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BIOLOGICAL CONCEPTS

The cell cycle and its regulation (receptors, growth factors)

Levels of organization (cells, tissues)

Gene expression and regulation (cell differentiation in embryos and adult animals, potentiality, stem cells)

Embryonic development

Interaction of genotype and environment (carcinogens, mutagens, diet)

Scaling (ratio of surface area to volume)

Health and disease (homeostasis, risk)

Molecular biology of cancer (oncogenes, tumor suppressor genes)

Cancer is now the second leading cause of death in most industrialized countries, second only to heart disease. In the Netherlands and some other countries, cancer ranks first. Cancer is also one of the most dreaded illnesses, and people who learn that they have cancer often suffer additionally from fear of the disease.

Cancer is actually not one disease, but rather a collection of many diseases that exhibit the same fundamental features; it is therefore more accurate to speak of cancers in the plural. **Cancers** of all types result from one central problem: cell division that is out of control. Cancerous cells no longer respond to the signals that normally limit the frequency of cell division. These signals keep normal cells functioning in an integrated manner, a necessity in all multicellular organisms, whose cells are organized into specialized tissues. The activity of normal cells in tissues is sometimes compared to the behavior of animals in social groups: like the behavior of one animal, the behavior of one cell influences that of others. In contrast with normal cells, cancer cells do not behave in an integrated, social way. Instead, they grow chaotically, under their own direction, gradually pushing normal cells aside or growing right over them. Research on cancer has taught us much about the biology of normal cells, including stem cells, which are cells that have the ability to develop into many different kinds of tissues. The basic rules of normal cellular behavior are common to all multicellular species, and processes that regulate cell division are even more universal, encompassing all eucaryotic cells. In this chapter, we first consider how cell growth, division, and behavior are regulated in normal cells. We then discuss what goes wrong in cancer and how it develops. We will also consider how we can most effectively reduce our risks for various cancers.

MULTICELLULAR ORGANISMS ARE ORGANIZED GROUPS OF CELLS AND TISSUES

All living organisms are composed of one or more compartments called **cells**. This tenet is part of the **Cell Theory** that was put forth in the early 1800s by early cell biologists who observed the compartmentalized structure of many different organisms under microscopes. Organisms larger than a certain microscopic threshold size are subdivided into many cells. The first organisms consisted of individual cells performing all life functions in one single compartment, with little or no spatial separation among their functions. Over time, some of these ancestral cells evolved to have greater complexity, dividing their functions among multiple membrane-bound organelles, and establishing the eucaryotic branch of the tree of life. Bacteria and single-celled eucaryotes like yeasts and amoebas continue to live very successfully in a unicellular lifestyle. So why should multicellular life forms have evolved at all? The answer is that **compartmentalization**—whether into the multiple organelles of a single-celled eucaryote or, further, into the many different types of cells that make up a multicellular organism—offers a number of advantages.

Compartmentalization

Compartmentalization of various life processes into specialized organelles allows those processes to happen more efficiently, since the molecules involved are concentrated into a smaller space and can interact with each other more readily. Similarly, compartmentalization of most organisms into multiple cells permits those organisms to become much larger than they could be as single cells because the ratio of a cell's surface

area to its volume imposes a physical restriction. The requirements for energy and the production of wastes both increase in proportion to the volume of an organism, while the organism's ability to absorb nutrients and to release wastes varies with its surface area. However, as an organism enlarges, its volume grows faster than its surface area. Look at the cubes shown in Figure 10.1. What are the volumes of the two cubes, A and B? What are their surface areas? When you calculated the volume of cube A, you multiplied three numbers: height times width times depth. (Another way to say this is that volume is proportional to length cubed.) When you calculated surface area, you multiplied only two numbers—height times width—and then added up the products for each of the cube's faces. (Another way to say this is that area is proportional to length squared.) As you found out, the volume of cube B is 27 times the volume of cube A, yet the surface area of cube B is only 9 times the surface area of cube A. By subdividing a cube, we make more surface area. Subdivided cube C has the same volume as cube B, but three times the surface area.

Just as with the cubes in Figure 10.1, compartmentalization of the interior volume of an organism into cells keeps the volume the same but increases the effective surface area. This maintains an efficient ratio of surface area to volume so that nutrient intake stays balanced with metabolism. Again, in Figure 10.1, the ratio of surface area to volume of cube A is 6, but for the larger cube B, the ratio is only 2. Subdividing cube C returns the ratio of surface area to volume to 6, the same as it was for the small cube A. Subdividing an organism into cells achieves the same effect and ensures that sufficient surface area is maintained to allow for efficient exchange of materials between the cell and its surroundings.

Specialization

Even unicellular organisms sometimes form multicellular “communities” or "aggregates" under certain environmental conditions. Examples include bacterial **biofilms** that can form on many different surfaces, including rocks in natural bodies of water, on medical devices (such as catheters), and on your teeth (as plaque) (see Figure 6.4). In these communities, individual cells take on different patterns of gene expression that allow for different morphological features (shapes) and functions, and the different types of cells are often separated from each other in layered structures. An advantage for both multicellular aggregates and multicellular organisms is that not every cell needs to perform every function. This allows for specialization, which again increases efficiency, similar to the concept of division of labor in a factory, where, for example, certain employees may be highly skilled in machining the parts for a product, others in assembling the product, and still others in adding decorative touches.

In sponges (kingdom Animalia, phylum Porifera), cells are specialized but are not organized into tissues (see Figure 6.14, p. 000). The cells of all other animals do form **tissues**, which are distinct structures consisting of similar cells and their products that function together to execute a particular biological process. Thus, tissues are both *structurally* and *functionally* integrated. The inner and outer cell layers of animals in the phylum Cnidaria, also called coelenterates, which include jellyfish, sea anemones, and corals, (see Figure 6.14, p. 000), are separate tissues with different functions. Although the cells in the two layers are different, each cell is still changeable, so that the Cnidaria are able to regenerate an entire organism from a small piece. The cellular flexibility of Cnidaria (and other organisms capable of regeneration from parts) contrasts with the

situation in more complex multicellular organisms. In these organisms, the fate of a cell becomes more and more restricted as it divides, through a process called **differentiation**. Initially, a cell is capable of performing a variety of functions, but as the cell differentiates (literally, 'becomes different'), it progressively loses some of its abilities and becomes specialized at doing only a few things very well. Some cells, such as human muscle or nerve cells, lose so many important abilities during differentiation that they may become incapable of further cell division. Other cells, such as those of human bone marrow and adult stem cells, retain a good deal of the flexibility characteristic of cells in developing organisms (i.e. embryos). How cells 'know' what type of tissue to become has long been one of the major questions in biology. Much of what we currently know about normal cell division and differentiation has been aided by the comparison of normal cells and cancer cells, as we will discuss later in this chapter.

Cooperation and homeostasis

The organization of cells into tissues allows for the same type of efficiency that was previously described for compartmentalization of individual cells into organelles and of organisms into individual cells. For specialization to be beneficial, however, the behavior of one type of cell must be integrated with the behavior of other cells (just as the behaviors of individual organelles within a cell must be coordinated). Tissues are further integrated into organs and organ systems, in which two or more types of tissues coordinate to perform more complex functions, such as reproduction (see Chapter 8), digestion (see Chapter 9), external sensing (see Chapter 11), respiration, circulation or excretion (see Chapter 13). A multicellular organism can thus be considered a complex ecosystem. A human being, for example, is a complex ecosystem of some ten trillion individual cells! In

the course of evolution, specialization based on cooperation worked well and was favored by natural selection. All multicellular organisms—fungi, plants, and animals—have continued this basic strategy.

The proper functioning of the whole organism depends on the continued integration and cooperation of all the cells. When this integration is functioning properly, that is, when the ecosystem of cells is stable, we may consider that organism to be in a state of health. According to French physiologist Claude Bernard (1813–1878), cells are responsible for maintaining a 'milieu intérieur' (an internal environment) within each cell and within the body as a whole. Good health is defined as the maintenance of more or less constant conditions within this internal environment, a process that Bernard named **homeostasis**. This does not mean that during homeostasis there are no changes within the organism. Just the opposite is true: molecules and cells are constantly being made and being broken down, but these changes occur around a balance point. Homeostasis is the ability to return to that balance point. Disruption of homeostasis produces illness. As we will see, cancer is a disruption of the cellular homeostasis in which cell division is no longer in balance with cell death.

Regulating gene expression and protein function

In order to maintain cooperation and homeostasis, multicellular organisms need to regulate both gene expression and protein function. When biologists say that a process is **regulated,** it means that there are natural mechanisms that cause either more or less of the process to occur in a given time period, based on some sort of informational input. All cells regulate their activities by controlling how much of a given gene product (usually a

protein, but sometimes an RNA) is present at a given time and/or whether or not that gene product is active at that time. In procaryotic cells, both steps of gene expression, transcription of DNA to mRNA and translation of mRNA to protein, occur in one compartment, and translation can actually begin before transcription has finished. Therefore, there primary way that procaryotes regulate gene expression is by controlling when and how rapidly transcription occurs. Eucaryotic cells have many more options for regulation because the two steps in gene expression are compartmentalized, with transcription taking place in the nucleus, where the DNA is located, and translation happening in the cytoplasm, where ribosomes are found.

Procaryotic and euaryotic cells regulate transcription in the same basic way, although the details are more complicated in eucaryotes. The regulation of transcription is summarized in Figure 10.2. Transcription begins when an enzyme called RNA polymerase binds to a special DNA sequence known as a **promoter** sequence (see Figure 10.2A). Each eucaryotic gene has its own promoter, whereas, in procaryotes, multiple genes that function in the same process are often organized next to each other in the genome and transcribed from a single promoter. Proteins needed only in small amounts are expressed by genes present as single copies in the genome and controlled by promoters that only weakly bind RNA polymerase. When the polymerase enzyme falls off the promoter, transcription stops. A protein needed in very large amounts is often coded for by multiple copies of the same gene, controlled by a promoter that strongly binds the polymerase enzyme. When the polymerase stays attached to the promoter longer, more copies of messenger RNA (mRNA) are transcribed.

On the DNA near a promoter (or, in eucaryotes, sometimes as many as thousands of nucleotides away from the promoter), there are regulatory gene sequences that can either activate or repress transcription by changing how RNA polymerase binds to the promoter. If an activator binds to one of these regulatory sequences, RNA polymerase binds more strongly, and more copies of mRNA are transcribed from that gene (see Figure 10.2B). On the other hand, if a repressor binds, RNA polymerase is blocked from the promoter, and transcription is halted (see Figure 10.2C).

Activators and repressors themselves are also regulated (see Figure 10.3). There are many possible ways in which this can occur. For example, an activator might require a signaling molecule to bind to it, causing a shape change that allows the activator to bind to DNA and increase transcription (Figure 10.3A); alternatively, an enzyme might add a chemical modification that prevents an activator from binding DNA, thus decreasing the rate of transcription (Figure 10.3B). Similarly, a inhibitory signaling molecule might bind to a repressor and change its shape, thus preventing the repressor from binding to DNA and allowing transcription to continue (Figure 10.3C), or an enzyme might add a chemical modification to a repressor that in turn allows it to bind DNA, thus repressing transcription (10.3D). Note that all types of regulation are possible: it is just as likely that an activator signal binding or chemical modification can either activate or inactivate any type of regulatory protein. Furthermore, mutations that alter the structures of activator or repressor proteins themselves can render these regulators non-functional or, in rarer cases, hyperactive. We will see that such mutations in proteins that regulate transcription of genes related to cell division or cell death can promote uncontrolled cell division, contributing to cancer.

We have just seen the general strategy by which transcription is regulated (Figures 10.2 and 10.3). As previously mentioned, this is the most prevalent type of regulation in procaryotic cells (Figure 10.4, step 1), but, eucaryotic cells often also regulate gene expression at several additional steps after transcription (Figure 10.4, steps 2 through 6). As noted above, in eucaryotic cells, the mRNA synthesized during transcription must leave the nucleus before it can be translated into proteins because the ribosomes are in the cytoplasm. Many mRNAs must be chemically modified before they can leave the nucleus (Figure 10.4, step 2). One example of an mRNA modification is the removal of the non-coding regions (introns) by the process of RNA splicing, as we saw in Chapter 4. This is crucial, since an unspliced mRNA does not have the correct sequence to code for a functional protein. The two ends of the mRNA are also modified, with a chemical "cap" (a modified G nucleotide, 7mG) being added to one end and a long series of "A" nucleotides being added to the other, forming a poly-A "tail." These end modifications stimulate efficient translation once an mRNA reaches the cytoplasm. The fact that mRNAs cannot be "exported" from the nucleus to the cytoplasm until splicing and end-modifications have occurred is a regulatory mechanism that ensures that only mature mRNAs that are actually ready for translation can reach the translation machinery.

Once the processed mRNA is in the cytoplasm, the rate of translation can also be regulated: mRNAs contain sequences that bind to proteins that can influence how often a ribosome initiates translation on that particular mRNA. Rapid translation produces more copies of a protein, while slow translation produces less (Figure 10.4, step 3).

Further regulation often occurs after translation. The amino acid sequence first produced in translation may not be the sequence of the final protein. Some amino acids

may need to be removed, or chemical groups may need to be added to certain amino acids before the protein can fold properly into its functional shape. Without this processing, functional proteins are not produced (Figure 10.4, step 4).

Finally, as introduced previously in the context of transcriptional regulators, the activity of any mature protein can be regulated in several ways. Other molecules, called effector molecules, can bind to a protein and change its shape, either slowing down or speeding up the activity of the protein; also, chemical modifications can also be added to and removed from a folded protein to regulate its activity (Figure 10.4, step 5). Lastly, a protein can be targeted for destruction at certain times but not at others, causing that protein to accumulate in the cell only when it is needed (Figure 10.4, step 6). The last regulatory mechanism, regulated protein destruction, is especially important for regulation of the cell division cycle.

