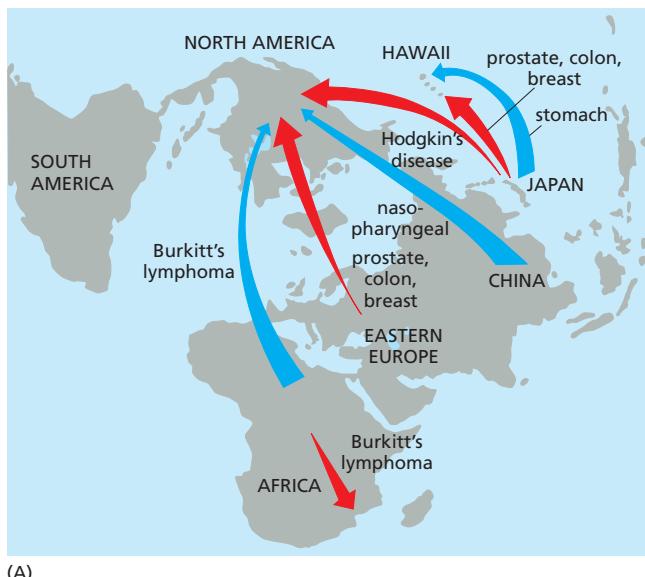
Nevertheless, environmental factors seem to play a large part in determining the risk for cancer. This is demonstrated most clearly by a comparison of cancer incidence in different countries: for almost every cancer that is common in one country, there is another country where the incidence is much lower. Because migrant populations tend to adopt the pattern of cancer incidence typical of their new host country, the differences are thought to be due mostly to environmental, not genetic, factors. From such findings, it has been suggested that 80–90% of cancers should be avoidable, or at least postponable (**Figure 20–37**).

Unfortunately, different cancers have different environmental risk factors, and a population that escapes one such danger is usually exposed to another. This is not, however, inevitable. There are some human subgroups whose way of life substantially reduces the total cancer death rate among individuals of a given age. Under the current conditions in the United States and Europe, approximately one in five people will die of cancer. But the incidence of cancer among strict Mormons in Utah—who avoid alcohol, coffee, cigarettes, drugs, and casual sex—is only about half the incidence for non-practicing members of the same family or for Americans in general. Cancer incidence is also low in certain relatively affluent populations in Africa.

Although such observations on human populations indicate that cancer can often be avoided, it has been difficult in most cases—with tobacco as a striking exception—to pinpoint the specific environmental factors responsible for these large population differences or to establish how they act. Nevertheless, several important classes of environmental cancer risk factors have been identified (Figure 20–37B). One thinks first of mutagens. But there are also many other influences—including the amount of food we eat, the hormones that circulate in our bodies, and the irritations, infections, and damage to which we expose our tissues—that are no less important and favor development of the disease in other ways.

### Sensitive Assays Can Detect Those Cancer-Causing Agents that Damage DNA

Many quite disparate chemicals are carcinogenic when they are fed to experimental animals or painted repeatedly on their skin. Examples include a range



| cause                         | cancers caused<br>(percent of total) | number of deaths in US<br>(annual) | magnitude of reduction<br>possible<br>(percent) |
|-------------------------------|--------------------------------------|------------------------------------|---|
| smoking                       | 33                                   | 189,000                            | 75  |
| diet, overweight, and obesity | 25                                   | 143,000                            | 50  |
| lack of exercise              | 5                                    | 28,600                             | 85  |
| viruses                       | 5                                    | 28,600                             | 100   |
| alcohol                       | 3                                    | 17,200                             | 50  |
| UV and ionizing radiation     | 2                                    | 11,400                             | 50  |
| occupational carcinogens      | 5                                    | 28,600                             | 50  |

**Figure 20–37** Cancer incidence is related to environmental influences.

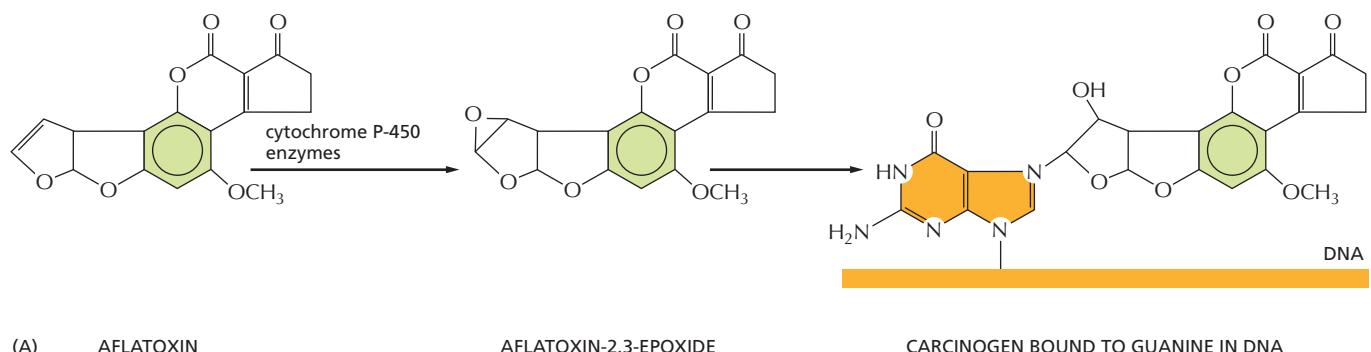
(A) This map of the world shows the rates of cancer increasing (red arrows) or decreasing (blue arrows) when specific populations move from one location to another. Such observations suggest the importance of environmental factors, including diet, in dictating cancer risk. (B) Some estimated effects of environment and lifestyle on cancer in the United States (US). The table shows both the yearly deaths in the US attributable to each cancer and the estimated percentage of that cancer that could be eliminated through prevention. (B, data from G.A. Colditz, K.Y. Wolin and S. Gehlert, *Sci. Transl. Med.* 4:127rv4, 2012.)

of aromatic hydrocarbons and derivatives of them such as aromatic amines, nitrosamines, and alkylating agents such as mustard gas. Although these **chemical carcinogens** are diverse in structure, a large proportion of them have at least one shared property—they cause mutations. In one common test for mutagenicity (the *Ames test*), the carcinogen is mixed with an activating extract prepared from rat liver cells (to mimic the biochemical processing that occurs in an intact animal). The mixture is then added to a culture of specially designed test bacteria and the bacterial mutation rate measured. Most of the compounds scored as mutagenic by this rapid and convenient assay in bacteria also cause mutations or chromosome aberrations when tested on mammalian cells.

A few of these carcinogens act directly on DNA. But generally the more potent ones are relatively inert chemically; these chemicals become damaging only after they have been converted to a more reactive molecule by metabolic processes in the liver, catalyzed by a set of intracellular enzymes known as the *cytochrome P-450 oxidases*. These enzymes normally help to convert ingested toxins into harmless and easily excreted compounds. Unhappily, their activity on certain chemicals generates products that are highly mutagenic. Examples of carcinogens activated in this way include *benzo[a]pyrene*, a cancer-causing chemical present in coal tar and tobacco smoke and the fungal toxin *aflatoxin B1* (Figure 20–38).

### Fifty Percent of Cancers Could Be Prevented by Changes in Lifestyle

Tobacco smoke is the most important carcinogen in the world today. Even though many other chemical carcinogens have been identified, none of these appear to be responsible for anything like the same numbers of human cancer deaths. It is sometimes thought that the main environmental causes of cancer are the products of a highly industrialized way of life—the rise in pollution, the enhanced use of food additives, and so on—but there is little evidence to support this view. The idea may have come in part from the identification of some highly carcinogenic materials used in industry, such as 2-naphthylamine and asbestos. Except for the increase in cancers caused by smoking, however, age-adjusted death rates for most common human cancers have stayed much the same over the past half-century, or, in some cases, have declined significantly (Figure 20–39). Survival rates, moreover, have improved. Thirty years ago, less than 50% of patients lived more than five years from the time of diagnosis; now, more than two-thirds do so.

**Figure 20–38** Some known carcinogens. (A) Carcinogen activation.

A metabolic transformation must activate many chemical carcinogens before they will cause mutations by reacting with DNA. The compound illustrated here is *aflatoxin B1*, a toxin from a mold (*Aspergillus flavus oryzae*) that grows on grain and peanuts when they are stored under humid tropical conditions. *Aflatoxin* is an important cause of liver cancer in the tropics. (B) Different carcinogens cause different types of cancer. (B, data from Cancer and the Environment: Gene Environment Interactions, National Academies Press, 2002.)

## • VINYL CHLORIDE:

liver angiosarcoma

## • BENZENE:

acute leukemias

## • ARSENIC:

skin carcinomas, bladder cancer

## • ASBESTOS:

mesothelioma

## • RADIUM:

osteosarcoma

(B)

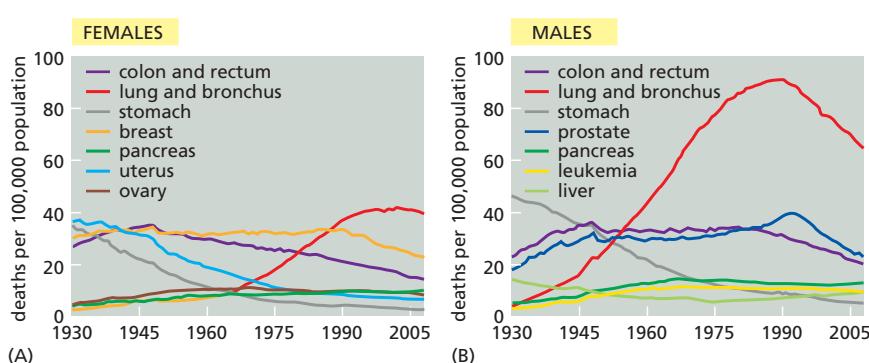
Most of the carcinogenic factors that are known to be significant are by no means specific to the modern world. The most potent known carcinogen, by certain assays at least, is *aflatoxin B1* (see Figure 20–38). It is produced by fungi that naturally contaminate foods such as tropical peanuts and is an important cause of liver cancer in Africa and Asia.