Cell signaling

Maintaining cooperation and homeostasis in a multicellular organism requires communication, or transfer of signals, among the various cells. Cells receive many different signals from their surroundings that are transmitted across the cell membrane to cause changes in their behavior, their shape, and their internal composition. One crucial process that is regulated by external signals is cell division. Normal cells do not divide unless they receive signals to do so, whereas cancer cells ignore this requirement. Before considering how external signals regulate normal cell division and what goes wrong in cancer, we will first describe the general mechanisms by which cells receive and respond to signals.

Most signals are small molecules released (secreted) by other cells. These signals may affect other cells in the same area, in a process called **paracrine** signaling (Figure 10.5A), or they may enter the bloodstream to travel to other parts of the body and affect cells at a distance, which is known as **endocrine** signaling (Figure 10.5B). Endocrine signals are called 'hormones' (Chapter 8). Signals in one class, the steroid hormones (including the sex hormones testosterone and estrogen) are able to cross the cell membrane and cause immediate changes in gene expression inside the cells (Figure 10.6A). In contrast, most other signals are blocked from entry into cells because of their chemical character. Therefore, these signals must cause changes from outside the cell. How do they do this? The multistep process of communication across the cell membrane, shown in Figure 10.6B, is called 'signal transduction'. Because many steps are involved, with each leading to the next, such a signaling pathway is often called a 'signaling cascade'.

In the first step of signal transduction, the signal molecule binds to the extracellular (outside) portion of a specific receptor protein (Figure 10.6B, step 1). The term *specific* means that a given receptor can bind to only one particular type of molecule: the signal and its receptor match each other in terms of their shape and their ability to form chemical bonding interactions. Binding of a signal to the extracellular portion of its specific receptor changes the shape of the intracellular portion, the part of the receptor molecule that is inside the cell. This allows the intracellular part of the receptor to interact with other signaling proteins and, in turn, to cause changes in those proteins as well, thus propagating the signal through the multiple steps of a signaling cascade (Figure 10.6B, step 2). Some of the intracellular proteins that are altered as a result of a cell-surface receptor being 'activated' (bound by a signal) are enzymes. Once activated, these enzymes can catalyze chemical reactions that result in the production of internal signaling molecules called

second messengers (Figure 10.6B, step 3). These second messengers go on to transmit the original signal to still other proteins, some of which will cause changes in the cell that constitute the end result of the multistep signaling pathway (Figure 10.6B, step 4). An external signal that carries the message for a cell to divide is typically called a **growth factor**. When a growth factor activates its specific cell-surface receptor, it triggers a signaling cascade, as was just described, that ultimately results in the cell initiating a sequence of events that lead to its division into two cells. The growth factor itself remains outside the cell, but *information* is transmitted across the cellular membrane and into the cell nucleus, stimulating cell division.

As previously mentioned, the defining feature of cancer is too much cell division. Therefore, it should be apparent that either too much of a growth factor or excessive activity of a growth factor receptor or other members of the signaling cascade could contribute to cancer. In some cases, cancer cells themselves begin to produce their own growth factors and then respond to them, eliminating the need for neighboring cells to secrete the signaling molecules and increasing the local concentration of the signals. One signal, epidermal growth factor (EGF), is commonly overproduced in this way by a wide range of different types of cancer cells, and, similarly, an intracellular signaling protein called Ras, that is part of many growth factor signaling cascades, is hyperactive in about 25% of all human cancers. Furthermore, too many copies of a cell-surface growth factor receptor called HER2 are present in certain types of breast cancer. As we will see later, knowing the specific signaling defects in cancer cells has allowed scientists to develop cancer drugs that target those particular defects.

THOUGHT QUESTIONS

1. When the weight of some material in solution remains the same, how do changes in cell volume influence the concentration of the material?
2. What would an organism be like if all of its cells were the same?
3. Many animals, including insects, fishes, amphibians, and reptiles, do not maintain a constant internal temperature but allow their internal temperature to change with the external temperature. Are these animals in homeostasis? Why or why not? (Many of these animals can regulate their temperature behaviorally by moving to different locations.)
4. How could multicellularity in animals or plants have evolved through natural selection?
5. Why do eucaryotic cells have so many more ways or regulating gene expression and protein function, compared to procaryotic cells?

CELL DIVISION IS CLOSELY REGULATED IN NORMAL CELLS

As we just discussed, cell division (or production of more cells, often called cell *proliferation*) is one cellular process that is regulated by signals from outside the cell. In Chapter 2, we learned about mitosis, which is the division of a cell's nucleus and the genetic material contained in it. Mitosis is one step of a larger process of cell division called the **cell cycle**. Scientists have found that the molecular signals that regulate cell division and the cell cycle are remarkably similar in highly diverse eucaryotic organisms,

from fungi to humans. (Note that this similarity does not extend to procaryotes, and is thus shared only by cells with nuclei.) Just as abnormal growth factor signaling can lead to cancer, defects in the machinery that regulates the actual process of cell division are also seen in tumor cells, and, in fact, studying cancer cells has taught scientists a lot about the normal features of cell cycle regulation.

The cell cycle

Normal cells only grow a small fraction of the time. They continually make new proteins and other cellular chemicals to replace ones that have been used or damaged, but most of the time they do not increase in size. When cells do grow, they soon reach the size at which their surface area-to-volume ratio makes them inefficient. Instead of becoming more inefficient, the cells divide. When we talk about how fast cells grow, we usually mean how frequently they divide, not how fast they enlarge.

The series of phases that make up the eucaryotic cell cycle is shown in Figure 10.7. The cell cycle begins with a phase called G_1 in which protein synthesis is increased. Then, if the cell receives signals, it enters the synthesis, or S, phase, marked by DNA synthesis and the replication (copying through complementary base pairing) of both DNA strands (see Figure 2.15, p. 000). When DNA synthesis is complete, the cell enters the G_2 phase in which preparations are made for mitosis. Mitosis itself (see Chapter 2, pp. 000) constitutes M phase, at the end of the cell cycle, when the duplicated DNA is evenly split between two nuclei. Division of the cytoplasm, or **cytokinesis**, usually follows close on the heels of M phase, yielding two so-called daughter cells.

After cytokinesis, one or both of the resulting daughter cells can re-enter the cell cycle and divide again. Most of the time, however, both daughter cellsenter into a resting

stage, called G_0, that constitutes a pause between cell cycles. During the resting stage, other cellular metabolic processes proceed, but the cell does not re-enter the cell cycle to divide again unless it is signaled to do so. The duration of the cell cycle (G_1 through M) is fairly constant within a species, but the duration of G_0 varies greatly. For single-celled eucaryotic organisms, such as yeast, which have been key model organisms for studying the cell cycle, the length of time in the resting stage depends on the availability of nutrients. The length of G_0 in multicellular organisms varies with the developmental stage. When an animal or plant is developing as an embryo, the rate of increase in the number of cells can be very rapid, and cells spend little or no time in G_0. In most species, most types of cells spend more time in G_0 once adulthood is reached and will only re-enter the cell cycle when they receive an external signal to do so. For example, tissue damage might cause release of a signal that triggers cell division in neighboring cells within the tissue to repair the damage. Replacement cells are obviously needed if there has been an injury, but even in uninjured tissues some cells die and others divide to replace them, so there is typically a low baseline rate of cell division. This rate depends on the tissue: in skin, where surface cells slough off every day, the baseline rate of division is much higher than in an organ like the adult heart or brain.

Regulation of cell division

The cell cycle (and thus cell division) is a tightly regulated process in all types of eucaryotic organisms, both single-celled and multicellular. In order for two viable daughter cells to be produced, the stages of the cell cycle and the events of mitosis must occur in the correct order, and each step must be completed properly before the next step begins. Consider, for example, what would happen if a cell did not go through G_1 (the

growth period) before it divided: multiple rounds of omitting G_1 would lead to smaller and smaller cells, eventually leading to a non-viable size. Alternatively, imagine what would happen if mitosis began before DNA synthesis was complete, or if anaphase of mitosis (when duplicated chromosomes separate from each other) began before all of the chromosomes were attached to the cytoskeletal filaments of the mitotic spindle: either case would result in daughter cells with the wrong sets of genetic material. Because it is so important for the events of the cell cycle to occur in the correct order and at the proper time, regulatory mechanisms called **checkpoints** exist that serve as barriers, or brakes, to block cell progression until all conditions are favorable to proceed to the next step (see Figure 10.7).

The primary regulators of the cell division cycle are **cyclin dependent kinases** (CDKs). A kinase is an enzyme (catalytic protein) that can add phosphate groups to certain amino acids in other proteins, a process called **phosphorylation**. As their name suggests, cyclin dependent kinases require a partner protein called a **cyclin** in order to be active (Figure 10.8A). Cyclins earned their name because scientists studying sea urchin embryos noticed that the amounts of certain proteins increased and decreased in a characteristic manner with each round of cell division: that is, the levels of these proteins *cycled* up and down with each cell division cycle. Different cyclin proteins reach their peak levels at different times in the cell cycle, and it is the activity of the partner CDK that each cyclin activates that triggers progression into the next phase of the cell cycle (Figure 10.8B). For example, when the amount of cyclin D in a cell reaches a peak level, the cyclin D-CDK becomes active and phosphorylates other proteins that eventually cause the cell to enter into S phase. On the other, hand, when cyclin B reaches its peak, the cell enters mitosis (M

phase). Although the CDK proteins are always present in the cell, they are only active when their partner cyclins are also present.

The cell cycle checkpoints mentioned above influence the timing of the rises and falls in CDK activity. Checkpoint proteins bind to a cyclin-CDK complex and block its enzymatic activity, preventing target proteins from being phosphorylated and activated. This action pauses the cell cycle to allow for problems to be fixed before the cell moves on to the next stage. For example, the cell might pause to allow for all of its chromosomes to become attached to the mitotic spindle or to give DNA repair proteins time to fix DNA damage so that mutations are not passed on to the daughter cells. Thus, if cyclin-CDK complexes are the "gas pedals" of the cell cycle, checkpoint proteins are the "brakes." In relation to cancer, both improper activation of the "gas pedals" and inactivation of the "brakes" contributes to the excessive cell division that produces tumors.

For cell division to take place in normal tissues, a number of conditions must be met: (1) as we have seen, signals must communicate the need for the cell to exit G_0 and re-enter the cell cycle; (2) there must be available space for the new cell in the tissue, and (3) the dividing cell must be attached to a surface (except in the case of blood cells, which naturally exist in liquid suspension). In adult organisms, cells do not divide unless an existing cell has died or been damaged, opening a space for a new cell. Contact with neighboring cells suppresses cell division in normal cells, a condition called **contact inhibition**.

Most cells have an additional requirement called **anchorage dependence:** they divide only when they are attached to a surface. In multicellular organisms, cells attach to complex organic molecules outside the cells (the **extracellular matrix** in some tissues, or the basement membrane in others). When cells are isolated and grown in a laboratory

tissue culture, they attach to the plastic dish or to a coating on the dish (Figure 10.9A), forming a relatively flat layer. A normal cell may be prevented from dividing if it loses its ability to adhere to such an external structure, or if the structure changes in a way that prevents adherence.

All three criteria listed above must be met in order for a normal cell to divide, but cancer cells ignore all of these rules. Thus, in culture, cancer cells have a rounded, unattached appearance and form mounds of cells stacked one on top of each other (Figure 10.9B). Such mounds or heaps are called **tumors** when they form inside organisms.

Limits to cell division

Normal cells of most tissues seem to have a limit to the number of times that they can divide. After a certain number of divisions, the cells stop dividing and enter a terminal "aged" state (eventually dying), even when optimal conditions exist. In the 1960s, Leonard Hayflick discovered that normal cells would only divide about 40-60 times before ceasing division in a process called senescence. This number of cell divisions came to be called the "Hayflick limit" and spurred further research in the field of cell aging, including the search for a biological clock of some sort that keeps track of the number of cell divisions.

In 2009, a trio of American scientists, Elizabeth Blackburn, Carol Greider, and Jack Szostak, were awarded a Nobel Prize for their studies of the molecular nature of this cellular lifespan clock. They and others found that, in most eukaryotic cells, structures at the end of chromosomes called **telomeres** shorten with each round of cell division; once a cell's telomeres reach a threshold level of shortness, the cell no longer divides. Telomeres consist of thousands of repeats of a short DNA sequence (TTAGGG in humans). This

repeat-containing DNA segment folds back on itself and associates with a number of proteins to form a sort of cap that protects the chromosome ends from damage (Fig. 10.10A); to describe this protective function, telomeres are often compared to the plastic caps on the end of shoelaces that keep them from fraying. During DNA replication (see Ch. 2), one strand of a DNA molecule cannot be copied all the way to the end because of limitations of the replication enzymes. This is what causes the telomeres to shorten with each round of cell division. An enzyme called **telomerase** is capable of extending telomere ends to counteract this shortening, thus preventing cell "aging" (see Figure 10.10B). Only cells in developing embryos and small populations of stem cells in organisms after birth normally express telomerase and thus avoid the aging process.

Cancer cells also escape the aging process, becoming "**immortal**," which means that they will go on proliferating indefinitely. Reactivation of telomerase expression is a major way that cancer cells achieve immortality. The immortal nature of cancer cells is reflected in the title of the book "The Immortal Life of Henrietta Lacks" by Rebecca Skloot, which tells the story of an African American woman who died from an especially aggressive form of cervical cancer in 1951. Cells from the tumor that was surgically removed from Ms. Lacks, named HeLa cells, have been proliferating in laboratories all over the world since her death. In the 1950s, patient consent regulations were not in place, so it was not until the 1970s that Ms. Lacks' family learned of the fate of their deceased relative's cells, raising significant ethical issues.

Programmed cell death

In addition to the production of new cells being tightly regulated, cell death can also be a regulated process. Maintaining tissue homeostasis and a healthy organism requires the proper balance of cell division and cell death. One type of cell death, **necrosis**, occurs when a tissue is physically injured. As described previously, in such a case, signals are released that stimulate division of neighboring cells to replace the damaged cells. However, what if a cell undergoes internal damage, such as damage to its DNA or infection with a virus? In this case, the damaged cell itself can initiate an internal signaling cascade that causes it to die (essentially to 'commit suicide') through a specialized process called **apoptosis**, or 'programmed' cell death. It is called 'programmed' because proteins encoded in the genome initiate the events that lead to cell death, rather than it being caused by outside forces. When a cell dies by necrosis due to physical damage, its membrane usually breaks open, spilling the cellular contents into the tissue and causing inflammation. In contrast, a cell dying by apoptosis essentially collapses inward and does not affect other cells in the tissue. When apoptosis is initiated by signals within the cell, enzymes begin cutting up the DNA and altering the membrane structure of cell so that it shrinks and shrivels up, detaching from other cells in the tissue. Specialized cells of the immune system, called **phagocytes** (literally 'cell eaters') clean up the remains of cells that have died by apoptosis, internalizing the contents by wrapping a membrane around the debris and bringing the contents into internal compartments called 'vacuoles,' where the remains are then digested. Figure 10.11 contrasts cell death by necrosis vs. apoptosis.

Apoptosis is an essential process in normal tissues. During mammalian embryonic development, the digits of the hands and feet are initially webbed, and apoptosis removes the intervening cells, shaping the fingers and toes. In the developing brain, more neurons are formed than are ultimately needed, and a process called neural pruning eliminates the

extra brain cells via apoptosis. Interestingly, defects in this process have been associated with autism. As mentioned previously, cells infected with viruses can be triggered to undergo apoptosis, usually when stimulated by an immune cell that can recognize the infected cell based on its expression of 'foreign' (e.g., virus-induced) proteins; thus, apoptosis is a natural defense mechanism.

Lastly, apoptosis represents a powerful barrier to cells becoming cancerous. As we will learn later in this chapter, cancer occurs when a cell sustains multiple changes (mutations) to its DNA. Thus, DNA damage can promote cancer. If cells with damaged DNA die by apoptosis rather than continuing to proliferate with accumulated mutations, this risk is minimized. For this reason, defects in the process of apoptosis (e.g. due to mutations in genes that encode proteins that function in apoptosis) will actually promote cancer.

THOUGHT QUESTIONS

1. Why is it an adaptive advantage for an organism to have certain proteins such as insulin produced by just one type of cell rather than produced by all cells throughout the organism?
2. Does a cell's DNA determine what type of cell it becomes? What other factors, if any, are involved?
3. In what way might research on cancer also lead to a better understanding of the aging process? How might it further our understanding of birth defects?

DEVELOPMENT BEGINS WITH UNDIFFERENTIATED CELLS CALLED EMBRYONIC STEM CELLS

A fertilized egg (zygote) is a single cell whose cellular descendants are capable of forming all the different cell types within the body. This capability is referred to as "totipotency." The long list of possible fates for **totipotent** cells includes skin cells, muscle cells, glandular cells, bone cells, liver cells, and so forth. In placental mammals, like humans, totipotent cells can also give rise to the placenta, which is required to nourish the embryo (see Chapter 8).

Within a developing multicellular organism, as cells proliferate, they also become different. **Differentiation** takes place in steps. At each successive cell division and differentiation, the range of possible future identities for that cell lineage is progressively narrowed, until it narrows to a single cell type. Once a cell lineage has differentiated in the direction of muscle cells, for example, all progeny cells are committed to being muscle cells. A few groups of cells, however, remain undifferentiated, and these are called **stem cells.**

Beyond the first few divisions of a human embryo, cells can no longer give rise to the placenta (which is an organ that is separate from the embryo; see Chapter 8), but can give rise to all embryonic cell types. These **embryonic stem cells** are referred to as **pluripotent**. At still later stages of development and in adult organisms, populations of stem cells remain, but their capabilities are more restricted than those of embryonic stem cells; these stem cells are called **multipotent** and can only develop into certain subsets of cells. For example, hematopoietic stem cells exist in the bone marrow throughout a person's life; they are capable of generating all types of blood cells, but not the cells of other tissues.

Like cell division, differentiation is tightly regulated by the control of gene expression. The light-sensing rod cells in the eyes, the smooth muscle cells in the

intestines, and the insulin-secreting beta-cells in the pancreas are very different in both structure and function, yet they contain the same set of genes; the difference is that these three cell types express different *subsets* of that same genome (see Figure 10.12). Thus, differentiation is largely about turning off certain genes while keeping others on or activating some genes at a higher level. Much of what we know about cell differentiation has come from embryology, the study of the development of an organism from a zygote, and from the study of stem cells. Studies of normal differentiation have also taught us much about the abnormal conditions that exist in cancer. As it turns out, cancer cells share many similarities with stem cells; in other words, the path by which a normal cell progresses to become a cancer cell partly involves a process of "de-differentiation."

Cellular differentiation and tissue formation

As described above, the list of possible types that a cell may become is called its **potentiality**. The zygote has maximum potentiality because it gives rise to all cell types. The potentiality of cells has been investigated by transplanting cells from the embryos of experimental animals. Up until the eight-cell stage in a mammalian embryo, each of the cells—called **embryonic stem cells**—could develop into a complete organism. As the cells continue to divide, they first form a hollow ball called a **blastula** (see Figure 6.16, p, 000). Cells then begin to differentiate and form tissue layers (ectoderm, mesoderm, and endoderm), a process that begins at a landmark called the dorsal lip. The differentiation process takes place with the help of **organizers,** secretions that promote differentiation (Figure 10.13). As a result, cells in each layer are restricted to becoming certain types of tissues. At the stage where the embryo begins to form differentiated cell layers, it is called a **gastrula**. These early steps in embryonic development are shown in Figure 10.14A.

A group of cells removed from the ectodermal layer of the embryo at the gastrula stage and transplanted elsewhere on the same embryo can form various tissue types, but only types that are ectodermal. Their potentiality is still quite broad, but not as broad as that of the zygote. As shown in Figure 10.14B, each of the gastrula cell layers is destined to become certain types of cells. Cells transplanted at a later time have a further narrowed potentiality. An ectodermal cell is restricted to one of two groups, epidermal cells or cells of the nervous system (see Figure 10.14B). Finally, at a still later stage, the fate of these cells is completely **determined**, so that eye lens cells, for example, can form only eye lens tissue (see Figure 10.14B).

We have seen that, as a cell becomes differentiated, its potentiality becomes restricted, and that the cell somehow seems to 'know' what these restrictions are. In the 1960s, a British cell biologist named John Gurdon asked the question of whether these restricted potentialities result from a loss of genes as a cell differentiates. To do this, he exposed some frog eggs (phylum Chordata, class Amphibia) to ultraviolet radiation. Because ultraviolet radiation is absorbed by DNA, a sufficient dose of ultraviolet can be used to destroy the egg nucleus without damaging its cytoplasm. Gurdon then carefully inserted into each of these eggs the nucleus of a differentiated cell type, such as a skin cell. The resulting cell thus had cytoplasm from an egg but a nucleus from a differentiated cell. Gurdon was able to show that this cell, like a zygote, was able to produce an entire tadpole (Figure 10.15). This landmark experiment proved that the various types of cells of the body do not differ in the genes that they contain. Each nucleus usually keeps its full genome, and it is thus not the loss of genes that restricts potentiality, but rather which genes are expressed and which are not expressed (silenced) in a given cell type (see Figure 10.12).

Spatial distribution of signaling molecules drives differentiation. As cells in a developing organism lose potentiality and become more specialized, how do they "know" which genes to turn on and off? The answer lies in a complicated pattern of spatial signals to which different cells are exposed. At each round of cell division during development, a given cell receives chemical signals that determine whether it will differentiate and what type of cell it will form. From the very beginning, at the single-cell stage (zygote), some type of asymmetry exists that drives a cascade of differentiation, like a single domino setting off a branching chain reaction. In some organisms, the unfertilized egg is already asymmetrical due to the process by which it matures. For example, in fruit flies (Drosophilidae), cells called nurse cells surround one side of a developing egg in a structure called an egg chamber; these nurse cells (and other cells called follicle cells) transport certain proteins and mRNAs (which will later give rise to proteins via translation) into specific domains within the egg that will allow the two cells produced in the very first cell division to be different from one another (see Figure 10.16A). These distinct domains within the egg define *axes of polarity* that will distinguish the head (anterior) from the tail (posterior) and the front (ventral side) from the back (dorsal side) in the developing embryo. In some other organisms, such as frogs, a different mechanism results in the same basic outcome: internal signals (second messengers) are released inside the cell at the point where the sperm passes through the egg's membrane upon fertilization, and the presence of these signals on one side of the resulting zygote but not the other again creates an axis of polarity that specifies the ventral vs. the dorsal side of the embryo (see Figure 10.16B). The mechanisms that generate polarity in mammalian

embryos are more complicated, but the same requirement for asymmetry must be met at a very early stage in development.

Once an initial state of asymmetry is established in the zygote, it sets up a chain reaction like the falling dominoes that leads to the cascade of differentiation. The specific set of signals received by each cell as rounds of cell division proceed turn on certain genes and silence others through signal transduction pathways like those illustrated in Figure 10.6. With many such rounds of signaling as cells divide, very different patterns of gene expression are established in the cells that go on to become distinct tissues. These disparate patterns of gene expression are significant for cancer: while all types of cancer arise from excessive cell proliferation, and mutations in certain genes are shared among many different types of cancers, the specific type of cell that begins to proliferate out of control has a distinct pattern of gene expression that will influence the behavior of the cancer cells, including how they may respond to different treatments. Therefore, understanding differences between cells and identifying the specific type of cell that initiates a tumor (the tumor **cell of origin**), is important to determine the best treatment plan for a particular cancer.

In the development of normal tissues, cells often do not originate in their end location; tissue formation relies on many migrating cells that travel through the organism until they find their 'proper' location, where they adhere and join the tissue. These cells are generally partly differentiated at the time of their migration and become fully differentiated when exposed to growth factors in their new microenvironment. Such molecular 'addresses' can take the form of membrane receptors that bind specifically to molecules expressed only on certain types of tissues. Abnormalities in cellular adhesion

and cell migration are pertinent to the spread of cancer to other tissues, a process called **metastasis**, which is responsible for 90% of all deaths from cancer (see Figure 10.22). During metastasis, tumor cells stop making certain cell-surface proteins that normally attach them tightly to other cells in their tissue of origin and begin to express protein that give them the ability to move away from their starting site in the body. These migrating tumor cells can enter the blood and lymphatic vessels and move through the circulatory system; at some point, they may exit the circulation and make attachments to a new tissue, where they can eventually form a secondary, or metastatic, tumor. Cancer that has metastasized is referred to as stage IV, the most advanced stage of cancer. At this stage, it is much more difficult to treat than at earlier, localized stages, since there are tumor cells in circulation that can continue to form new tumors at different sites throughout the body.

Stem cells

Stem cells are cells that are: (1) in an undifferentiated state; (2) able to differentiate into more committed cell types; and (3) able to renew themselves by cell division. They can be derived either from embryonic cells or from adult tissue.

The discovery that stem cells can be induced to grow into many different cell types in tissue culture has led to the idea that they may someday be used therapeutically when replacement cells are needed. The idea is that a person's own stem cells might be removed, isolated, grown in tissue culture, and stimulated with the proper signaling molecules to cause them to differentiate into the needed replacement cell types as a cure for certain diseases. For example, insulin-producing beta cells might be generated as a treatment for diabetes (see Chapter 9), or new neurons might be produced to treat

Parkinson's disease (see Chapter 13). Stem cells may hold the key to a new era of regenerative medicine.

Embryonic stem cells. We have just seen how embryonic stem cells give rise to all of the tissues and organs of a new organism. Embryonic stem cells were first isolated in the 1970s from early mouse embryos called blastocysts. In 1998, this process was copied in the laboratory using human blastocysts. A blastocyst is a hollow, ball-shaped cluster of about 60 to 200 mammalian cells from an early stage in development—the stage when implantation to the uterine wall takes place (see Figure 8.12). Blastocysts maintained in culture in the laboratory for six months or more and without showing any signs of differentiating are referred to as 'embryonic stem cell lines.' They can be stored frozen in liquid nitrogen for use at a later date. Under the influence of a growth medium containing different signaling molecules functioning as 'organizers', they can be induced to differentiate into various types of cells.

Research is under way to see if embryonic stem cells transplanted into adult organisms can successfully replace damaged or degenerated tissues. Success has been demonstrated in mice where mouse embryonic stem cells were directed to differentiate *in vitro* into the neurons normally defective in Parkinson's disease. When transferred to the mouse brain, the stem cell-derived neurons improved motor function. Human embryonic stem cells have similarly been induced to form neurons in the laboratory, but no clinical trials have yet attempted to transplant these into human patients, in part due to concerns that residual, undifferentiated cells could form tumors. Several clinical trials have been performed in which human fetal tissue that presumably contained embryonic stem cells was transplanted into human Parkinson’s patients. One Swedish study conducted in the

1990s reported remarkable benefits to some patients, but these results were questioned when two NIH-funded studies in the U.S. failed to replicate these results and also reported some significant, negative side-effects. Different transplantation procedures may possibly explain these different results; a new European Union-funded study is currently investigating this possibility. Although progress has been slow, the potential benefits of stem cell therapy continue to lend great hope to the human sufferers of many diseases, including Parkinson's.

Despite its tantalizing therapeutic potential, human embryonic stem-cell research is a highly controversial area because it involves the destruction of a human embryo. Virtually all of the existing human embryonic stem cell lines were derived from excess embryos from *in vitro* fertilization clinics. As we saw in Chapter 8, infertile couples can sometimes successfully conceive a child when their eggs and sperm are mixed in a dish, incubated in the laboratory, and the embryo implanted into the woman's uterus. In all cases, more embryos are created than are ever implanted. The extra embryos sometimes remain frozen and, with the couple's informed consent, can be used for research; in other cases they are discarded. In 2001, president George W. Bush authorized the use of 64 already existing human embryonic stem cell lines for further research, but declared that no U.S. federal funding could go toward developing any new lines. Many scientists were concerned by this restriction because the existing cell lines were not well characterized, and they feared that banning the development of additional stem cell lines could hamper progress on potentially life-saving and life-transforming regenerative medicine techniques. While this ban was in place, development of new embryonic stem cell lines continued in other countries and in U.S. labs supported by non-federal funding sources. In a bold move, the state of California passed legislation authorizing a $3 billion bond measure to fund

embryonic stem cell research in 2004. Just five years later, the Bush-era ban was overturned by President Barack Obama as one of the earliest actions of his presidency, restoring U.S. federal funding for a broad spectrum of embryonic stem cell investigations. New restrictions, imposed in 2019, limit the use of human fetal tissue from elective abortions in federally funded research.