Except for tobacco, chemical toxins and mutagens are of lesser importance as contributory causes of cancer than other factors that are more a matter of personal choice. One important factor is the quantity of food we eat: as mentioned earlier, the risk of cancer is greatly increased in people who are obese. In fact, it is estimated that as many as 50% of all cancers could be avoided by simple, identifiable changes in lifestyle (see Figure 20–37B).

### Viruses and Other Infections Contribute to a Significant Proportion of Human Cancers

Cancer in humans is not an infectious disease, and most human cancers do not have any infectious cause. However, a small but significant proportion of human cancers, perhaps 15% in the world as a whole, are thought to arise by mechanisms that involve viruses, bacteria, or parasites. Evidence for their involvement comes partly from the detection of viruses in cancer patients and partly from epidemiology. Thus, cancer of the uterine cervix is associated with infection with a papillomavirus, while liver cancer is very common in parts of the world (Africa and Southeast Asia) where hepatitis-B viral infections are common. Chronic infection

**Figure 20–39** Age-adjusted cancer death rates, United States, 1930–2008. Selected death rates, adjusted to the age distribution of the US population, are plotted for (A) females and (B) males. Note the dramatic rise in lung cancer for both sexes, following the pattern of tobacco smoking, and the fall in deaths from stomach cancer, thought to be related to a fall in rates of infection with *Helicobacter pylori*. Recent reductions in other cancer death rates may correspond to improvements in detection and treatment. Age-adjusted data like these are needed to compensate for the inevitable increase in cancer as people live longer, on average. (Adapted from Cancer Facts and Figures, 2012. Data from U.S. Mortality Volumes 1930 to 1959, U.S. Mortality Data 1960 to 2008, National Center for Health Statistics, Centers for Disease Control and Prevention. © 2012, American Cancer Society, Inc., Surveillance Research.)



| TABLE 20-2 Viruses Associated with Human Cancers  |  |                                 |
|---|--|---------------------------------|
| Virus   | Associated cancer                            | Areas of high incidence         |
| <b>DNA viruses</b>  |  |                                 |
| <i>Papovavirus family</i>   |  |                                 |
| Papillomavirus (many distinct strains)  | Warts (benign)                               | Worldwide                       |
|   | Carcinoma of the uterine cervix              | Worldwide                       |
| <i>Hepadnavirus family</i>  |  |                                 |
| Hepatitis-B virus   | Liver cancer (hepatocellular carcinoma)      | Southeast Asia, tropical Africa |
| <i>Herpesvirus family</i>   |  |                                 |
| Epstein–Barr virus  | Burkitt's lymphoma (cancer of B lymphocytes) | West Africa, Papua New Guinea   |
|   | Nasopharyngeal carcinoma                     | Southern China, Greenland       |
| Human herpesvirus 8   | Kaposi's sarcoma                             | Central and Southern Africa     |
| <b>RNA viruses</b>  |  |                                 |
| <i>Retrovirus family</i>  |  |                                 |
| Human T-cell leukemia virus type I (HTLV-1)   | Adult T-cell leukemia/lymphoma               | Japan, West Indies              |
| Human immunodeficiency virus (HIV, the AIDS virus)  | Kaposi's sarcoma (via human herpesvirus 8)   | Central and Southern Africa     |
| <i>Flavivirus family</i>  |  |                                 |
| Hepatitis-C virus   | Liver cancer (hepatocellular carcinoma)      | Worldwide                       |
| For all these viruses, the number of people infected is much larger than the number who develop cancer: the viruses must act in conjunction with other factors. As described in the text, different viruses contribute to cancer in different ways. |  |                                 |

with hepatitis-C virus, which has infected 170 million people worldwide, is also clearly associated with the development of liver cancer.

The main culprits, as shown in **Table 20-2**, are the DNA viruses. The **DNA tumor viruses** cause cancer by the most direct route—by interfering with controls of the cell cycle and apoptosis. To understand this type of viral carcinogenesis, it is important to review the life history of viruses. Many DNA viruses use the host cell's DNA replication machinery to replicate their own genomes. However, to produce a large number of infectious virus particles within a single host cell, the DNA virus has to commandeer this machinery and drive it hard, breaking through the normal constraints on DNA replication and usually killing the host cell in the process. Many DNA viruses reproduce only in this way. But some have a second option: they can propagate their genome as a quiet, well-behaved passenger in the host cell, replicating in parallel with the host cell's DNA (either integrated into the host genome, or as an extrachromosomal plasmid) in the course of ordinary cell-division cycles. These viruses will switch between two modes of existence according to circumstances, remaining latent and harmless for a long time, but

then proliferating in occasional cells in a process that kills the host cell and generates large numbers of infectious particles.

Neither of these conditions converts the host cell to a cancerous character, nor is it in the interest of the virus to do so. But for viruses with a latent phase, accidents can occur that prematurely activate some of the viral proteins that the virus would normally use in its replicative phase to allow the viral DNA to replicate independently of the cell cycle. As described in the example below, this type of accident can switch on the persistent proliferation of the host cell itself, leading to cancer.

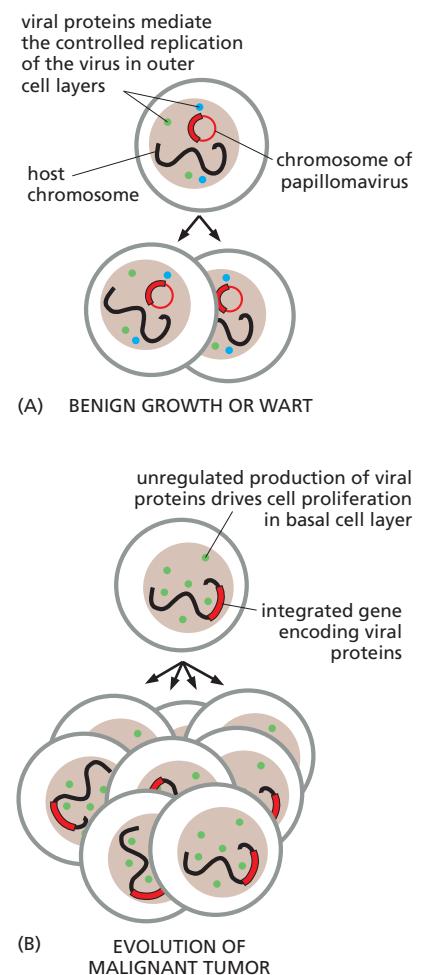
### Cancers of the Uterine Cervix Can Be Prevented by Vaccination Against Human Papillomavirus

The **papillomaviruses** are a prime example of DNA tumor viruses. They are responsible for human warts and are especially important as a cause of carcinoma of the uterine cervix: this is the second commonest cancer of women in the world as a whole, representing about 6% of all human cancers. Human papillomaviruses (**HPV**) infect the cervical epithelium and maintain themselves in a latent phase in the basal layer of cells as extrachromosomal plasmids, which replicate in step with the chromosomes. Infectious virus particles are generated through a switch to a replicative phase in the outer epithelial layers, as progeny of these cells begin to differentiate before being sloughed from the surface. Here, cell division should normally stop, but the virus interferes with this cell-cycle arrest so as to allow replication of its own genome. Usually, the effect is restricted to the outer layers of cells and is relatively harmless, as in a wart. Occasionally, however, a genetic accident causes the viral genes that encode the proteins that prevent cell-cycle arrest to integrate into the host chromosome and become active in the basal layer, where the stem cells of the epithelium reside (see Figure 22–10). This can lead to cancer, with the viral genes acting as oncogenes (**Figure 20–40**).

The whole process, from initial infection to invasive cancer, is slow, taking many years. It involves a long intermediate stage when the affected patch of cervical epithelium is visibly disordered but the cells have not yet begun to invade the underlying connective tissue—a phenomenon called *intraepithelial neoplasia*. Many such lesions regress spontaneously. Moreover, at this stage, it is still easy to cure the condition by destroying or surgically removing the abnormal tissue. Fortunately, the presence of such lesions can be detected by scraping off a sample of cells from the surface of the cervix and viewing it under the microscope (the “Pap smear” technique).

Better still, a vaccine has now been developed that protects against infection with the relevant strains of human papillomavirus. This vaccine, given to girls before puberty and thus before they become sexually active, has been shown to greatly reduce their risk of ever developing cervical cancer. Because the virus spreads through sexual activity, it is now recommended that both young males and young females be routinely vaccinated. Mass immunization programs have begun in several countries.

**Figure 20–40** How certain papillomaviruses are thought to give rise to cancer of the uterine cervix. Papillomaviruses have double-stranded circular DNA chromosomes of about 8000 nucleotide pairs. These chromosomes are normally stably maintained in the basal cells of the epithelium as plasmids (red circles), whose replication is regulated so as to keep step with the chromosomes of the host. (A) Normally, the virus perturbs the host cell cycle only when the virus is programmed to produce infectious progeny, in the outer layers of an epithelium. This is relatively harmless. (B) Rare accidents can cause the integration of a fragment of such a plasmid into a chromosome of the host, altering the environment of the viral genes in the basal cells of an epithelium. This can disrupt the normal control of viral gene expression. The unregulated production of certain viral proteins (E6 and E7) interferes with the control of cell division in the basal cells, thereby helping to generate a cancer (bottom).