Most progress toward human therapy has remained incremental, but a milestone advance was reported in October 2014: embryonic stem cell transplants into the eyes of legally blind patients suffering from macular degeneration were shown to partially restore their sight. Meanwhile, Doug Melton, a Harvard developmental biologist, decided to direct all of his research efforts toward a cure for diabetes when both of his children were diagnosed with Type 1 diabetes (see Chapter 9). After years of work, Dr. Melton's team has succeeded in coaxing embryonic stem cells to become pancreatic β-cells with the right combination of signaling molecules, but they are still working out the best way to deliver the cells to patients and avoid their destruction by the immune system, which is a central feature of the pathology of diabetes.

Adult stem cells. Stem cells do not only exist in embryos but are also found in some adult tissues and are important for normal tissue function. Very few types of cells are permanent: some cells die and must be replaced by cell division and differentiation throughout the lifetime of the organism (Table 10.1). **Adult stem cells** are partly differentiated cells present in some tissues of adult organisms whose normal function is to divide and replace cells that are lost through routine physiological processes. These stem cells are located in several areas where cells are continually being lost: skin, gut lining, uterine cervix, bone marrow, and many glands. When a stem cell divides normally, one

daughter cell usually remains undifferentiated as a stem cell and the other differentiates and is therefore less likely to continue dividing (Figure 10.17). These processes are tightly coordinated in adult organisms, so that cells lost from specific tissues are replaced by the correct number and type of cells.

One of the first locations in the body in which adult stem cells were identified was the bone marrow, the porous interior of the major bones. Throughout a person's life, bone marrow stem cells give rise to both red blood cells, which carry oxygen (see Chapter 9), and white blood cells, which are part of the immune system (see Chapter 14). New blood cells are produced to replace blood cells that have been lost through injury or have reached the end of their lifespan. Both the types and numbers of new cells produced are tightly regulated. If regulation of this process breaks down, the result can be leukemia or myeloma, which are blood cell cancers that develop in the bone marrow. Because the bone marrow contains stem cells, bone marrow transplants can reestablish blood cells and a complete immune system in individuals lacking them. Transplantation of bone marrow from one person to another requires the exact matching of a set of inherited cell-surface proteins; otherwise, the recipient's body may reject the transplanted cells, which can lead to a life-threating complication called graft-versus-host disease. In some cases, a person's own bone marrow cells can be removed and later transplanted back, for example after the person's own immune cells have been killed by cancer therapy. This works very well because a person's own cells will not trigger graft-versus-host disease.

Although adult stem cells are not as undifferentiated as embryonic stem cells, they are still capable of forming many types of cells in addition to the type found in the tissue from which they were derived. In tissue culture, bone marrow stem cells can develop into other types of cells, including muscle or nerves, given the right set of signaling molecules.

Transplants of these adult stem cells are being investigated for their future potential to regenerate other types of tissue in addition to blood cells. It appears that, in tissue culture, it may be possible to bring adult stem cells back to full potentiality, that is, the ability to differentiate into any and all kinds of cells. This is not yet known for certain, however.

Induced pluripotent stem cells. Because of the ethical issues regarding ESCs and the possible limitations of adult stem cells (including difficulties obtaining them from some adult tissues), scientists have sought ways to generate cells with stem-like properties. In 2006, a Japanese biologist Shinya Yamanaka, succeeded in “reprogramming” skin cells from adult mice, returning the cells to a pluripotent state by engineering them to express four transcription activator proteins. The following year, Yamanaka and colleagues repeated the accomplishment using human skin cells. The reprogrammed cells are called 'induced pluripotent stem cells'. Like embryonic stem cells, if given the proper signals, these induced stem cells can develop into a wide range of cell types. A major benefit of this strategy for regenerative medicine is that, theoretically, any cell type needed could be generated from a patient’s own skin cells, which can be easily obtained and not provoke any immune reactions. In 2010, Yamanaka and British biologist John Gurdon (mentioned earlier in this chapter) were jointly awarded the Nobel Prize in Physiology or Medicine for their discoveries regarding the reversible nature of the differentiation process.

It is not yet possible to know for sure if induced pluripotential stem cells have a potentiality as broad as embryonic stem cells, as the signals required to specify all cell types have not yet been identified. However, in 2009, mice were born from induced pluripotential stem cells using a technique called tetraploid complementation. These embryos had a low rate of implantation in recipient mothers, and some had physical

abnormalities, but some did survive and go on to reproduce, suggesting that the induced pluripotential stem cells were able to give rise to all necessary cell types. Such an experiment would not be considered ethical in humans.

Cloning

The term **cloning** means the asexual production of a group of genetically identical cells or organisms. We saw the term in Chapter 4, where the cloning of asexually reproducing bacterial cells was described in the context of genetic engineering. Here the usage of the term is somewhat different because it is cells or individuals of species that normally reproduce sexually that are being produced asexually. In cloning, there is no genetic recombination, so the genotype of the progeny is identical to the starting genotype.

There are two basic purposes for which cloning may be used. Therapeutic cloning is asexual cell growth whose outcome is the production of cells or tissues that might be used in treating illness, injury or disability. Reproductive cloning is any asexual cell growth whose purpose is the making of a complete individual. The two types of cloning use the same method to initiate the process, but in therapeutic cloning the end point is reached when stem cells are produced, and in reproductive cloning the end point is the birth of a new individual.

Therapeutic cloning. One barrier to the use of embryonic stem cell lines for regenerative medicine is that they themselves express the cell-surface molecules that are the barriers to all types of tissue and organ transplantation. Thus, they could only be transplanted into a recipient who has the same set of these molecules to avoid rejection by the recipient's immune system (see Chapter 14). This is one reason why many scientists disagreed with

the previous U.S. ban on establishing more embryonic stem cell lines. The 60 or so that existed at the time of the ban expressed a very limited sample of these cell-surface molecules that form the barriers to transplantation.

As described above, induced pluripotent stem cells derived from a patient's own skin cells are one potential way to overcome the problem of transplantation barriers; therapeutic cloning is another. In the method used currently, the nucleus of a cell from a patient is transferred into an enucleated egg from a donor. Under certain conditions in the laboratory, the egg will begin to divide, as though it were a fertilized embryo, developing up to the blastocyst stage. At that point, the inner cell mass is removed, and these embryonic stem cells are allowed to divide further in tissue culture. The cell-surface molecules they express will be the same set coded for by the genome that was donated by the patient. These embryonic stem cells have full potentiality for regeneration of cells or tissues needed by the patient, and because their cell-surface molecules will match perfectly, the cells or tissues derived from them will not be rejected when they are transplanted back into the patient.

This procedure is a form of cloning because the embryonic cells produced are genetically identical to the patient. Some researchers prefer the term *nuclear transfer for tissue replacement*, both because it is a more accurate description of the method, and because it may be less controversial than the word 'cloning'.

Reproductive cloning. Reproductive cloning uses the same kind of nuclear transfer as therapeutic cloning; however instead of the cells from the blastocyst being allowed to grow *in vitro* in tissue culture, the blastocyst is implanted into the uterus of a surrogate mother, where it will continue developing into a complete new individual with the genome

of the individual that donated the nucleus. The resulting individual is therefore a clone of the nuclear donor. Gurdon's experiment, in which a nucleus from a differentiated frog cell replaced the nucleus of a frog egg, was the first example of reproductive cloning.

Gurdon successfully cloned frogs in the 1960s, but this process is much more technically difficult in mammals. In 1997, Scottish researchers announced the birth of 'Dolly' the sheep, the first cloned mammal. They achieved this by incubating differentiated cells from a sheep's udder in conditions that relaxed the differentiation signals on their DNA. The nucleus from one of these cells was then put into an egg cell whose own nucleus had been destroyed. The resulting egg cell was allowed to divide in tissue culture to the blastocyst stage and then was implanted in a surrogate mother sheep who subsequently gave birth to Dolly, who was genetically identical to the animal that donated the nucleus.

Although Dolly appeared normal at birth and developed normally, she seemed to age prematurely. She died in February of 2003 at the age of six, although the normal life expectancy for a sheep is as much as 16 years. Even before her birth, scientists had hypothesized that Dolly might age prematurely because the donor nucleus likely had chromosomes with shortened telomeres, typical of the donor's age, so that Dolly's cells had already used up some of their limit of cell divisions.

Subsequent to the birth of Dolly, a number of other mammalian species have been cloned by the same type of nuclear transfer procedure. These include cow, cat, deer, dog, horse, mule, ox, rabbit, and rat. Since 2006, a company in South Korea has produced cloned dogs at the price of $100,000 for people who cannot bear the thought of losing a beloved pet. Interestingly, despite having identical genomes, cloned individuals do not

always look or behave alike, which demonstrates the role of the environment and of chance in determining an individual's characteristics.

Ethical and scientific questions

The ethical questions raised by stem cell research center mostly around the derivation of embryonic stem cells. Among the opponents of stem cell research are religious conservatives who argue that embryos should be given the moral status of a human being and not be destroyed even in the interest of scientific research. Other people hold that a new genome is not yet a unique individual. Biological evidence in support of this view includes the fact that an embryo may split to become twins, and thus become two individuals. Also, within the first two weeks in the uterus, two embryos may fuse and result ultimately in one individual. Such uncommon fusions are called chimeras, and the resultant individual will have some cells with one genome and some with the other. Human chimeras can have cells that are genetically two different skin colors or two different sexes. In this view, humanness develops later, making the use of a blastocyst ethically acceptable. (See Chapter 8 pp. 000-000, where we discuss the concept of what qualifies as humanness.) Proponents of stem cell research share this view, including many scientists, patients and people involved in the biotechnology industry.

Interestingly, healing and the promotion of health are part of all religious traditions. Therefore, most people see the use of adult stem cells for therapy as a potential good. We do not yet know whether adult stem cells will have the full potentiality of embryonic stem cells, so many people are reluctant to restrict the research only to adult stem cells. Some, though certainly not all, people of all religions have accepted *in vitro* fertilization as a method of infertility treatment, and people embark on such treatments knowing that more

embryos will be created than are used. Ethicists from most major religions have accepted the use of the excess embryos for stem cell research. The question of whether or not it is acceptable to create an embryo expressly for the production of stem cells, either though *in vitro* fertilization or therapeutic cloning, again usually depends on a person's view of whether or not an embryo is considered to have full human status from the point of conception. This varies among religions and religious sects, but several ethicists also oppose therapeutic cloning on the grounds that the embryo is being used "as a means only".

Apart from religious or moral objections, other people urge caution in pursuing stem cell therapies and therapeutic cloning because there are so many still unanswered scientific questions about these processes and whether there might be unintended consequences. For example, some scientists wonder whether transplanted stem cells could become cancerous cells in the recipient. In the next sections, we will examine the characteristics of cancer cells and their similarities to stem cells that make some scientists cautious.

Most people, including most scientists, find the idea of reproductive cloning of humans to be ethically and/or morally repugnant. In 2004, a group led by Woo-Suk Hwang in South Korea published an article claiming to have created a cloned human embryo through nuclear transfer in a test tube. They stopped short of attempting to implant the embryo in a woman's uterus. Eventually, the claim was found to have been falsified, and the paper was retracted. Hwang was largely discredited as a biomedical researcher, but he went on to found the dog cloning company mentioned above. The incident meanwhile raised red flags for many people regarding the creation and enforcement of ethical standards related to reproductive cloning. Currently, there are no laws banning human cloning in the U.S., although several bills have been introduced in Congress. In contrast, a

number of European countries, plus Canada, Japan, Russia, Israel, and others have passed laws banning human reproductive cloning, and some have also outlawed therapeutic cloning as well.

THOUGHT QUESTIONS

1. Should cloning of humans be allowed? Why or why not?
2. Should a human clone and his or her DNA donor be identical just because they have identical DNA? Identical twins share the same genome. Are they identical people?
3. What effect does the cytoplasm have on gene expression? If two identical nuclei were transferred into denucleated eggs from different individuals, would the clones develop differently?

CANCER RESULTS WHEN CELL DIVISION IS UNCONTROLLED

Now that we have seen how signal transduction, cell division, and differentiation are controlled in normal cells, we can examine these processes in cancer cells. Cancer is not just excessive proliferation of otherwise normal cells; cancer cells have escaped from many types of controls that operate in normal cells and exhibit a wide range of abnormal behaviors. Cancers can arise in any tissue whose cells are dividing, and all multicellular organisms can develop cancer. In this section we primarily discuss human cancers, although much of what follows also applies to cancer in other species, and animal cancer

models are crucial for understanding human cancers and developing new ways of treating them.

Properties of cancer cells

Cancer can be described as 'a genetic disease that arises over a person's lifetime'. This is because cancer begins when a *single cell* (the **cell of origin**) accumulates a sufficient number of mutations (DNA changes) in its genome to break down the normal regulatory mechanisms that enforce its 'good behavior' in the tissue (or cell community) of its origin. Thus, development of cancer is an evolutionary process that happens within the population of cells that makes up an individual, instead of a population of individuals. This change from being a normal, 'law-abiding' cell to being a 'rogue' cancer cell is called **transformation**.

Transformed cells grow and divide more rapidly than normal cells, even in the absence of any cell division signals; they also exhibit many other characteristics that differ from those of normal cells (Table 10.2). Cancer cells continue to divide indefinitely, and are therefore called 'immortal.' As in the case of the HeLa cells mentioned earlier, many cancer cell lines have been maintained in tissue culture for decades. In many cases, cancer cells are less differentiated than the cells from which they arose. The membrane transport systems of transformed cells carry nutrient molecules into the cell at a higher rate. In the body, this gives transformed cells a competitive advantage over normal cells. Transformed cells are not inhibited by contact with other cells. In tissue culture, their growth does not stop when they have formed one-cell-thick monolayers, but instead continues, forming piles of cells growing over and on top of each other (see Figure 10.9B,C). Cancer cells grow this way inside organisms, and the growing piles of cells are called **tumors**.

Transformed cells also grow without the need to be attached (see Figure 10.9B); in fact, this is the characteristic that best predicts whether a cell growing in culture will form a tumor if put into an animal. Changes back and forth from attached growth to unattached growth are believed to spread some tumors to new locations.

Cells become transformed when they are dividing; therefore, cells that are terminally differentiated and will never again divide, such as nerve cells in the brain and muscle cells of the heart, cannot become transformed. In contrast, many types of cancers arise from the transformation of stem cells. When a stem cell divides normally, one daughter cell retains the stem cell identity and remains undifferentiated, while the other divides multiple times to generate a population of cells, called 'transit amplifying cells,' that will ultimately differentiate (Figure 10.17). A transformed stem cell, unlike a normal stem cell, divides into two daughter cells that both remain undifferentiated, and each can continue to proliferate. Both stem cells and cancer cells share the property of immortality, or the unlimited potential to divide.

Transit amplifying cells may also be the cells of origin for some cancers if they undergo transformation and thus take on additional features of stem cells such as immortality. Interestingly, not all cells in a tumor are equivalent; a small subset of tumor cells has the potential to continue propagating the cancer or to found new tumors, while the rest of the tumor cells do not. The former cell population is referred to as ‘cancer stem cells.’ It is thought that treatment strategies that specifically eradicate cancer stem cells may be much more effective than those that target cancer cells in general. One concept of experimental therapy for some cancers is to give drugs that promote differentiation to the terminal, non-dividing state. In the other direction, one of the possible problems resulting from stem cell transplants or therapeutic cloning is that the normal transformation-

protective process may not operate in these cells and the cells meant to be therapeutic may divide without control and thus give rise to cancer.

The genetic basis of cancer cells

Some genetic diseases are caused by mutation of a single gene, and a person who inherits two mutated copies of the gene (or sometimes just one, depending on the disease) will be nearly guaranteed to have the disease. An example of such an inherited disease is cystic fibrosis, which is the most common inherited disease among Caucasians. In contrast, the majority of mutations that cause cancer arise in somatic (body) cells during a person's lifetime instead of being passed on from a person's parents. A person can inherit a predisposition to developing cancer, which we will discuss below. However, first, we will consider the types of genes involved in cancer.

The normal growth regulatory genes fall into two categories: genes that encode proteins that promote cell division (**proto-oncogenes**) and genes whose protein products normally inhibit cell division (**tumor suppressor genes**). The proto-oncogenes can be thought of as the 'gas pedal' of a car, and the tumor suppressor genes as the 'brakes.' In addition to directly promoting cell division, proto-oncogenes can also encode proteins that inhibit cell death or differentiation (both processes that counteract cell proliferation), and tumor suppressor genes can encode proteins that promote these processes. Mutations in either type of regulatory gene increase the probability that cancer will arise.

Oncogenes and proto-oncogenes. Proto-oncogenes include normal genes that encode components of the signal transduction pathways that promote cell division, such as growth factors, receptors, and internal signaling proteins, as well as the cell cycle proteins such as

cyclins that respond to those signals (Figure 10.18). When a proto-oncogene becomes mutated such that its gene product is hyperactive (like a stuck gas pedal), it becomes a cancer-promoting **oncogene**. American cell biologists J. Michael Bishop and Harold Varmus received the 1989 Nobel Prize for their discovery of the relationship between proto-oncogenes and oncogenes, leading to a new era in our knowledge of both cell division and cancer.

Oncogenes can differ from proto-oncogenes in any of three basic ways: the timing of their expression, the level of their expression, or the structure of their protein products (see Figure 10.18). For example, the expression of a proto-oncogene might be silenced by a transcription repressor protein unless conditions are right for cell division, whereas the oncogene might be expressed all the time due to a mutation in the repressor binding site. Another type of mutation results in an increased number of copies of a proto-oncogene in the genome (gene amplification), which in turn leads to more protein being expressed and therefore more activity. Mutations to gene regulatory sequences can also result in increased expression. Finally, a mutation that alters the protein product of an oncogene by as little as a single amino acid compared to the protein encoded by the corresponding proto-oncogene can sometimes be enough to hyperactivate the protein, eliminating normal regulatory mechanisms. This is true for the most common mutation in the Ras protein, an internal signaling protein that was one of the first oncogene products discovered and is implicated in about 25% of all human cancers. Whereas the normal Ras protein can turn itself off, the single amino acid change in the oncogenic form of Ras keeps it stuck in the 'on' state, constantly sending the signal for a cell to divide.

While the most common cancer-causing mutation in the *Ras* gene is very subtle (a single letter change in the DNA), other types of mutations can also generate oncogenes.

Genes moved to another chromosome or another part of a chromosome as a result of abnormal DNA crossovers (see Chapter 2, p. 00) can also create oncogenes by placing proto-oncogenes in new locations within the genome. In its new location, a gene may no longer be under the control of the regulatory elements that operated at its previous location in the genome. In rarer circumstances, an abnormal DNA crossover may cause the coding sequences of two genes to fuse, creating a new, hybrid gene that encodes a novel protein with hyperactive growth-promoting properties. This is the case for the *bcr-abl* gene fusion that is implicated in a particular type of leukemia. Interestingly, a drug designed specifically to inhibit the hybrid Bcr-Abl protein was one of the first successes of so-called 'designer' cancer drugs, as discussed later in this chapter.

Tumor suppressor genes. The protein products of tumor suppressor genes normally repress cell division or promote cell death (apoptosis). Mutations that inactivate these genes will promote cell transformation. In most cases, *both* copies of a tumor suppressor gene must be inactivated, resulting in the complete loss of function of that gene. This is different from the case for oncogenes. Because the types of mutations that convert a proto-oncogene to an oncogene result in a gain of function (hyperactivity), a single mutated copy is sufficient to promote cancer. In genetic terms, tumor suppressor mutations are recessive (two mutant copies needed for the effect to be observed), while oncogene mutations are dominant (only one mutant copy needed to affect the outcome) (Figure 10.19).

One crucial tumor suppressor gene, *p53*, is mutated in as many as 55% of human cancers. The p53 protein has been given the nickname 'guardian of the genome.' When something goes wrong inside a cell, such as the DNA being damaged, the normal p53 protein delays cell cycle progression by activating a cell cycle checkpoint (see Figure 10.7); this gives the cell time to try to fix the problem (e.g. to repair the damaged DNA). If the problem is severe enough that the damage cannot be repaired, p53 eventually causes the damaged cell to die via apoptosis. When the *p53* gene is mutated, the altered p53 protein does not halt cell division, and apoptosis is not triggered; therefore, cells with damaged DNA continue to live and divide, passing on their accumulated mutations to their progeny cells. Some of these cells may be so damaged that they will not survive, but others will have acquired additional mutations that promote the evolutionary process of cancer progression. Many cancer therapies rely on damaging cells such that apoptosis is triggered. Because the p53 protein is so important for signaling apoptosis to occur, the loss of p53 function in many cancers represents a challenge for cancer treatment. In fact, p53 is so central to cancer biology that the status of the *p53* gene in a given tumor is often ascertained by DNA sequencing to inform treatment plans.

Accumulation of many mutations

Fortunately for us, transformation of cells requires a combination of mutations in several proto-oncogenes and tumor suppressor genes rather than a single mutation. If single mutations were able to cause cancer, the overall likelihood of developing cancer would be much higher, and the rate of incidence for new cancers would be the same for individuals of every age. That is clearly not the case for most cancers; rather, rates of most cancers increase exponentially with age (Figure 10.20). Based on mathematical modeling of

cancer incidence as a function of age, it is estimated that five or six cancer-promoting mutations must occur *in a single cell* before it becomes transformed to a cancer cell. There are a few exceptions that can be explained by the unique biology of the particular tissues affected. For example, retinoblastoma, a tumor of the eye, occurs most often in children five years and younger, and only very rarely in adults. This is because the cells of the eye's retina become terminally differentiated and stop dividing after this point. Another type of cancer with an unusual age-dependence is testicular cancer. It is most common in males aged 15-40 years. This pattern is likely due to the effects of male sex hormones, such as testosterone, on the progression of this particular cancer.

Most cancers arise in somatic cells (body cells), rather than in gametes (eggs or sperm), and are referred to as **sporadic** rather than heritable cancers. Somatic mutations are passed along to the progeny cells in that individual but are not passed on to the individual's offspring. Some types of cancer do run in families, but an individual does not actually inherit cancer *per se*; rather, they inherit a predisposition to develop cancer. In these hereditary, or familial, cancers, a mutated cancer-causing gene is passed on to sons or daughters through the gametes, and the mutated gene is therefore present in all cells of the body. This means that all cells are one step closer to accumulating the set of mutations needed to become fully transformed and to progress to cancer. Hereditary cancers are usually associated with inheritance of one inactivated copy of a tumor suppressor gene. This increases the likelihood that both copies of that gene will become inactivated in some cell in the body and that the function of that tumor suppressor gene will be lost entirely, thus directing that cell down the pathway of transformation. It is rare that an individual will inherit two inactive copies of a tumor suppressor gene, as the functions of many of these genes are required for normal embryonic development. One common familial cancer

is breast cancer associated with inheritance of a mutated copy of either the BRCA1 or BRCA2 genes. Inherited mutations in these genes are more common in families of Ashkenzi Jewish descent than in other populations, so such individuals should be especially vigilant in screening for breast cancer. Another, less common, example is families that pass down a mutated copy of the *p53* gene. This is associated with a familial cancer susceptibility called Li-Fraumeni syndrome, in which family members have an increased risk of developing a number of different types of cancer.

What causes the mutations that generate oncogenes from proto-oncogenes or inactivate tumor suppressor genes? One source of mutations comes from the natural process of copying DNA every time a cell divides. The enzyme that copies DNA, DNA polymerase, makes a mistake about every 10^5 nucleotides. Fortunately, most mistakes are quickly fixed, either by the inherent 'proofreading' activity of DNA polymerase itself or by other 'spell-checking' proteins that can identify improper base-pairing (see Figure 10.21). These mechanisms lower the error rate of DNA replication to only about one mistake in 10^{10} nucleotides copied. However, given that an estimated 10^{16} cell divisions occur in a human lifetime, this low rate of mutations from DNA replication cannot be ignored. An even more important source of mutations comes from environmental exposure to chemicals and radiation that can react chemically with DNA bases or cause physical damage to DNA. Such chemicals and radiation that can alter DNA are referred to as **mutagens**. The cell's 'spell-checking' enzymes and other types of DNA repair proteins can help to minimize damage from mutagens, but some mistakes slip through the cracks. The genes that encode proteins that repair single nucleotide changes and other types of DNA damage are one class of tumor suppressor gene. Importantly, if both copies of a DNA repair gene become inactivated due to mutations, the overall rate of accumulation of

mutations will increase, and the risk of cancer will be elevated. The BRCA1 and BRCA2 genes that are implicated in familial breast cancer are two examples of DNA repair proteins with important tumor suppressor function.

Mutations caused by DNA replication errors or mutagens occur randomly throughout the genome. To contribute to transformation, uncorrected mutations must be in a proto-oncogene or a tumor suppressor gene. Because these genes represent a tiny fraction of the whole genome, the probability that one of them will receive an uncorrected mutation is low. Cells that suffer a significant level of DNA damage will be signaled to die by apoptosis (if the function of the p53 protein is intact). Additionally, for cancer to arise, recall that mutations in at least 5-6 growth regulatory genes must all accumulate in the same cell. Therefore, the barriers to cancer development are robust; nonetheless, the cancer rate statistics indicate that these barriers are surmounted at some point in many people's lifetimes.

Progression to cancer

We see the characteristics of transformed cells when we study them at the cellular level. However, cancer occurs in whole, multicellular organisms, not in isolated cells. As mentioned earlier, an organism can be considered an ecological system of many billions of cells. The interacting growth and differentiation signals keep the cellular ecosystem stable. After cells are transformed, progression to a tumor depends on many ecological factors. Mutated progeny cells may be killed, or they may not outgrow the normal cells and thus never progress to a tumor. Alternatively, the transformed cell and its progeny may continue to divide, taking up space and nutrients required by their neighbors, passing on their mutations to each new progeny cell, and usually accumulating even more mutations.

Normal cells begin to die off, not because they are killed outright by cancer cells, but because they are deprived of space and nutrients. With the decline in the number of normal cells comes a reduction of their normal function, and the organism begins to show signs of illness. The particular symptoms depend on the type of cancer and the types of normal cells that are lost.

Transformed cells within organs may form solid tumors within those organs (Figure 10.22, middle right). For a tumor to be visible by X-ray, the original transformed cell must divide repeatedly until there are about 10^8 cells in the tumor. For a tumor to be large enough to be felt (about 1 cm in diameter), approximately 10^9 cells are needed. Long before tumors are this size, they have begun to influence their environment. One major influence they exert is to stimulate the growth of new blood vessels, a process called **angiogenesis**. The tumor cells secrete signals called angiogenic growth factors that induce nearby blood vessels to develop new branches that grow into the tumor. This is crucial for continued tumor growth, as all cells required a constant supply of oxygen from the blood, and the distance limit for oxygen diffusion through tissues is only about 0.2 mm. Thus, tumors contain other types of cells besides cancer cells, including the cells that make up blood vessels as well as connective tissue cells and various types of immune cells. In recent years, much research has focused on the interactions between cancer cells and other, normal cell types that are intermixed with cancer cells in tumors. Together, the various types of cells in a tumor define the tumor microenvironment, the features of which can significantly affect the rate of tumor growth and its progression to increased stages of disease. Dampening pro-growth signals in the tumor microenvironment is one strategy for developing new cancer drugs. For example, one class of cancer drugs inhibits the process

of angiogenesis, with the goal of restricting a tumor's blood supply and essentially suffocating it.

A tumor is said to be **benign** if it is contained in one location and has not broken through the **basement membrane** to which normal cells are attached. Benign tumors, as their name suggests, often cause no health problems for the individual. Benign tumors can become large enough to interrupt the functioning of normal tissues, but their removal by surgery is generally successful because they have not intermingled with normal tissue. Tumor cells that invade normal tissues, rather than just pushing them out of the way, are said to be **malignant** (see Figure 10.22, bottom). For cells to be invasive, they must produce protein-degrading enzymes such as collagenase, an enzyme that dissolves the collagen connective tissue that holds groups of cells together (see Table 10.2). The term 'cancer' is generally reserved for malignant tumors.