## Infectious Agents Can Cause Cancer in a Variety of Ways

In papillomaviruses, the viral genes that are mainly to blame are called *E6* and *E7*. The protein products of these viral oncogenes interact with many host-cell proteins, but, in particular, they bind to two key tumor suppressor proteins of the host cell, putting them both out of action and so permitting the cell to replicate its DNA and divide in an uncontrolled way. One of these host proteins is Rb; the other is p53. Other DNA tumor viruses use similar mechanisms to inhibit Rb and p53, underlining the central importance of inactivating both of these tumor suppressor pathways if a cell is to escape the normal constraints on proliferation.

In other cancers, viruses have indirect tumor-promoting actions. The hepatitis-B and C viruses, for example, favor the development of liver cancer by causing chronic inflammation (hepatitis), which stimulates an extensive cell division in the liver that promotes the eventual evolution of tumor cells. In AIDS, the human immunodeficiency virus (HIV) promotes development of an otherwise rare cancer called Kaposi's sarcoma by destroying the immune system, thereby permitting a secondary infection with a human herpesvirus (HHV-8) that has a direct carcinogenic action. By causing severe inflammation, chronic infection with parasites and bacteria can also promote the development of some cancers. For example, chronic infection of the stomach with the bacterium *Helicobacter pylori*, which causes ulcers, appears to be a major cause of stomach cancer; dramatic falls in the incidence of stomach cancer over the last half-century (see Figure 20–39) correlate with a decline in the incidence of *Helicobacter* infections.

## The Search for Cancer Cures Is Difficult but Not Hopeless

The difficulty of curing a cancer is similar to the difficulty of getting rid of weeds. Cancer cells can be removed surgically or destroyed with toxic chemicals or radiation, but it is hard to eradicate every single one of them. Surgery can rarely ferret out every metastasis, and treatments that kill cancer cells are generally toxic to normal cells as well. Moreover, unlike normal cells, cancer cells can mutate rapidly and will often evolve resistance to the poisons and irradiation used against them.

In spite of these difficulties, effective cures using anticancer drugs (alone or in combination with other treatments) have already been found for some formerly highly lethal cancers, including Hodgkin's lymphoma, testicular cancer, choriocarcinoma, and some leukemias and other cancers of childhood. Even for types of cancer where a cure at present seems beyond our reach, there are treatments that will prolong life or at least relieve distress. But what prospect is there of doing better and finding cures for the most common forms of cancer, which still cause great suffering and so many deaths?

## Traditional Therapies Exploit the Genetic Instability and Loss of Cell-Cycle Checkpoint Responses in Cancer Cells

Anticancer therapies need to take advantage of some molecular peculiarity of cancer cells that distinguishes them from normal cells. One such property is genetic instability, reflecting deficiencies in chromosome maintenance, cell-cycle checkpoints, and/or DNA repair. Remarkably, the most widely used cancer therapies seem to work by exploiting these abnormalities, although this was not known by the scientists who first developed the treatments. Ionizing radiation and most anticancer drugs damage DNA or interfere with chromosome segregation at mitosis, and they preferentially kill cancer cells because cancer cells have a diminished ability to survive the damage. Normal cells treated with radiation, for example, arrest their cell cycle until they have repaired the damage to their DNA, thanks to the cell-cycle checkpoint responses discussed in Chapter 17. Because cancer cells generally have defects in their checkpoint responses, they may continue to divide after irradiation, only to die after a few days because the genetic damage remains unrepaired. More generally, most cancer cells are physiologically deranged to a stressful degree: they live dangerously. Even though the cells

in a tumor have evolved to be unusually tolerant of minor DNA damage, they are hypersensitive to the much greater amount of damage that can be created by radiation and by DNA-damaging drugs. A small increase of genetic damage can be enough to tip the balance between proliferation and death.

Unfortunately, while the molecular defects present in cancer cells often enhance their sensitivity to cytotoxic agents, they can also increase their resistance. For example, where a normal cell might die by apoptosis in response to DNA damage, thanks to the stress response mediated by p53, a cancer cell may escape apoptosis because its p53 is lacking. Cancers vary widely in their sensitivity to cytotoxic treatments, some responding to one drug, some to another, probably reflecting the particular kinds of defects that a particular cancer has in DNA repair, cell-cycle checkpoints, and the control of apoptosis.

### New Drugs Can Kill Cancer Cells Selectively by Targeting Specific Mutations

Radiotherapy and traditional cytotoxic drugs are rather weakly selective: they hurt normal cells as well as the cancer cells, and the safety margin is narrow. The dose often cannot be raised high enough to kill all the cancer cells, because this would kill the patient, and curative treatments, where achievable, generally require a combination of several cytotoxic agents. The side effects can be harsh and hard to endure. How can we do better?

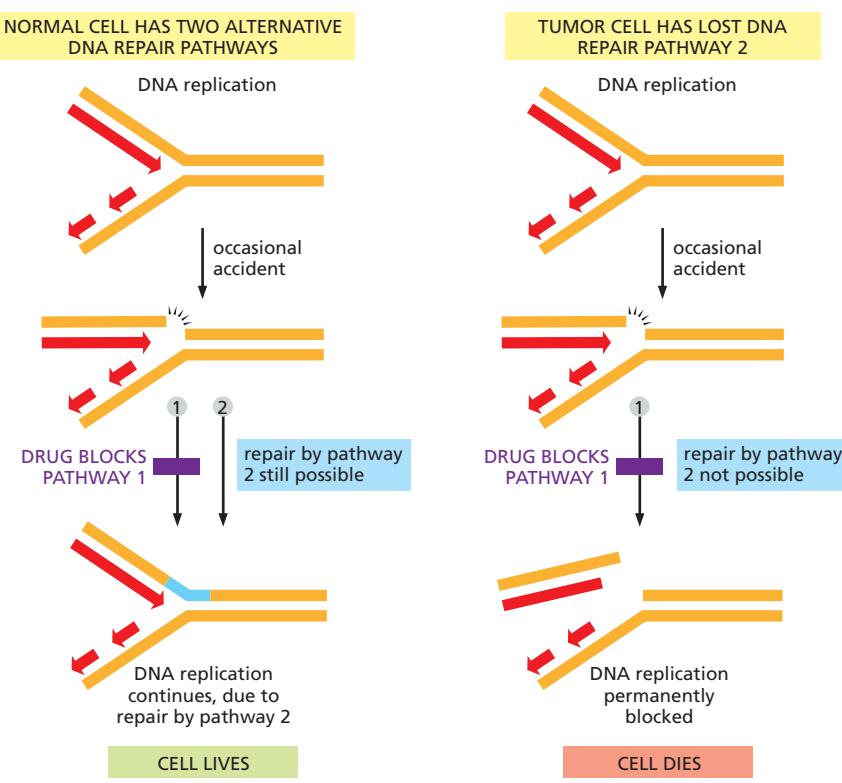
An ideal treatment is one that is cell-lethal in combination with some lesion that is present in the cancer cells, but harmless to cells where this lesion is absent. Such a treatment is said to be *synthetic-lethal* (from the original sense of the word *synthesis*, meaning “putting together”): it kills only in partnership with the cancer-specific mutation. As we become increasingly able to pinpoint the specific alterations in cancer cells that make them different from their normal neighbors, new opportunities for such precisely targeted treatments are coming into view. We end this chapter with some examples of new treatments of this type that are already being put into practice.

### PARP Inhibitors Kill Cancer Cells That Have Defects in *Brca1* or *Brca2* Genes

As we have emphasized, the genetic instability of cancer cells makes the cells both dangerous and vulnerable—dangerous because of the enhancement in their ability to evolve and proliferate, and vulnerable because treatment that leads to still more extreme genetic disruption can take them over the brink and kill them. In some cancers, genetic instability results from an identified fault in one of the many devices on which normal cells depend for DNA repair and maintenance. In this case, a drug is tailored to block a complementary part of the DNA repair machinery can lead to such severe genetic damage that the cancer cells die.

Detailed studies of the mechanisms for DNA maintenance discussed in Chapter 5 reveal a surprising amount of apparent redundancy. Thus, knocking out a particular pathway for DNA repair is generally less disastrous than one might expect, because alternate repair pathways exist. For example, stalled DNA replication forks can arise when the fork encounters a single-strand break in a template strand, but cells can avoid the disaster that would otherwise result either by directly repairing these single-strand breaks, or, if that fails, repairing the broken fork that results by homologous recombination (see Figure 5–50). Suppose that the cells in a particular cancer have become genetically unstable by acquiring a mutation that reduces their ability to repair broken replication forks by homologous recombination. Might it be possible to eradicate that cancer by treating it with a drug that inhibits the repair of single-strand breaks, thereby greatly increasing the number of forks that break? The consequences of such drug treatment might be expected to be relatively harmless for normal cells, but lethal for the cancer.

This strategy appears to work to kill the cells in at least one class of cancers—those that have inactivated both copies of either their *Brca1* or their *Brca2* tumor



**Figure 20–41** How a tumor's genetic instability can be exploited for cancer therapy. As explained in Chapter 5, the maintenance of DNA sequences is so critical for life that cells have evolved multiple pathways for repairing DNA damage and reducing DNA replication errors. As illustrated, a DNA replication fork will stall whenever it encounters a break in a DNA template strand. In this example, normal cells have two different repair pathways that help them to avoid the problem, pathways 1 and 2. They are therefore not harmed by treatment with a drug that blocks repair pathway 1. But, because the inactivation of repair pathway 2 was selected for during the evolution of the tumor cell, the tumor cells are killed by the same drug treatment.