Because malignant tumors produce enzymes that allow them to invade other tissue, they often spread to new locations, a process known as **metastasis**. In this process, one or more of the transformed cells lose their attachment to the other cells of the tumor, break through the basement membrane, and spread via the circulation to other areas of the body (see Figure 10.22, bottom right). In the new location they regain attachment and continue to divide, forming new tumors. The new tumors are of the same type as the original tumor and thus when viewed with a microscope are seen to be different from the cells around them. Cancers that have begun to metastasize are far more serious and more resistant to treatment than those that have not, because no amount of surgery can eliminate all the cancerous cells that have spread.

THOUGHT QUESTIONS

1. How do stem cells differ from transformed cells? How do stem cells differ from muscle cells or blood cells?
2. In what ways are benign and malignant tumors the same and in what ways are they different?
3. What does the statement "Cancer is a disease of the genes, but it is not an inherited disease" mean?

CANCERS HAVE COMPLEX CAUSES AND MULTIPLE RISK FACTORS

The study of disease at the population level constitutes the science of **epidemiology**. The basic epidemiological data for various forms of cancer have been compiled for the United States since 1950. The incidence for various cancers in the United States is given in Table 10.3.

Epidemiology uses descriptive statistics to find patterns in the incidence of diseases. Those patterns indicate possible risk factors that can suggest hypotheses that can be further tested in other ways. In general, and with a number of exceptions, the causes of adult cancers seem to be mainly environmental, not genetic. Evidence to support this conclusion comes from epidemiological data for the United States and several European countries, showing in each case a marked increase in cancer rates throughout the twentieth century. Most of this increase in cancer pertains to a single type, cancer of the lung (Figure

10.23A). The increase coincided with advancing industrialization and other changes in the environment, but very little change in the gene pool, suggesting environmental or lifestyle causes were primarily responsible. In the case of lung cancer, the death rate showed a striking parallel with increases in the consumption of cigarettes, with a lag of about 15–20 years (Figure 10.23B). Women adopted the habit of cigarette smoking later than men, and their increase in lung cancer deaths also occurred later. Since 1960, cigarette consumption by males has decreased, and in 1990, the lung cancer death rate among males began to decrease. Cancers of the pancreas and large intestine increased more slowly over the same period, while stomach and rectal cancers declined. The rapid change and irregular pattern of change both conform much better to the hypothesis of environmental causes than the alternative hypothesis of a genetic cause or causes. Interestingly, although media coverage of cancer has dramatically increased in the past decades, cancer death rates in the U.S. have been declining for the past 25 years, due in large part to improved treatment and implementation of regular screening as normal part of medical care. For some cancers, such as breast cancer, this decrease in mortality has occurred despite coincident increases in cancer incidence. Part of the increased incidence in breast cancer may be due to environmental factors, but increased screening (and thus identification of more cases that may not be life threatening) may also play a role.

Human cancers are named according to the type of cell from which the cancer is derived (the cell of origin). A cancer that arises in epithelial cells (cells forming sheet-like tissues that line body cavities or glandular tissues) is called a **carcinoma;** a cancer that arises in connective tissue is called a **sarcoma.** Blood cell cancers such as leukemias and lymphomas make up a third class of cancers, and cancers that arise in cells of the nervous system define the fourth major class. Carcinomas account for the vast majority of cancers,

largely because cells in epithelial tissues naturally turn over more frequently than cells in other tissues, and more cell division means more opportunities for accumulation of mutations. Cells in many epithelial tissues also come into contact with more environmental mutagens; for example, lung epithelia are exposed to substances in the air we breathe, and intestinal epithelia are exposed to chemicals in the food we eat. In addition to the major classes of tumors, there are also tumor subtypes: a liposarcoma, for example, is a sarcoma derived from fat cells, and the cells of origin of squamous cell carcinomas are skin cells called keratinocytes.

Cancers are rare in children, adolescents, and young adults, but certain cancers do occur in these populations. In particular, blood cancers (leukemias) occur much more frequently in children than do other types of cancers, making up about 26% of all childhood cancers. It is important to note, however, that adults still make up the vast majority of people who develop leukemia. Approximately 75% of childhood leukemias are acute lymphocytic leukemias that arise from stem cells in the bone marrow. Although these are very aggressive, they have a good cure rate because children still have many normal cells to take over after therapy.

About 85% of adult cancers are carcinomas, including cancers of the lungs, breast, colon, rectum, pancreas, skin, prostate, and uterus (see Table 10.3). The incidence of these cancers (and many others) increases with age, so that cancers become more and more significant as causes of death with advancing age (see Figure 10.20). Most of these adult cancers are believed to be caused mainly by environmental or lifestyle factors. On the basis of epidemiological evidence, the following factors have been suspected of causing at least one type of cancer or of increasing the rate at which at least some cancers occur: genes, increasing age, viruses, ionizing radiation, ultraviolet radiation, diet, stress, mental

state, weak immune systems, unsafe sexual behavior, hormones, alcohol, tobacco, and some chemical substances.

In this section we examine the evidence from epidemiological studies and animal studies that have suggested the many possible causes of cancer. We will see how these seemingly disparate causes may be working by very similar pathways at the cellular and molecular levels. Keep in mind that when we speak of 'causes' of cancer we often mean factors that are associated in epidemiological studies with increased incidence in populations. As such, these factors are more properly called 'risk factors,' not causes. A multitude of factors contributes to whether any particular person gets cancer. We generally cannot say that one thing 'caused' a particular cancer. As Clark Heath of the American Cancer Society has said, "Cancer cases are clinically nonspecific—you can't look at a leukemia case clinically and say, 'Ah, this a radiation-caused leukemia.' " (*Scientific American*, September 1996, p. 86.)

Inherited predispositions for cancers

As described previously, cancer is not inherited, but a predisposition for some cancers can be. Some of the genes implicated in familial cancers and the types of cancers associated with inheriting mutant alleles of these genes are listed in Table 10.4. As mentioned earlier, retinoblastoma is a rare cancer of the eye that primarily affects young children (see Figure 10.24A). The presence of a growing tumor in the eye causes a white spot in photographs, and a cell phone app has been developed to help detect retinoblastoma at an early stage when it can be treated without loss of vision. Most people who develop retinoblastoma inherit one defective allele of the gene for a protein called pRb, which is a checkpoint protein required for monitoring the entry of cells back into the

cell division cycle from G_0, the resting state. Of the people who inherit a defective allele, 80–90% develop retinoblastoma. The rapid cell division in the developing eye during early childhood seems to make it quite likely that the second functional RB1 gene is lost, setting a cell on the pathway to becoming cancerous. Surgery, often paired with additional therapies such as radiation and chemotherapy, can cure up to 95% of retinoblastoma cases. Notably, however, people who have familial retinoblastoma are at higher risk for other types of cancer later in life, reflecting the central role of the pRb protein in cell cycle control.

Another rare cancer is xeroderma pigmentosum (XP), a condition that results in very high rates of skin cancer due to a defect in one of several proteins that function in a pathway that repairs DNA damage caused primarily by ultraviolet radiation (e.g. from the sun). In the case of XP, affected individuals inherit two defective alleles of one of the genes that functions in the repair pathway. Children who are known to have inherited XP alleles are counseled to take extreme precautions to avoid sun exposure (see Figure 10.24B). With such precautions, it is possible for some XP patients to live a relatively normal lifespan, but patients also exhibit neurological problems that may decrease their lifespan independent of threats from cancer.

The APC gene encodes a tumor suppressor protein that promotes cell differentiation to a non-dividing state rather than continued cell proliferation. Inheritance of one defective copy of the APC gene causes a condition called familial adenomatous polyposis, or FAP, which manifests as very high numbers of benign tumors, or polyps, in the colon, or large intestine (see Figure 10.24C). While the polyps themselves are not cancerous, there is a very high chance (nearly 100%) that additional mutations will occur that cause cells in one or more polyp to become transformed, leading to malignant colon

cancer. The presence of intestinal polyps can be identified by colonoscopy, where a camera mounted on a flexible tube is inserted into the colon through the anus. In the past 20 years, in the U.S., it has become standard medical procedure to use colonoscopy to screen all people over age 50 every 10 years for polyps and cancerous lesions in the colon. Persons known to be at high risk based on family history are screened as frequently as every two years. During a colonoscopy, isolated polyps can often be removed by a simple clipping procedure, thus ensuring that they do not progress into malignant tumors. In patients with FAP, however, the number of polyps is usually so great by the time they reach their mid-thirties that the polyps cannot all be removed in this way, and the vast majority will eventually develop colon cancer. The APC gene functions in a number of tissues besides the colon, and individuals with FAP do have increased rates of some other cancers, but the risk is much lower than for colon cancer. The reason for this discrepancy is unknown. In addition, while retinoblastoma and xeroderma pigmentosum are quite rare, colon cancer is one of the most common cancers. The vast majority of people who develop colon cancer have not inherited a mutant APC allele and therefore have sporadic, rather than heritable colon cancer. However, loss-of-function mutations in the APC do seem to occur at an early stage in the development of sporadic colon cancers. The mutations associated with colon cancer progression are especially well characterized because of the availability of various stages of tumor tissue removed from patients during colonoscopies. (Note that any research using tissue samples from patients must now abide by informed consent laws, unlike when the HeLa cell line was derived from Henrietta Lacks' cervical tumor in the 1950s.)

Retinoblastoma, XP, and FAP occur in almost all people who inherit the relevant mutations, and the associated cancers often occur quite early in life. In contrast, mutations in the *BRCA1* and *BRCA2* genes (so-named for breast cancer 1 and 2) confer a breast cancer risk that is less extreme: by age 80, about 72% of women who carry a mutated *BRCA1* gene and 69% who carry a mutated *BRCA2* gene will develop breast cancer, whereas only about 12% of non-carriers will develop cancer in their lifetimes. Although the risks associated with the *BRCA1* and *BRCA2* mutations are less certain than for the other familial cancers we have discussed, some women who know they are carriers, including actress Angelina Jolie, choose to have preventive mastectomies (breast removal). Others do not choose this option but are vigilant with regular breast self-exams and mammograms. Despite the prevalence of news stories about the 'breast cancer genes,' only five to ten percent of all breast cancers are associated with a genetic predisposition. Environmental factors play a much larger role in the overall prevalence of breast cancer, as is also the case for colon cancer.

Increasing age

Far more cancers seem to be environmentally caused than genetically caused, even after taking into account genetic predispositions for some cancers. The more common type of breast cancer, a late-onset disease of postmenopausal women, is not linked to inheritance. The strongest risk factor for these breast cancers is age. The incidence of all cancers increases with age, presumably because there has been more time for environmental exposures to produce accumulated mutations. In fact, one of the reasons for the present-day higher incidence of cancers (and chronic diseases such as heart disease) is that people are living much longer because mortality from infectious diseases is lower.

From data on the incidence of cancers, the probability of acquiring cancer at different ages can be calculated. Table 10.5 gives data for breast cancer. As can be seen, the probability increases with age. Because age is such a strong factor in all health studies, epidemiologists must 'control for age;' that is, they must either compare groups of the same ages, or use mathematical formulas to 'age-adjust' the data.

Cancer data are often shown as the probability of acquiring cancer by age 75. Examples are shown in Figure 10.25. Here data of the type shown in Table 10.5 have been added up to give the 'lifetime probability' of acquiring cancer by age 75. Note that these numbers do not mean, for example, that a 75 year-old white woman's chance of acquiring breast cancer while she is 75 is 10%. The chances for each age group are the numbers shown in Table 10.4, which are much lower.

Data of the type shown in Figure 10.25 are useful in comparing the probabilities for different types of cancers. They are also useful in comparing the probabilities for different segments of the population. In the United States, health statistics are summarized by sex and by race. These are data for populations and do not mean that any individual's probability is the number shown. Individual risk is increased or decreased by all of the factors mentioned in this section.

Viruses

Several cancers are known to be associated with viruses and other infectious agents. In 1911, American pathologist Peyton Rous showed that a tumor of connective tissues (a sarcoma) in chickens was caused by a virus that was later named Rous sarcoma virus. (Rous received a Nobel Prize for this work, but not until 1966.) Chickens infected with

this virus develop sarcomas at a high rate. Viruses seem to be associated with cancer in at least two different ways. First, a viral infection may cause a decrease in the activity of the immune system. Decreased immunity increases the likelihood that a transformed cell will progress to cancer, highlighting the role of the immune system in eliminating cancer cells, which have some abnormal features that can make them seem "foreign". An example of this type is Kaposi's sarcoma, which occurs in people with AIDS (see Chapter 15, p. 000). Second, some viruses carry genes that, when inserted into the host DNA, cause the host cell to become transformed into a cancerous cell. These genes are, therefore, oncogenes. The viruses do not cause the mutation; rather, they carry an entire mutated gene into the cell they infect. The 1989 Nobel Prize awarded to Bishop and Varmus honored their discovery of the fact that the oncogene in Rous sarcoma virus was actually a cellular gene that had been 'picked up' by the virus and mutated to an onocogenic form that could then trigger transformation when reintroduced into cells via infection. This relationship between cellular proto-oncogenes and viral oncogenes is illustrated in Figure 10.26.

The incidence of liver cancer is high in third-world countries. A very high proportion of the people who develop liver cancer have previously had hepatitis B, a viral infection of the liver. Some people, after recovering from the acute symptoms of hepatitis, remain infected carriers of the virus. Over 250 million people worldwide are carriers of hepatitis B virus; carriers have a 100-fold higher risk of developing liver cancer, although this may occur as long as 40 years after hepatitis infection. Hepatitis C virus is also associated with liver cancer, particularly in Japan. Worldwide, as many as 80% of liver cancers are caused by viral infections. The means by which the hepatitis viruses cause liver cancer are not as clear as for the cancer-causing mechanism of the Rous sarcoma virus, but research in this area is ongoing.