In the actual case that underlies this example, the function of repair pathway 1 (requiring the PARP protein discussed in the text) is to remove persistent, accidental breaks in a DNA single strand before they are encountered by a moving replication fork. Pathway 2 is the recombination-dependent process (requiring the Brca2 and Brca1 proteins) for repairing stalled replication forks illustrated in Figure 5–50. PARP inhibitors have promise for treating cancers with defective *Brca2* or *Brca1* tumor suppressor genes.

suppressor genes. As described in Chapter 5, Brca2 is an accessory protein that interacts with the Rad51 protein (the RecA analog in humans) in the repair of DNA double-strand breaks by homologous recombination. Brca1 is another protein that is also required for this repair process. Like *Rb*, the *Brca1* and *Brca2* genes were discovered as mutations that predispose humans to cancer—in this case, chiefly cancers of the breast and ovaries (though unlike *Rb*, they seem to be involved in only a small proportion of such cancers). Individuals who inherit one mutant copy of *Brca1* or *Brca2* develop tumors that have inactivated the second copy of the same gene, presumably because this change makes the cells genetically unstable and speeds tumor progression.

While Brca1 and Brca2 are needed for the repair of DNA double-strand breaks, single-strand breaks are repaired by other machinery, involving an enzyme called PARP (polyADP-ribose polymerase). This understanding of the basic mechanisms of DNA repair led to a striking discovery: drugs that block PARP activity kill *Brca*-deficient cells with extraordinary selectivity. At the same time, PARP inhibition has very little effect on normal cells; in fact, mice that have been engineered to lack PARP1—the major PARP family member involved in DNA repair—remain healthy under laboratory conditions. This result suggests that, while the repair pathway requiring PARP provides a first line of defense against persistent breaks in a DNA strand, these breaks can be repaired efficiently by a genetic recombination pathway in normal cells. In contrast, tumor cells that have acquired their genetic instability by the loss of Brca1 or Brca2 have lost this second line of defense, and they are therefore uniquely sensitive to PARP inhibitors (Figure 20–41).

PARP inhibitors are still under clinical trial, but they have produced some striking results, causing tumors to regress in many Brca-deficient patients and delaying progression of their disease, with relatively few disagreeable side effects. These drugs also appear to be applicable to cancers with other mutations that cause defects in the cell's homologous recombination machinery—a small, though significant, proportion of cancer cases.

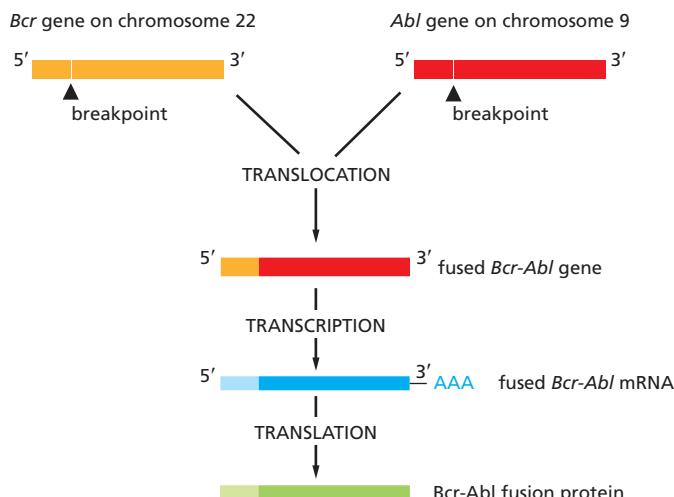
PARP inhibition provides an example of the type of rational, highly selective approach to cancer therapy that is beginning to be possible. Along with other new treatments to be discussed below, it raises high hopes for treating many other cancers.

### Small Molecules Can Be Designed to Inhibit Specific Oncogenic Proteins

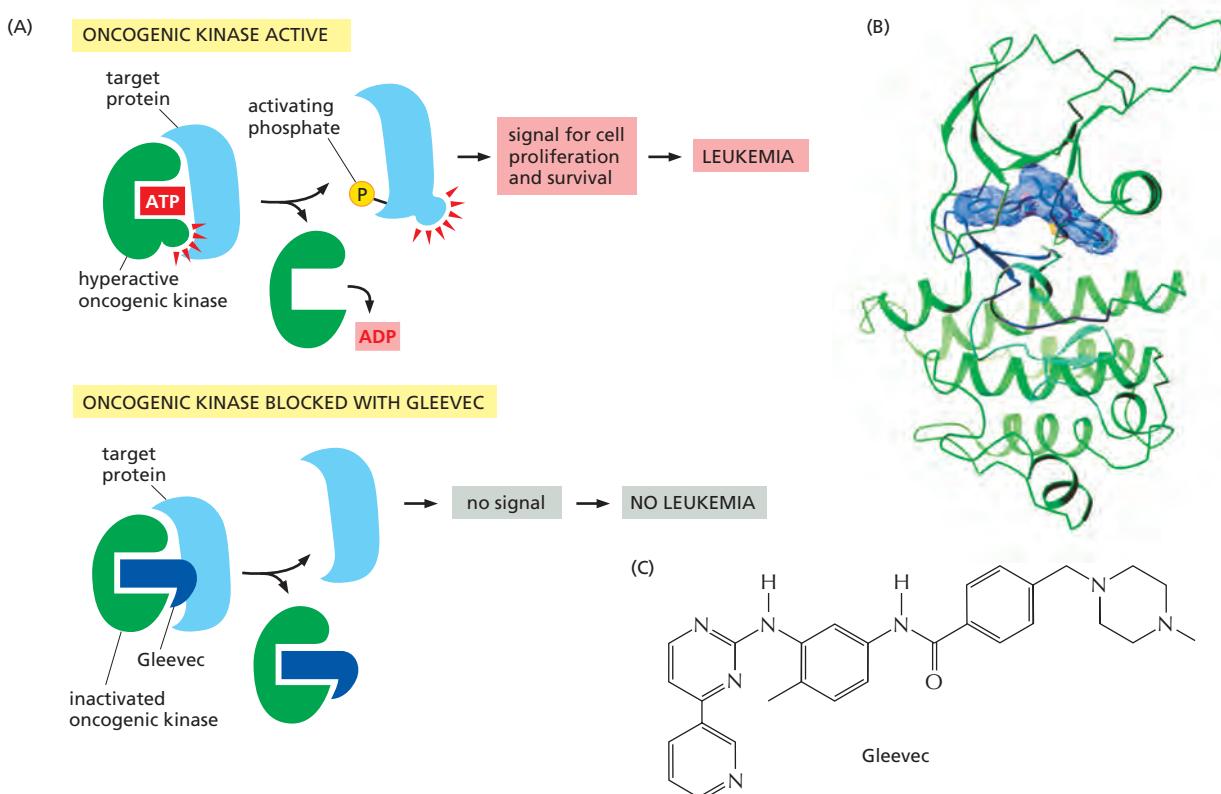
An obvious tactic for treating cancer is to attack a tumor expressing an oncogene with a drug designed to specifically block the function of the protein that the oncogene produces. But how can such a treatment avoid hurting the normal cells that depend on the function of the proto-oncogene from which the oncogene has evolved, and why should the drug kill the cancer cells, rather than simply calm them down? One answer may lie in the phenomenon of *oncogene dependence*. Once a cancer cell has undergone an oncogenic mutation, it will often undergo further mutations, epigenetic changes, or physiological adaptations that make it reliant on the hyperactivity of the initial oncogene, just as drug addicts become reliant on high doses of their drug. Blocking the activity of the oncogenic protein may then kill the cancer cell without significantly harming its normal neighbors. Some remarkable successes have been achieved in this way.

As we saw earlier, chronic myelogenous leukemia (CML) is usually associated with a particular chromosomal translocation, visible as the Philadelphia chromosome (see Figure 20–5). This results from chromosome breakage and rejoining at the sites of two specific genes, *Abl* and *Bcr*. The fusion of these genes creates a hybrid gene, called *Bcr-Abl*, that codes for a chimeric protein consisting of the N-terminal fragment of *Bcr* fused to the C-terminal portion of *Abl* (Figure 20–42). *Abl* is a tyrosine kinase involved in cell signaling. The substitution of the *Bcr* fragment for the normal N-terminus of *Abl* makes it hyperactive, so that it stimulates inappropriate proliferation of the hemopoietic precursor cells that contain it and prevents these cells from dying by apoptosis—which many of them would normally do. As a result, excessive numbers of white blood cells accumulate in the bloodstream, producing CML.

The chimeric *Bcr-Abl* protein is an obvious target for therapeutic attack. Searches for synthetic drug molecules that can inhibit the activity of tyrosine kinases discovered one, called *imatinib* (trade name Gleevec®), that blocks *Bcr-Abl* (Figure 20–43). When the drug was first given to patients with CML, nearly all of them showed a dramatic response, with an apparent disappearance of the cells carrying the Philadelphia chromosome in over 80% of patients. The response appears relatively durable: after years of continuous treatment, many patients have not progressed to later stages of the disease—although imatinib-resistant cancers emerge with a probability of about 5% per year during the early years.



**Figure 20–42** The conversion of the *Abl* proto-oncogene into an oncogene in patients with chronic myelogenous leukemia. The chromosome translocation responsible joins the *Bcr* gene on chromosome 22 to the *Abl* gene from chromosome 9, thereby generating a Philadelphia chromosome (see Figure 20–5). The resulting fusion protein has the N-terminus of the *Bcr* protein joined to the C-terminus of the *Abl* tyrosine protein kinase; in consequence, the *Abl* kinase domain becomes inappropriately active, driving excessive proliferation of a clone of hemopoietic cells in the bone marrow.

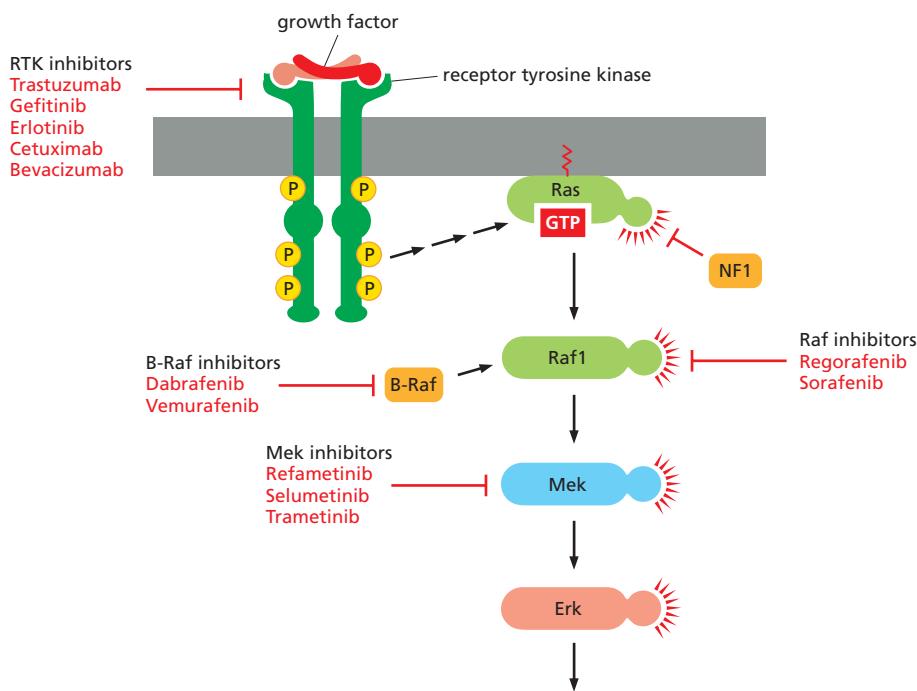


**Figure 20-43** How imatinib (Gleevec) blocks the activity of Bcr-Abl protein and halts chronic myelogenous leukemia. (A) Imatinib sits in the ATP-binding pocket of the tyrosine kinase domain of Bcr-Abl and thereby prevents Bcr-Abl from transferring a phosphate group from ATP onto a tyrosine residue in a substrate protein. This blocks transmission of a signal for cell proliferation and survival. (B) The structure of the complex of imatinib (solid blue object) with the tyrosine kinase domain of the Abl protein (ribbon diagram), as determined by x-ray crystallography. (C) The chemical structure of the drug. It can be given by mouth; it has side effects, but they are usually quite tolerable. (B, from T. Schindler et al., *Science* 289:1938–1942, 2000. With permission from AAAS.)

Results are not so good for those patients who have already progressed to the more acute phase of myeloid leukemia, known as blast crisis, where genetic instability has set in and the march of the disease is far more rapid. These patients show a response at first and then relapse because the cancer cells develop a resistance to imatinib. This resistance is usually associated with secondary mutations in the part of the *Bcr-Abl* gene that encodes the kinase domain, disrupting the ability of imatinib to bind to Bcr-Abl kinase. Second-generation inhibitors that function effectively against a whole range of imatinib-resistant mutants have now been developed. By combining one or more of these new inhibitors with imatinib as the initial therapy (see below), it seems that CML—at least in the chronic (early) stage—may be on its way to becoming a curable disease.

Despite the complications with resistance, the extraordinary success of imatinib is enough to drive home an important principle: once we understand precisely what genetic lesions have occurred in a cancer, we can begin to design effective rational methods to treat it. This success story has fueled efforts to identify small-molecule inhibitors for other oncogenic protein kinases and to use them to attack the appropriate cancer cells. Increasing numbers are being developed. These include molecules that target the EGF receptor and are currently approved for the treatment of some lung cancers, as well as drugs that specifically target the B-Raf oncogene in melanomas.

Protein kinases have been relatively easy to inhibit with small molecules like imatinib, and many kinase inhibitors are being produced by pharmaceutical companies in the hope that they can be effective as drugs for some forms of cancer. Many cancers lack an oncogenic mutation in a protein kinase. But most tumors contain inappropriately activated signaling pathways, for which a target



**Figure 20–44** Some anticancer drugs and drug targets in the Ras-MAP-kinase signaling pathway. Each of the signaling proteins in this diagram has been identified as a product of a cancer-critical gene, with the exception of Raf1 and Erk. This Ras-MAP-kinase signaling pathway is triggered by a variety of receptor tyrosine kinases (RTKs), including the EGF receptor (see Figures 15–47 and 15–49). Those drugs that are antibodies end in “mab,” while those that are small molecules end in “nib.” (Adapted from B. Vogelstein et al, *Science* 339:1546–1558, 2013.)

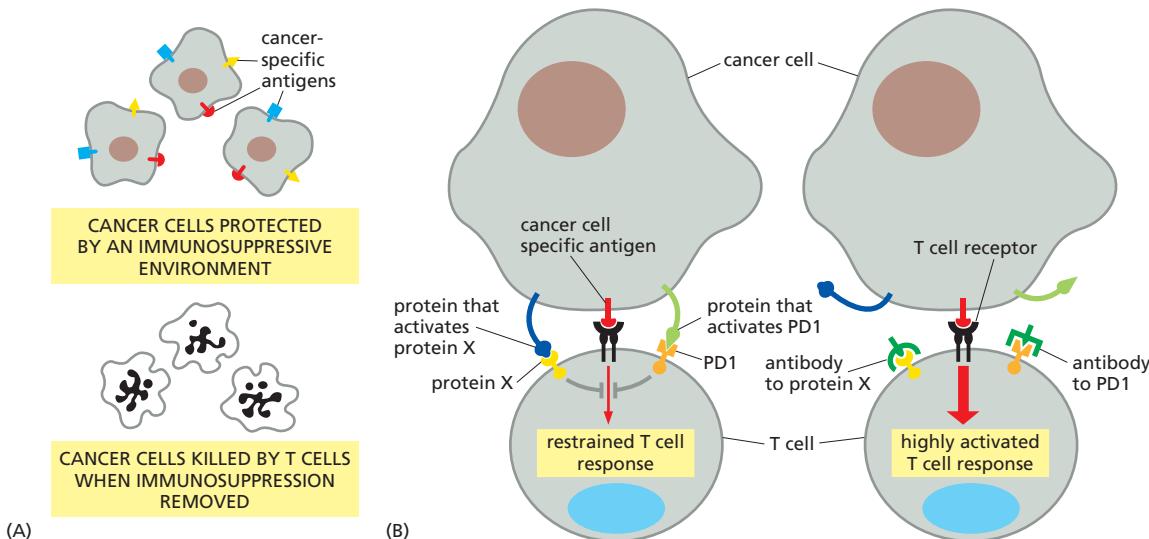
somewhere in the pathway can hopefully be found ([Movie 20.7](#)). As an example, **Figure 20–44** displays some of the anticancer drugs and drug targets that are currently being tested for a pathway frequently activated in cancers.

### Many Cancers May Be Treatable by Enhancing the Immune Response Against the Specific Tumor

Cancers have complex interactions with the immune system, and its various components may sometimes help as well as hinder tumor progression. But for more than a century it has been a dream of cancer researchers to somehow harness the immune system in a controlled and efficient way to exterminate cancer cells, just as it exterminates infectious organisms. There are finally signs that this dream may one day be realized, at least for some forms of cancer.

The simplest type of immunological therapy, conceptually at least, is to inject the patient with antibodies that target the cancer cells. This approach has had some successes. About 25% of breast cancers, for example, express unusually high levels of the Her2 protein, a receptor tyrosine kinase related to the EGF receptor that plays a part in the normal development of mammary epithelium. A monoclonal antibody called *trastuzumab* (trade name *Herceptin*®) that binds to Her2 and inhibits its function slows the growth of breast tumors in humans that overexpress Her2, and it is now an approved therapy for these cancers (see Figure 20–44). A related approach uses antibodies to deliver poisons to the cancer cells. Antibodies against proteins that are abundant on the surface of a particular type of cancer cell but rare on normal cells can be armed with a toxin that kills those cells that bind the antibody molecule.

A great deal of current excitement centers around a different type of approach, based on the relatively recent recognition that the microenvironment in a tumor is highly immunosuppressive. As a result, the cancer victim’s immune system is prevented from destroying the tumor cells. Recall that, from the thousands of genome sequences thus far determined, we know that a typical cancer cell will contain on the order of 50 proteins with a mutation that alters an amino acid sequence, most of these being “passenger” mutations, as previously explained (see p. 1104). Many of these mutant proteins will be recognized by the patient’s immune system as foreign, but—to allow the cancer cells to survive throughout the course of tumor progression—the cancer cells have evolved a set of anti-immune defenses. These



defenses include the expression on the cancer cell surface of one or more proteins that bind to inhibitory receptors on activated T cells.

The normal immune system is subject to complex controls that keep its activity within safe bounds and prevent autoimmunity from developing. The inhibitory receptors that are expressed on the surface of activated T cells have an important normal function: they control the immune response by down-regulating the T cell response under appropriate circumstances. But in the context of a tumor, the down-regulation is inappropriate, because it prevents the organism from killing the cancer cells that are threatening its survival.