Cancers of the reproductive organs, especially cancer of the uterine cervix, are statistically related to both male and female sexual behavior. Incidence rates for cervical cancer are higher among women who were younger at the time of their first instance of sexual intercourse or who have had multiple sexual partners. The rates of cervical cancers are also high in those countries in which women tend to have few sexual partners and to marry as virgins but where a tradition of *machismo* often encourages men to seek multiple sexual partners. This epidemiological evidence argues that male promiscuity is an important risk factor for cervical cancer even though it is women who develop the disease. Sexually transmitted human papilloma viruses (HPV) often cause genital warts, but some types also cause cancer of the cervix, which is thus a sexually transmitted form of cancer. The HPV genome contains two genes whose protein products inactivate two of the cell's key tumor suppressors; unlike for the case of the Rous sarcoma virus, these oncogenes are virus-specific and are not related to normal cellular genes. Papilloma viruses account for more than 80% of cancers of the genitals and anus. The first vaccine against HPV was approved for use in the U.S. in 2006, targeting pre-adolescents through young adults. A study that analyzed cervical tumors diagnosed during the period from 2008 to 2014 found a statistically significant decrease in HPV infection, indicating that the rate of HPV-induced cancer decreased with the onset of widespread administration of the HPV vaccine. Even individuals who had not been vaccinated showed a decrease in HPV incidence, illustrating the concept of 'herd immunity' (see Chapter 14, p.000).

The epidemiological evidence for the association of each of these viruses with cancer has been verified by animal and tissue-culture experimentation. Although the incidence of virally induced tumors is low in the United States, such tumors account for 20% of all cancers worldwide.

Physical and chemical carcinogens

A large and growing number of external agents are known to cause cancer; such agents are called **carcinogens**. Evidence that carcinogens cause cancer comes from studies in which animals are exposed to them. Evidence also comes from epidemiological studies of occupationally exposed persons, such as industrial workers who handle the agents. Still other carcinogens are discovered by recognizing epidemiological clusters of persons with unusually high incidence rates for particular cancers living in the area surrounding an industrial plant or waste disposal site. There are two types of carcinogens: those that induce DNA mutations (**mutagens**, also called **tumor initiators**) and those that promote the progression of transformed cells into cancer by causing DNA damage (**tumor promoters**). Some mutagens are physical agents, energy sources with high enough power to damage DNA. Other mutagens are chemical agents; these also damage DNA through chemical reactions with the DNA bases or the DNA backbone (see Chapter 2, p. 000).

Physical carcinogens (radiation). Some carcinogens are physical agents, particularly certain types of energy sources. Exposure to ultraviolet (UV) radiation—most often from sunlight or tanning beds—is the a primary risk factor for developing a range of skin cancers. UV radiation causes abnormal chemical bonds to form between certain DNA bases in the same DNA strand, most often between two neighboring 'T' bases (Figure 10.27). This interferes with the normal base-pairing between those 'T' bases and the complementary 'A' bases on the opposite DNA strand, which can cause the introduction of mutations when the DNA is replicated. Cells have DNA repair mechanisms that can fix this type of damage, but the extent of damage can overwhelm the repair machinery, leading to mutations being passed on when skin cells divide. UV radiation can also

produce reactive oxygen species (ROSs) in cells, which can damage DNA in other ways (see below).

Skin cancer rates have been steadily rising in the United States during in recent years; currently, over 3 million people are diagnosed with some form of skin cancer in the U.S. each year. Most of these are squamous or basal cell carcinomas, which are among the less aggressive and more treatable skin cancers. In contrast, malignant melanoma is a cancer of the pigment cells (melanocytes) that is especially dangerous because melanoma cells readily spread around the body (metastasize), often before a person even realizes that they have a skin lesion. In 2019, nearly 200,000 Americans werediagnosed with malignant melanoma. Melanomas kill more women in their twenties than does breast cancer. Light-skinned people are more susceptible to skin cancers and should be especially vigilant about protecting themselves from UV radiation through the use of sunscreen and physical barriers to sun exposure. Ninety percent of skin cancers are attributable to UV radiation, particularly UV B rays. In contrast to UV A rays, UV B rays have shorter wavelengths and cannot penetrate as deeply into the skin; instead they are mostly absorbed by the surface layers of the skin, where they cause DNA damage. The adult incidence of skin cancer is correlated with the number of sunburns that a person received as a child. Epidemiological evidence shows that even exposures to natural levels of UV radiation increase the incidence rates for skin cancers, which are much higher in the southern half of the United States than in the northern half. In contrast to most skin cancers, one rare and especially deadly form of melanoma is not directly linked to UV exposure but is more determined by genetics. Dark-skinned people are more likely to develop this type of melanoma, particularly on the skin of their palms or the bottoms of their feet, and it is often diagnosed

late because people of color and their physicians are less attuned to being aware of skin cancer in darker-skinned populations.

Ionizing radiation, such as that produced by radioactive substances, was clearly shown to be carcinogenic by studies on the Japanese survivors of the 1945 bombings of Hiroshima and Nagasaki. Leukemia was the first type of cancer to show increased prevalence in survivors of the bombings, exhibiting an uptick just five years later, but increased rates of a variety of other cancers have also been linked to the wartime radiation exposure as survivors have been followed through the years. The French (Polish-born) chemist Marie Curie (1867–1934), a two-time winner of the Nobel Prize and pioneer in the study of radioactive elements, died of a leukemia induced by her frequent handling of these elements. X-ray machines also produce ionizing radiation, so the level of exposure is carefully controlled to minimize the exposure. Medical and dental diagnostic X-rays do not increase cancer incidence. The only medical uses of radiation associated with increased cancer risk are the very high radiation doses used in cancer therapy itself. Although these treatments are necessary to save a patient, they do induce DNA damage, from which new cancers may arise a decade or more later. Radiation treatments, like all medical treatments, are based on estimates of risk-benefit ratios. When someone is very sick and would die without treatment, the probable benefit from the treatment may make a higher level of risk acceptable to the patient and her or his physician.

Ionizing radiation associated with cancer is also caused by radon, a radioactive element that occurs naturally in certain types of rocks. When these rocks are uncovered, radon gas may be released. A person with long-term exposure to radon gas may develop lung cancer; however, radon accounts for less than 10% of lung cancers, whereas smoking

accounts for more than 85%. Proper ventilation removes virtually all of the risk from radon.

Any time that charged particles move, whether they are electrons in a power line or ions moving across cell membranes, electric and magnetic energy fields are created. Therefore, all living things are sources of electrical and magnetic energy because of the ions moving through them. Low-frequency electric and magnetic fields from power lines or household appliances and radio-frequency electromagnetic radiation from cell phones or microwaves are too low in energy to cause ionizing damage to DNA. Although media stories have sensationalized the potential risk from electric and magnetic fields produced by these sources, carefully controlled epidemiological studies have found no correlation between exposure to them and increased cancer rates. In fact, the ambient level of energy from a cell phone is less than one one-hundredth of the electromagnetic radiation given off by the person holding the phone.

Chemical carcinogens: tumor initators and tumor promoters. Other carcinogens are chemicals. As mentioned above, these make up two categories: **tumor initiators** and **tumor promoters**. Tumor initiators are agents that begin the process of transformation by causing permanent damage in the DNA; in other words, they are **mutagens**. Some chemicals are themselves harmless but become carcinogens when acted upon by metabolic enzymes in cells. Since the 1970s, the Ames test, described in Box 10.1, has been used as a simple method for screening chemicals for mutagenic activity. Although it is performed in bacteria, the Ames test incorporates a mechanism to allow for metabolic activation that mimics what would occur in human cells. When interpreting results from the Ames test, it is important to consider what concentration caused mutations and whether a person would

ever be exposed to that concentration in normal life; for example, certain compounds found naturally in celery give positive results in the Ames test, but that does not mean that eating celery will give you cancer because those compounds naturally occur at concentrations well below any level of concern. Compounds that warrant concern are those that cause mutations at low concentrations and might occur in contaminated drinking water or in polluted air, or compounds that could constitute realistic exposure concentrations for certain populations such as people in certain occupations.

In a cell whose DNA has been damaged by exposure to a mutagen, or tumor initiator, transformation can be completed by subsequent exposure to a tumor promoter. Tumor promoters by themselves do not cause mutation; therefore, they do not produce positive results in the Ames test. Instead, tumor promoters induce cell division, either directly, by affecting cell signaling, or, more commonly, by causing tissue damage or inflammation that triggers cell division to repair the tissue. If a dividing cell contains a mutation from an earlier exposure to a tumor initiator, its chance of acquiring additional mutations that lead to complete transformation are increased. Because the DNA damage from an initiator is permanent, a tumor promoter can have its effect years after exposure to the initiator. Tumor promoters include alcohol, phorbol esters (often used as tumor promoters in laboratory studies), natural hormones such as estrogen, dioxins (by-products of industrial bleaching processes), saccharin, and asbestos.

Tobacco smoke contains dozens of known carcinogens including nitrosamines, formaldehyde, arsenic, nickel, cadmium, and several compounds called polycyclic aromatic hydrocarbons (PAHs). These are also present in smokeless tobacco (chewing tobacco and snuff). Many of the carcinogens in tobacco undergo metabolic activation to generate mutagens, and some are tumor promoters. A substance that is both a tumor

initiator and a tumor promoter is referred to as a 'complete carcinogen.' Tobacco fits this description, and exposure to the chemicals in tobacco smoke, including second-hand smoke, is the largest single risk factor for cancer in the industrialized world. People who begin to smoke when they are teenagers or in college are more than ten times more likely to develop lung cancer than people who have never smoked. Both smoked and smokeless tobacco also greatly increase the risk of cancers of the mouth and throat (oral cavity and pharynx) due to direct exposure of these tissues to the carcinogens when these products are used. Although a person's risk of cancer decreases after he or she stops smoking, the risk never drops as low as that for people who have never smoked. The danger of second-hand smoke is evident from the fact that nonsmoking women whose husbands smoke have higher cancer rates than nonsmoking women with nonsmoking husbands.

A large number of industrial chemicals are carcinogens, including vinyl chloride (used in the making of many plastics), formaldehyde, asbestos, nickel, arsenic, benzene, chromium, cadmium, and polychlorinated biphenyls (PCBs) (Table 10.6). People can become exposed to these chemicals through factory work or industrial contamination of the water supply. Some chemicals in herbicides and insecticides used in agriculture are also carcinogens, and people can similarly be exposed to these through farm work or through the water supply. Even when a company is known to have released carcinogens into the environment, it is nearly impossible to know whether any individual case of cancer was caused by environmental exposure to those chemicals. Instead, probable cause can be attributed to environmental contamination when a statistically significant increase in cancer cases is observed in a discrete area near the proposed site of release (a cancer 'cluster'). This invariably involves long, expensive lawsuits. Consequently, there have been relatively few instances in the United States when a company has been found

responsible for a cancer cluster and has been required to compensate victims or their families. Two notable cases were made into Hollywood films: the case against W.R. Grace in Woburn, MA, which was the subject of the film "A Civil Action", and the case against Pacific Gas and Electric in Hinkley, CA, which was the subject of "Erin Brockovich." In the former, the chemicals in question were trichloroethylene and perchloroethylene, and in the latter, it was chromium-6. Recently, concerns have emerged over release of ethylene oxide gas into the air by two medical technology companies in Georgia. Ethylene oxide is used for sterilization, as well as for the synthesis of other chemicals. In late 2016, the U.S. Environmental Protection Agency raised its risk assessment on this chemical, concluding that it is indeed a human carcinogen, whereas it was previously categorized as a 'possible carcinogen.' It is estimated that additional anti-pollution measures being undertaken at the plants in question will reduce the cancer risk from ethylene oxide exposure to two cases in one million people from the current estimate of 100 per one million people. It can be argued that even the higher risk is quite low compared to the risk associated with some other exposures that are more under a person's individual control (such as smoking or not wearing sunscreen), but this is provides little comfort to a person who believes his or her cancer was caused by industrial pollution.

The popular weed-killer Roundup® contains glyphosate, an herbicide implicated in causing non-Hodgkin lymphoma and several other cancers, and listed as a possible carcinogen by the World Health Organization and the International Agency for Research on Cancer, among others. Its manufacturer, Monsanto (now owned by Bayer), has been sued by multiple users of the herbicide who later developed cancer, and $2 billion has been awarded (through 2019) in various legal cases.

Dietary factors

The American Cancer Society states that "for the majority of Americans who do not use tobacco products, dietary choices and physical activity are the most important modifiable determinants of cancer risk." Evidence that dietary factors contribute to the development of cancer is best established for cancers of the digestive tract, including the colon and rectum. The evidence comes from laboratory studies of animals exposed to experimentally controlled diets, from clinical studies on human patients, and from epidemiological studies of large populations.

Dietary fiber and fats. Diets high in fiber and low in fats are associated with a lower incidence of cancers of the intestinal tract (including the colon and rectum) and also those of the pancreas and breast. In countries where fiber consumption is high and fat consumption is very low, as in most of equatorial Africa, incidence rates of colon and rectal cancer are only a fraction of what they are in the industrialized world. Australia, New Zealand, and the United States, where diets are lower in fiber and higher in fats, have high rates of colon and rectal cancers. In fact, diet is much more strongly correlated with cancer incidence than is industrial pollution. Studies comparing the cancer rates in Iceland and New Zealand, where diets are similar to those in the United States but where there is far less industrialization, have shown that the cancer incidence is the same in these countries as it is in the United States. In contrast, cancer rates overall, and for many specific types of cancer, are much lower in Japan, which, like the United States, is an industrialized nation, but one in which dietary fat intake is very low. The incidence of cancers among Seventh-Day Adventists who are vegetarian and do not smoke or drink is much lower than the incidence in their neighbors, despite both groups' living in the same

conditions and being exposed to the same environmental pollutants. Several studies have also shown that eating fresh vegetables, particularly those rich in vitamins A, C, E, and beta-carotene (a vitamin A precursor), reduces the incidence of many cancers. Epidemiological data on people who migrate from one country to another further support the idea that diet and lifestyle factors have a more significant role in cancer risk than genetic factors for most cancers. One study compared cancer incidences for ethnic Japanese people living in Osaka, Japan vs. those who had migrated to Hawaii. The Japanese typically have a low risk of breast cancer but a higher risk of stomach cancer compared to Americans; the ethnic Japanese living in Hawaii were found to have cancer incidence profiles more similar to their fellow Americans than to those in Japan, suggesting that their adoption of American dietary and lifestyle habits altered their patterns of cancer risk.