In its attack on infectious organisms, the natural immune system usually eliminates every last trace of infection and maintains this immunity in the long term. The challenge is to find ways of recruiting the immune system to attack cancers with similar efficiency and specificity, hunting the cancer cells down by virtue of the tumor-specific antigens that they express. With this aim, a new type of anti-cancer therapy focuses on overcoming the immunosuppressive environment in a tumor through the use of specific antibodies that prevent the tumor cells from engaging with the inhibitory receptors on T cells. As illustrated in Figure 20-45A, blocking the action of the immune suppressors with such treatments should unleash an immune attack on the cancer cells. Importantly, multiple antigens are recognized as foreign; thus, the cancer cells cannot escape through the mutational loss of a single antigen, making it difficult for the tumor to escape from the T cell attack.

This is a potentially dangerous strategy. If one provokes the immune system to recognize the cancer cells as targets for destruction, there is a risk of autoimmune side effects with dire consequences for normal tissues of the body, since the cancer cells and the normal cells are close cousins and share most of their molecular features. Nevertheless, several recent successes seem to hold great promise for the future.

One of the many molecules involved in keeping the activity of the normal immune system within safe bounds is a protein called CTLA4 (cytotoxic T-lymphocyte-associated protein 4), which functions as an inhibitory receptor on the surface of T cells. If the function of CTLA4 is blocked, the T cells become more reactive and may mount an attack on cells that they would otherwise leave in peace. In particular, the T cells may attack tumor cells that are recognizably abnormal but whose presence was previously tolerated. With this in mind, cancer immunologists developed a monoclonal antibody, called *ipilimumab*, that binds to CTLA4 and blocks its action. Injected repeatedly into patients with metastatic melanoma, this antibody increases their median lifespan by several months and, in one large trial, enabled as many as a quarter of them to survive for five years

**Figure 20-45** Therapies designed to remove the immunosuppressive microenvironment in tumors. (A) The cells in tumors will produce many mutant proteins. As described in Chapter 24, peptides from these proteins will be displayed on MHC complexes on the tumor-cell surface and would normally activate a T cell response that destroys the tumor (see Figure 24-42). However, as schematically illustrated, during the course of tumor progression, the cancer cells have evolved immunosuppressive mechanisms that protect them from such killing. (B) The cells in tumors often protect themselves from immune attack by expressing proteins on their surface that bind to and thereby activate the inhibitory receptors on T cells. As indicated, this makes the tumor susceptible to specific antibody therapies. In this diagram, two such inhibitory receptors are shown, PD1 and a hypothetical protein X. Different tumors are thought to protect themselves by activating different members of a large set of T cell inhibitory receptors, some of which are not yet well characterized.

or more—far beyond expectations for comparable patients without this treatment. Even more promising are recent clinical trials using a combination of two antibodies, one against CTLA4 and the other against PD1, a second cell-surface receptor on T cells that normally restrains their activity.

In clinical trials using such techniques, a substantial fraction of the patients can respond in a dramatic way, with their cancer being driven into remission for years, while the treatment fails to help others with the same type of cancer. One possible explanation is that, while most tumors express proteins that protect them from T-cell attack, these proteins are different for different tumors. Thus, while some tumors will respond dramatically when treated with an antibody that blocks a particular immunosuppressive agent, many others will not. If true, one can foresee an era of personalized immunotherapy, in which each patient's tumor is molecularly analyzed to determine its particular mechanisms of immunosuppression. The patient would then be treated with a specific cocktail of antibodies designed to remove these blocks (see Figure 20–45).

### Cancers Evolve Resistance to Therapies

High hopes have to be tempered with sobering realities. We have seen that genetic instability can provide an Achilles heel that cancer therapies can exploit, but at the same time it can make eradicating the disease more difficult by allowing the cancer cells to evolve resistance to therapeutic drugs, often at an alarming rate. This applies even to the drugs that target genetic instability itself. Thus, PARP inhibitors give valuable remission of illness, but in the long term the disease generally comes back. For example, *Brca*-deficient cancers can sometimes develop resistance to PARP inhibitors by undergoing a second mutation in an affected *Brca* gene that restores its function. By then, the cancer is already out of control and it may be too late to affect the course of the disease with additional treatments.

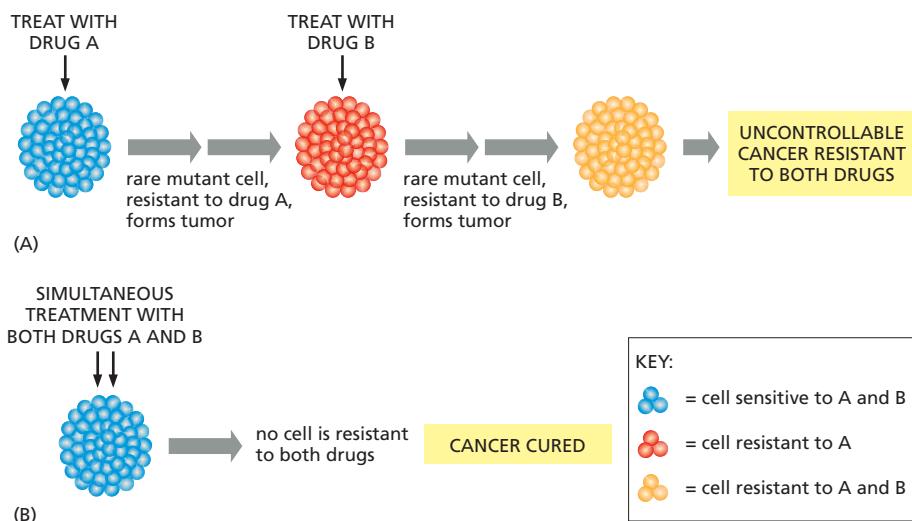
There are many different strategies by which cancers can evolve resistance to anticancer drugs. Often, a cancer will be dramatically reduced in size by an initial drug treatment, with all of the detectable tumor cells seeming to disappear. But months or years later the cancer will reappear in an altered form that is resistant to the drug that was at first so successful. In such cases, the initial drug treatment has evidently failed to destroy some tiny fraction of cells in the original tumor-cell population. These cells have escaped death because they carry a protective mutation or epigenetic change, or perhaps simply because they were lurking in a protected environment. They eventually regenerate the cancer by continuing to proliferate, mutating and evolving still further as they do so.

In some cases, cells that are exposed to one anticancer drug evolve a resistance not only to that drug but also to other drugs to which they have never been exposed. This phenomenon of **multidrug resistance** frequently correlates with amplification of a part of the genome that contains a gene called *Mdr1* or *Abcb1*. This gene encodes a plasma-membrane-bound transport ATPase of the ABC transporter superfamily (discussed in Chapter 11), which pumps lipophilic drugs out of the cell (see Movie 11.5). The overproduction of this protein (or some of its other family members) by a cancer cell can prevent the intracellular accumulation of many cytotoxic drugs, making the cell insensitive to them.

In the to-and-fro struggle between advanced metastatic cancer and the therapist, as current practice stands, the cancer usually wins in the end. Does it have to be so? As we discuss below, there is reason to think that by attacking a cancer with many weapons at once—instead of using them one after another, each until it fails—it may be possible to do much better.

### Combination Therapies May Succeed Where Treatments with One Drug at a Time Fail

Nowadays, cancers caught at an early stage can often be cured, by surgery, radiation, or drugs. For most cancers that have progressed and metastasized widely, however, cure is still beyond us. Treatments such as those described above can



**Figure 20-46** Why multidrug treatments can be more effective than sequential treatments for cancer therapy.

(A) Because tumor cells are hypermutable, two single-drug treatments that are given sequentially often allow for the selection of mutant cell clones that are resistant to both drugs. (B) Simultaneous treatment with both drugs can be more effective.

give valuable remissions, but sooner or later these are typically followed by relapse.

Nevertheless, for some relatively rare forms of advanced cancer, curative therapies have been developed. These generally involve a cocktail of several different anticancer agents: by trial and error, certain combinations of cytotoxic drugs have been found to wipe out the cancer completely. Discovering such combinations has hitherto involved a long, hard search. But now, armed with our new tools for identifying the specific genetic lesions that cancer cells contain, the prospects are better.

The logic of combination therapies is the same as that behind the current treatment of HIV-AIDS with a cocktail of three different protease inhibitors: whereas there may always be some cells in the initial population carrying the rare mutations that confer resistance to any one drug treatment, there should be no cell carrying the whole set of rare mutations that would confer resistance to several different drugs delivered simultaneously. In contrast, sequential drug treatments will allow the few cells resistant to the first drug to multiply to large numbers. Within this large population of cells resistant to the first drug, a small number of cells are likely to have arisen that are resistant to the next drug also; and so on (Figure 20-46).

### We Now Have the Tools to Devise Combination Therapies Tailored to the Individual Patient

Efficient, rational combination drug therapy requires three things. First, we have to identify multiple peculiarities of cancer cells that make them vulnerable in ways that normal cells are not. Second, we have to devise drugs (or other treatments) that target each of these vulnerabilities. Third, we have to match the combination of drugs to the specific set of peculiarities present in the cancer cells of the individual patient.

The first requirement is already partially met: we now have large catalogs of cancer-critical genes that are commonly mutated in cancer cells. The second requirement is harder, but attainable: we have described some remarkable recent successes, and for cancer researchers there is excitement in the air. It is becoming increasingly possible to use our growing knowledge of cell and molecular biology to design new drugs against designated targets. At the same time, efficient, high-throughput automated methods are available to screen large libraries of chemicals for any that may be effective against cells with a given cancer-related defect. In such searches, the goal is synthetic lethality: a cell death that occurs when and only when a particular drug is put together with a particular cancer cell abnormality. Through these and other approaches, the repertoire of precisely targeted anticancer drugs is rapidly increasing.

This brings us to the third requirement: the therapy—the choice of drugs to be given in combination—must be tailored to the individual patient. Here, too, the prospects are bright. Cancers evolve by a fundamentally random process, and each patient is different; but modern methods of genome analysis now let us characterize the cells from a tumor biopsy in exhaustive detail so as to discover which cancer-critical genes are affected in a particular case. Admittedly, this is not straightforward: the tumor cells in an individual patient are heterogeneous and do not all contain the same genetic lesions. With increased understandings of the pathways of cancer evolution, however, and with the experience gained from many different cases, it should become possible to make good guesses at the optimal therapies to use.

From the perspective of the patient, the pace of advance in cancer research can seem frustratingly slow. Each new drug has to be tested in the clinic, first for safety and then for efficacy, before it can be released for general use. And if the drug is to be used in combination with others, the combination therapy must then go through the same long process. Strict ethical rules constrain the conduct of trials, which means that they take time—typically several years. But slow and cautious steps, taken systematically in the right direction, can lead to great advances. There is still far to go, but the examples that we have discussed provide proof of principle and grounds for optimism.

From the cancer research effort, we have learned a great deal of what we know about the molecular biology of the normal cell. Now, more and more, we are discovering how to put that knowledge to use in the battle with cancer itself.

## Summary

*Our growing understanding of the cell biology of cancers has already begun to lead to better ways of preventing, diagnosing, and treating these diseases. Anticancer therapies can be designed to destroy cancer cells preferentially by exploiting the properties that distinguish cancer cells from normal cells, including the cancer cells' dependence on oncogenic proteins and the defects they harbor in their DNA repair mechanisms. We now have good evidence that, by increasing our understanding of normal cell control mechanisms and exactly how they are subverted in specific cancers, we can eventually devise drugs to kill cancers precisely by attacking specific molecules critical for the growth and survival of the cancer cells. In addition, great progress has recently been made through sophisticated immunological approaches to cancer therapy. And, as we become better able to determine which genes are altered in the cells of any given tumor, we can begin to tailor treatments more accurately to each individual patient.*

## PROBLEMS

Which statements are true? Explain why or why not.

**20–1** The chemical carcinogen dimethylbenz[a]anthracene (DMBA) must be an extraordinarily specific mutagen since 90% of the skin tumors it causes have an A-to-T alteration at exactly the same site in the mutant *Ras* gene.

**20–2** In the cellular regulatory pathways that control cell growth and proliferation, the products of oncogenes are stimulatory components and the products of tumor suppressor genes are inhibitory components.

**20–3** Cancer therapies directed solely at killing the rapidly dividing cells that make up the bulk of a tumor are unlikely to eliminate the cancer from many patients.

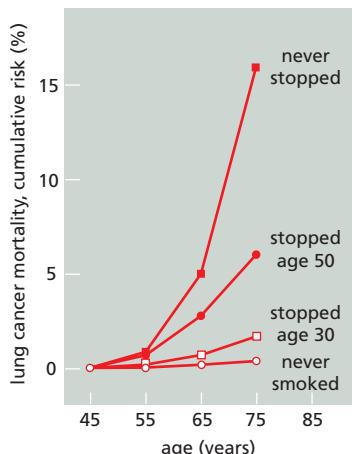
## WHAT WE DON'T KNOW

- What is required to enable a cancer cell to metastasize?
- How can the molecular analysis of an individual tumor be more effectively used to design effective therapies to kill it?
- Can we identify general features common to all cancer cells—such as their production of misfolded, mutated proteins—that can be used for the targeted destruction of many different types of cancers?
- Can sensitive and reliable blood tests be devised to detect cancers very early, before they have grown to a size where treatment with a single drug will generally be defeated by the survival of a preexisting resistant variant?
- How can the observed environmental effects on cancer rates be exploited to reduce avoidable cancers?
- Can new technologies be devised to reveal exactly how a quiescent micrometastasis converts to a full-blown metastatic tumor?

**20–4** The main environmental causes of cancer are the products of our highly industrialized way of life such as pollution and food additives.

### Discuss the following problems.

**20–5** In contrast to colon cancer, whose incidence increases dramatically with age, incidence of osteosarcoma—a tumor that occurs most commonly in the long bones—peaks during adolescence. Osteosarcomas are relatively rare in young children (up to age 9) and in adults (over 20). Why do you suppose that the incidence of osteosarcoma does not show the same sort of age-dependence as colon cancer?



**Figure Q20-1** Cumulative risk of lung cancer mortality for nonsmokers, smokers, and former smokers (Problem 20–6). Cumulative risk is the running total of deaths, as a percentage, for each group. Thus, for continuing smokers, 1% died of lung cancer between ages 45 and 55; an additional 4% died between 55 and 65 (giving a cumulative risk of 5%); and 11% more died between 65 and 75 (for a cumulative risk of 16%).

**20–6** Mortality due to lung cancer was followed in groups of males in the United Kingdom for 50 years. **Figure Q20-1** shows the cumulative risk of dying from lung cancer as a function of age and smoking habits for four groups of males: those who never smoked, those who stopped at age 30, those who stopped at age 50, and those who continued to smoke. These data show clearly that individuals can substantially reduce their cumulative risk of dying from lung cancer by stopping smoking. What do you suppose is the biological basis for this observation?

**20–7** A small fraction—2 to 3%—of all cancers, across many subtypes, displays a quite remarkable phenomenon: tens to hundreds of rearrangements that primarily involve a single chromosome, or chromosomal region. The breakpoints can be tightly clustered, with several in a few kilobases; the junctions of the rearrangements often involve segments of DNA that were not originally close together on the chromosome. The copy number of various segments within the rearranged chromosome was found to be either zero, indicating deletion, or one, indicating retention.

You can imagine two ways in which such multiple, localized rearrangements might happen: a progressive rearrangements model with ongoing inversions, deletions, and duplications involving a localized area, or a catastrophic model in which the chromosome is shattered into fragments that are stitched back together in random order by nonhomologous end joining (**Figure Q20-2**).

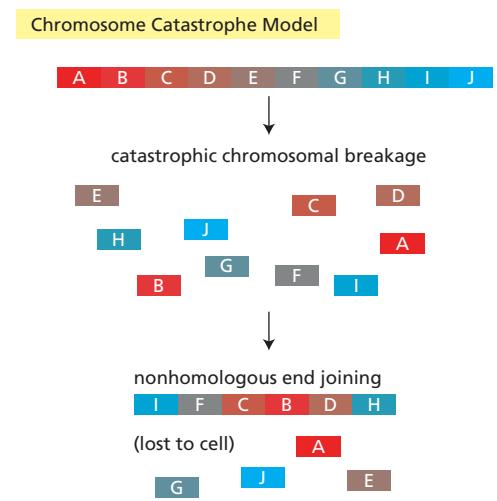
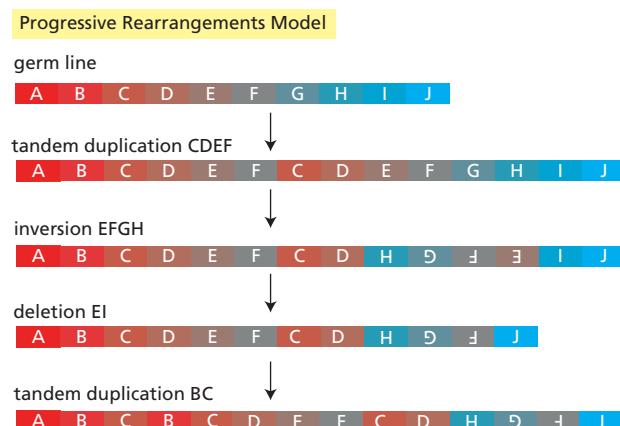
**A.** Which of the two models in Figure Q20-2 accounts more readily for the features of these highly rearranged chromosomes? Explain your reasoning.

**B.** For whichever model you choose, suggest how such multiple rearrangements might arise. (The true mechanism is not known.)

**C.** Do you suppose such rearrangements are likely to be causative events in the cancers in which they are found, or are they probably just passenger events that are unrelated to the cancer? If you think they could be driver events, suggest how such rearrangements might activate an oncogene or inactivate a tumor suppressor gene.

**20–8** Virtually all cancer treatments are designed to kill cancer cells, usually by inducing apoptosis. However, one particular cancer—acute promyelocytic leukemia (APL)—has been successfully treated with all-*trans*-retinoic acid, which causes the promyelocytes to differentiate into neutrophils. How might a change in the state of differentiation of APL cancer cells help the patient?

**20–9** One major goal of modern cancer therapy is to identify small molecules—anticancer drugs—that can be used to inhibit the products of specific cancer-critical genes. If you were searching for such molecules, would you design inhibitors for the products of oncogenes or the products of tumor suppressor genes? Explain why you would (or would not) select each type of gene.

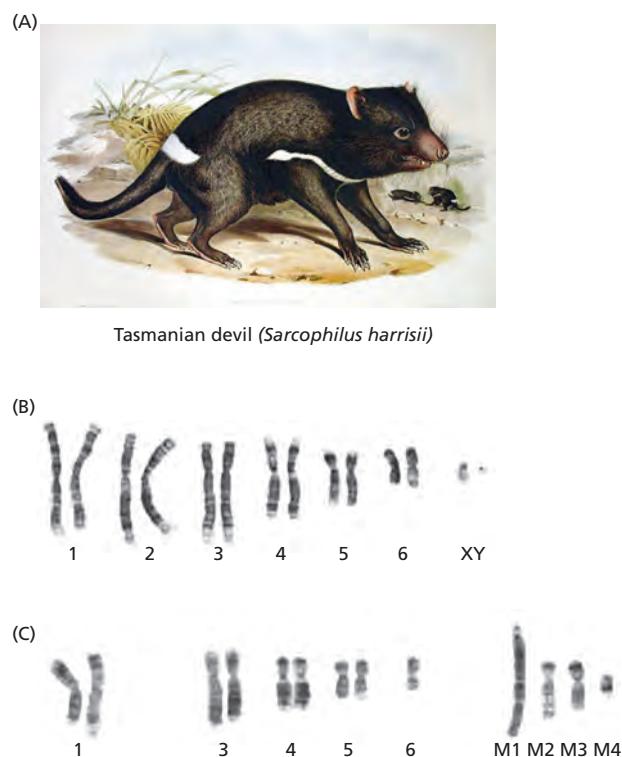


**Figure Q20-2** Two models to explain the multiple, localized chromosome rearrangements found in some cancers (Problem 20–7). The progressive rearrangements model shows a sequence of rearrangements that disrupts the chromosome, generating increasingly complex chromosomal configurations. The chromosome catastrophe model shows the chromosome being shattered into fragments that are stitched back together in random order by nonhomologous end joining.

**20–10** PolyADP-ribose polymerase (PARP) plays a key role in the repair of DNA single-strand breaks. In the presence of the PARP inhibitor olaparib, single-strand breaks accumulate. When a replication fork encounters a single-strand break, it converts it to a double-strand break, which in normal cells is then repaired by homologous recombination. In cells defective for homologous recombination, however, inhibition of PARP triggers cell death.

Patients who have only one functional copy of the *Brcal* gene, which is required for homologous recombination, are at much higher risk for cancer of the breast and ovary. Cancers that arise in these tissues in these patients can be treated successfully with olaparib. Explain how it is that treatment with olaparib kills the cancer cells in these patients, but does not harm their normal cells.

**20–11** The Tasmanian devil, a carnivorous Australian marsupial, is threatened with extinction by the spread of a fatal disease in which a malignant oral-facial tumor interferes with the animal's ability to feed. You have been called in to analyze the source of this unusual cancer. It seems clear to you that the cancer is somehow spread from devil to devil, very likely by their frequent fighting, which is accompanied by biting around the face and mouth. To uncover the source of the cancer, you isolate tumors from 11 devils captured in widely separated regions and examine them. As might be expected, the karyotypes of the tumor cells are highly rearranged relative to that of the wild-type devil (Figure Q20–3). Surprisingly, you find that the karyotypes from all 11 tumor samples are very similar. Moreover, one of the Tasmanian devils has an inversion on chromosome 5 that is not present in its facial tumor. How do you suppose this cancer is transmitted from devil to devil? Is it likely to arise as a consequence of an infection by a virus or microorganism? Explain your reasoning.



**Figure Q20–3** Karyotypes of cells from Tasmanian devils (Problem 20–11). (A) A Tasmanian devil. (B) Normal karyotype for a male Tasmanian devil. The karyotype has 14 chromosomes, including XY. (C) Karyotype of cancer cells found in each of the 11 facial tumors studied. The karyotype has 13 chromosomes, no sex chromosomes, no chromosome 2 pair, one chromosome 6, two chromosomes 1 with deleted long arms, and four highly rearranged marker chromosomes (M1–M4). (A, reproduced courtesy of Museum Victoria; B and C, from A.M. Pearse and K. Swift, *Nature* 439:549, 2006. With permission from Macmillan Publishers Ltd.)

## REFERENCES

### General

- Bishop JM (2004) How to Win the Nobel Prize: An Unexpected Life in Science. Cambridge, MA: Harvard University Press.  
 Hanahan D & Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646–674.  
 Vogelstein B, Papadopoulos N, Velculescu VE et al. (2013) Cancer genome landscapes. *Science* 339, 1546–1558.  
 Weinberg RA (2013) The Biology of Cancer, 2nd ed. Garland Science: New York.

### Cancer as a Microevolutionary Process

- Brown JM & Attardi LD (2005) The role of apoptosis in cancer development and treatment response. *Nat. Rev. Cancer* 5, 231–237.  
 Chambers AF, Naumov GN, Vantyghem S & Tuck AB (2000) Molecular biology of breast cancer metastasis. Clinical implications of experimental studies on metastatic inefficiency. *Breast Cancer Res.* 2, 400–407.  
 Chi P, Allis CD & Wang GG (2010) Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. *Nat. Rev. Cancer* 10, 457–469.  
 Fidler IJ (2003) The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. *Nat. Rev. Cancer* 3, 453–458.  
 Hoeijmakers JHJ (2001) Genome maintenance mechanisms for preventing cancer. *Nature* 411, 366–374.  
 Joyce JA & Pollard JW (2009) Microenvironmental regulation of metastasis. *Nat. Rev. Cancer* 9, 239–252.  
 Lowe SW, Cepero E & Evan G (2004) Intrinsic tumour suppression. *Nature* 432, 307–315.  
 Nowell PC (1976) The clonal evolution of tumor cell populations. *Science* 194, 23–28.  
 Stephens PJ, McBride DJ, Lin M-L et al. (2009) Complex landscapes of somatic rearrangement in human breast cancer genomes. *Nature* 462, 1005–1010.  
 Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. *Nat. Rev. Cancer* 2, 442–454.  
 Vander Heiden MG, Cantley LC & Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033.  
 Zink D, Fischer AH & Nickerson JA (2004) Nuclear structure in cancer cells. *Nat. Rev. Cancer* 4, 677–687.

### Cancer-Critical Genes: How They Are Found and What They Do

- Berdasco M & Esteller M (2010) Aberrant epigenetic landscape in cancer: how cellular identity goes awry. *Dev. Cell* 19, 698–711.
- Brognard J & Hunter T (2011) Protein kinase signaling networks in cancer. *Curr. Opin. Genet. Dev.* 21, 4–11.
- Eilers M & Eisenman R (2008) Myc's broad reach. *Genes Dev.* 22, 2755–2766.
- Feinberg AP (2007) Phenotypic plasticity and the epigenetics of human disease. *Nature* 447, 433–440.
- Garraway LA & Lander ES (2013) Lessons from the cancer genome. *Cell* 153, 17–37.
- Greaves M & Maley CC (2012) Clonal evolution in cancer. *Nature* 481, 306–313.
- Junttila MR & Evan GI (2009) p53—a Jack of all trades but master of none. *Nat. Rev. Cancer* 9, 821–829.
- Levine AJ (2009) The common mechanisms of transformation by the small DNA tumor viruses: the inactivation of tumor suppressor gene products: p53. *Virology* 384, 285–293.
- Lu P, Weaver VM & Werb Z (2012) The extracellular matrix: a dynamic niche in cancer progression. *J. Cell Biol.* 196, 395–406.
- Mitelman F, Johansson B & Mertens F (2007) The impact of translocations and gene fusions on cancer causation. *Nat. Rev. Cancer* 7, 233–245.
- Negrini S, Gorgoulis VG & Halazonetis TD (2010) Genomic instability—an evolving hallmark of cancer. *Nat. Rev. Mol. Cell Biol.* 11, 220–228.
- Nguyen DX, Bos PD & Massagué J (2009) Metastasis: from dissemination to organ-specific colonization. *Nat. Rev. Cancer* 9, 274–284.
- Radtke F & Clevers H (2005) Self-renewal and cancer of the gut: two sides of a coin. *Science* 307, 1904–1909.
- Rowley JD (2001) Chromosome translocations: dangerous liaisons revisited. *Nat. Rev. Cancer* 1, 245–250.
- Shaw RJ & Cantley LC (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* 441, 424–430.
- Suvà ML, Riggi N & Bernstein BE (2013) Epigenetic reprogramming in cancer. *Science* 339, 1567–1570.
- Weinberg RA (1995) The retinoblastoma protein and cell cycle control. *Cell* 81, 323–330.

### Cancer Prevention and Treatment: Present and Future

- Al-Hajj M, Becker MW, Wicha M et al. (2004) Therapeutic implications of cancer stem cells. *Curr. Opin. Genet. Dev.* 14, 43–47.
- Ames B, Durston WE, Yamasaki E & Lee FD (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Natl. Acad. Sci. USA* 70, 2281–2285.
- Bozic I, Reiter JG, Allen B et al. (2013) Evolutionary dynamics of cancer in response to targeted combination therapy. *eLife* 2, e00747.
- Doll R & Peto R (1981) The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* 66, 1191–1308.
- Druker BJ & Lydon NB (2000) Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *J. Clin. Invest.* 105, 3–7.
- Huang P & Oliff A (2001) Signaling pathways in apoptosis as potential targets for cancer therapy. *Trends Cell Biol.* 11, 343–348.
- Jain RK (2005) Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 307, 58–62.
- Jonkers J & Berns A (2004) Oncogene addiction: sometimes a temporary slavery. *Cancer Cell* 6, 535–538.
- Kalos M & June CH (2013) Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. *Immunity* 39, 49–60.
- Loeb LA (2011) Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat. Rev. Cancer* 11, 450–457.
- Lord CJ & Ashworth A (2012) The DNA damage response and cancer therapy. *Nature* 481, 287–294.
- Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12, 252–264.
- Peto J (2001) Cancer epidemiology in the last century and the next decade. *Nature* 411, 390–395.
- Sawyers C (2004) Targeted cancer therapy. *Nature* 432, 294–297.
- Schreiber RD, Old LJ & Smyth MJ (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 331, 1565–1570.
- Sliwkowski MX & Mellman I (2013) Antibody therapeutics in cancer. *Science* 341, 1192–1198.
- Varmus H, Pao W, Politi K et al. (2005) Oncogenes come of age. *Cold Spring Harb. Symp. Quant. Biol.* 70, 1–9.
- Ward RJ & Dirks PB (2007) Cancer stem cells: at the headwaters of tumor development. *Annu. Rev. Pathol.* 2, 175–189.