Salty and pickled foods. Cancer of the stomach follows a different epidemiological pattern correlated with a different set of dietary factors. This cancer is most frequent in Japan and in certain Latin American countries, where it seems to be correlated with the eating of very salty foods and pickled vegetables. The incidence of this cancer in Japanese immigrants to Hawaii and California decreases after a generation or two, while that for cancers of the colon, rectum, and breast increases. Among Japanese-Americans in Hawaii, the incidence of stomach cancers correlates closely with the retention of other aspects of Japanese culture: that segment of the Japanese-American population who maintain more of their traditional culture have higher rates of stomach cancer than those who adopt more Western cultural practices. This evidence suggests that diet has a larger role than genetics in the incidence of stomach cancer.

Alcohol. Ethyl alcohol has been identified as a risk factor for cancer by a number of researchers, but the increased cancer risk is largely confined to people who also smoke. The risk of developing a cancer of the mouth or throat, for example, is much higher in people who both smoke and drink (Figure 10.28). This is an example of a **synergistic effect**, meaning that the increased risk due to two causes is much more than the additive combination of their effects taken separately. Alcohol and tobacco are also synergistic in producing other forms of cancer. The reason for their synergy is that tobacco is a tumor initiator and alcohol is a tumor promoter.

Internal resistance to cancer

A good deal of evidence shows that people vary in their resistance to cancer. People with the same exposure to all known risks do not get cancer at the same rate. People also vary in their recovery rates once they get cancer. Individual variation in hormones, stress, mental outlook, and immune function may be involved.

Hormones. Hormones are implicated in several types of cancers, including uterine, ovarian, breast, prostate and testicular cancers. In addition to these cancers that have a clear relationship to female and male sex hormones, cancers for which obesity is a key risk factor also have links to hormones. Obesity causes chronic inflammation, which leads to elevated levels of the hormones insulin and estrogen (in both men and women). These in turn stimulate cell division, thus promoting a number of types of cancer.

The relationship between hormones (primarily estrogen) and cancer risk has been studied most extensively for breast cancer, but the results are complex. The risk of some breast cancers can be reduced by ovariectomy (removal of the ovaries) or by taking the estrogen-inhibiting drug tamoxifen. A five-year course of tamoxifen treatment is commonly prescribed for women whose breast cancer has been eradicated by surgery, radiation, and/or chemotherapy to prevent recurrence of the cancer. Breastfeeding causes hormonal changes that protect women from both breast and ovarian cancer; experts recommend that women breastfeed for at least six months to receive these benefits. In addition, children who were breastfed as infants have a lower risk of obesity, which results in them also having a lower risk of a number of types of cancer. A woman's reproductive history also influences her risk of breast cancer due to hormonal changes that occur during pregnancy; women who have a child before age 35 receive some long-term protection against breast cancer—although the risk of developing breast cancer is elevated for 10 years immediately after a first birth—and each successive birth adds a small amount of additional long-term protection.

Women who take oral contraceptives (birth control pills) have a slightly increased risk of breast cancer, but a *decreased* risk of ovarian cancer. Birth control pills contain two hormones, estrogen and progesterone (see Chapter 9). The slightly increased rate of breast cancer is associated with the increased dose of estrogen, while the decreased risk of ovarian cancer is due to the blockage of ovulation (release of eggs from the ovaries) by the combination of hormones used in birth control pills. Hormone replacement therapy to lessen symptoms of menopause is also linked to an increase in breast cancer and a smaller increase in ovarian cancer. A comprehensive report that reanalyzed data from more than

50 studies found a slight elevation in risk of breast cancer in women who took birth control pills. For every 10,000 women who are currently using the pill and who started using it between the ages of 25 and 29, 48.7 are expected to develop breast cancer in the next 10 years. Among women of the same age who have never used the pill, 44 out of 10,000 are expected to develop breast cancer in the next 10 years. Therefore the 'attributable risk' (the number that can be attributed to oral contraceptives) is 48.7 minus 44 or 4.7 cases per 10,000 women. Data such as these are often reported as 'relative risk' calculated as 48.7 divided by 44 or 1.16. Relative risk is generally what is reported to the public and would be stated as follows: women using oral contraceptives (who started at age 25–29) are 1.16 times as likely (or 16% more likely) to develop breast cancer in 10 years. Most experts focus on women with known genetic susceptibility to breast cancer (i.e. those with an inherited mutation in BRCA1 or BRCA2) as the population who should consider the additional risk posed by the pill when deciding on a method of birth control. The additional breast cancer risk from post-menopausal hormone replacement therapy is about double that due to oral contraceptives. Because there are other, safer ways of treating menopausal symptoms, many physicians counsel women against choosing hormone replacement.

The news media have paid significant attention to the possible breast cancer risks associated with plant estrogens that occur naturally in some foods, especially soybeans, as well as to so-called endocrine disruptors, which are chemicals that have structures similar to human hormones and can have hormonal effects in the body at certain concentrations. Because consumption of soy products is also known to have a number of health benefits, and Asian countries where soy products are frequently consumed have low levels of breast cancer, most experts believe the benefits of eating soy outweigh any possible risks.

Bisphenol-A (BPA) is an estrogen-like endocrine disruptor that is found in many plastics. It binds rather weakly to the estrogen receptor, so only high concentrations have hormone disrupting effects. However, it can also affect cells through a number of other mechanisms, including causing DNA damage (at lower concentrations), promoting inflammation, and interfering with intracellular signaling pathways. There has been a strong consumer backlash against BPA, but the true risk remains unclear.

Imbalances between estrogens and androgens (male hormones) are implicated in testicular cancer. In many cases, such hormonal imbalances may be genetically determined. Ongoing research is also examining the hypothesis that endocrine disruptors in the environment may promote development of testicular cancer. The rates of testicular cancer have been increasing worldwide over the past few decades, especially in developed countries. These epidemiological data support the existence of an environmental component to testicular cancer risk. In contrast, to testicular cancer, rates of prostate cancer have remained relatively steady in recent decades. Research has not identified a clear hormonal influence on the risk of developing prostate cancer; however, androgens are required for continued growth and development of prostate tumors, and differences in hormone levels among patients do seem to affect treatment outcomes, although the relationships are not yet clear.

Stress. There are two types of stress: acute stress, which is short-term and related to a specific event (such as having to give a presentation in class), and chronic stress, which is long-term and related to prolonged difficult life circumstances (such as poverty, a dysfunctional family, abuse, etc.). Chronic stress causes sustained changes in the levels of certain hormones that affect the nervous system, which are called neuroendocrine

hormones. These include cortisol (commonly referred to as the ‘stress hormone’) and catecholamines such as dopamine, norepinephrine, and epinephrine (formerly called adrenaline). Altered levels of these hormones can have wide-ranging effects on health. Most studies examining stress and cancer have found no solid correlation between chronic stress and cancer incidence; however, a 2017 report did find a slight increase in prostate cancer risk in men younger than 65 who reported chronic workplace stress versus those who did not. No physiological data (e.g. levels of stress hormones in blood) were examined to classify the men who were considered to be chronically stressed. In contrast to the relatively weak evidence for stress promoting cancer initiation, there is considerable evidence that cancer is more likely to progress more rapidly in individuals who are experiencing chronic stress relatively to those who have lower stress levels. In particular, stress seems to promote cancer metastasis, which is associated with much worse clinical outcomes than non-metastatic cancer. Thus, many cancer treatment facilities now offer support services such as counseling, meditation, yoga, and massage to lower stress levels in patients. Among women who have been treated for breast cancer, survival rates are significantly higher for those women who participate in social support groups (see Chapter 14).

Immune function. Evidence is increasing that people with weakened immune systems develop cancers more frequently. This factor is striking in conditions that severely damage the immune system, such as AIDS (see Chapter 16), but is also present in people whose immune systems are weakened less drastically from other causes, including chronic stress, sleep disorders, and so forth. Far more cells are mutated and transformed than ever develop into cancers. A healthy and active immune system eliminates most of these cells as they arise, in a process called immunosurveillance. Any weakening of the immune

system increases the number of transformed cells that grow and proliferate, and a higher rate of cancer is one of the results. People's immune systems also weaken with age, which is consistent with the finding that the incidence of new cancers increases with age.

Animal studies have shown that one component of the innate immune system (see Chapter 14, p. 000), natural killer (NK) cells, is especially important for combating cancer. NK cells recognize abnormal cell-surface features shared by many cancer cells (but not normal cells) and inject so-called 'cytotoxic granules' that contain proteins that kill the cancer cells. Genetically engineered mice that have defects in NK function were shown to develop spontaneous tumors at an increased frequency than normal mice and also developed tumors more rapidly when exposed to a chemical mutagen.

The adaptive (specific) immune system (see Chapter 14, p. 000) is also important for carrying out immunosurveillance and eliminating transformed cells that would otherwise proliferate to form tumors. Cancer arises from a person's own cells, so cancer cells are not 'foreign' in the sense that a virus or a bacterium is. However, because cancer cells contain numerous genetic mutations, they express proteins that are different from those that were present in the body early in life when the immune system was being 'trained' not to attack the body's own cells (see Chapter 14, p. 000). These new (novel) features of the mutated proteins expressed by cancer cells therefore function as antigens to attract the attention of antigen-specific B and T cells, activating immune responses that eliminate the cancer cells expressing the novel proteins. The novel proteins in cancer cells that stimulate a specific immune response need not be the same mutated proteins that are responsible for the cells' transformation (e.g. the proteins produced by oncogenes or inactivated tumor suppressor genes). In fact, one key feature of transformed cells is that they acquire new mutations at a much higher rate than normal cells (often because of loss-of-function mutations in DNA

repair genes). Many of these mutations are so-called ‘passenger’ mutations; they don’t ‘drive’ the cancerous progression but are just ‘along for the ride.’ Nevertheless, passenger mutations can code for novel proteins that target cancer cells for elimination by the adaptive immune system. The novel proteins produced by a given cancer can differ in the degree to which they activate B and T cells—that is, in their ‘antigenicity.’ Thus, some tumors might grow very slowly because they express mutated proteins that are highly antigenic, causing the cancer cells to constantly be attacked by immune cells, whereas others may express mutated proteins that are less antigenic and can ‘fly under the radar’ of the body’s immunosurveillance system. These differences in antigenicity of mutated proteins produced by cancer cells can have important implications for therapies that exploit the body’s immune system (immunotherapies), which are discussed later in this chapter.

Social, economic and racial factors

Socioeconomic status refers to a person’s income and education level. Together with race, socioeconomic status influences incidence rates and especially survival rates for various cancers. With regard to race, some differences likely relate to genetic susceptibilities: for example, African American women seem especially susceptible to developing a particularly aggressive form of breast cancer (triple negative breast cancer, so-named because the cancer cells do not express three cell-surface receptors common on breast cells). Other racial differences relate instead to socioeconomic forces more likely to be experienced by particular racial groups.

In the United States, data for the years 2011-2015 showed higher incidence rates for black (African American) men for all cancers combined compared to all races or to whites. Black women had lower rates of cancer incidence, but both black men and women had higher rates of death from cancer (mortality). The mortality rates for blacks are higher, in part, because they are less likely to receive routine medical exams that would detect the common cancers in their earliest and most treatable stages. These include screening measures such as mammograms, colonoscopies, Pap smears, and PSA tests, discussed elsewhere in this chapter.

From the 1950s to the 1980s, socioeconomic status was *directly* correlated with cancer incidence and mortality rates; in other words, people with *higher* income and education were *more* likely to develop and die of cancer than those with lower income and education. At the end of the 1980s, that trend reversed; it now shows an *inverse* correlation, such that *lower* socioeconomic status is now correlated with *increased* cancer incidence and mortality rates for all races. People of all races with lower socioeconomic status are more likely to exhibit behaviors that increase cancer risk, such as smoking, physical inactivity, and poor diets. Differences in socioeconomic status are associated with a number of disparities that can influence cancer rates and outcomes: access to affordable health insurance (and therefore access to regular, preventive care); access to transportation to be able to receive care; ability to take time off work (paid sick leave) or find child care for medical appointments; access to healthy foods; and education surrounding habits that can reduce cancer risk. In the United States, the Medicaid expansion that occurred in many states as a result of the Affordable Care Act passed by Congress in 2014 made health insurance more attainable for people with low incomes. While this helped all races, it was especially effective in narrowing the gap in the percentages of whites versus non-whites

who have health insurance. This is important progress toward ensuring healthcare access that can decrease cancer mortality rates; however, significantly higher percentages of non-whites remain uninsured compared to whites, especially for Hispanic and Native American individuals. Apart from healthcare access and the lifestyle factors already mentioned, chronic stress related to socioeconomic status may also play a role in cancer incidence rates and outcomes. Several recent studies have indicated that many non-white people who are not disadvantaged economically or educationally nevertheless experience higher levels of stress than their white counterparts due to real or perceived encounters with racial discrimination or stereotyping.

As we have seen, many factors may contribute to cancer incidence rates, including genetic predisposition, lifestyle, and exposure to environmental carcinogens. Estimates of the relative contributions of various biological causes is given in Table 12.6. These are rough averages for global cancer incidence. The percentage of cancers attributable to viruses is about 15% worldwide but is much lower in the United States. The percentage attributable to diet also varies from one part of the world to another, as does the percentage attributable to chemical carcinogens in the environment. With the exception of the low percentage of cancers that may be attributable to genetic predisposition, changes that can be made by individuals or by societies have the potential to significantly decrease incidence rates for most cancers, a topic we explore further in the next section.

THOUGHT QUESTIONS

1. Not everyone who smokes gets cancer. Does this mean that smoking is not a risk factor for cancer?

2. What is the difference between a risk factor and a cause? Can we say what caused cancer in a given individual?

3. Why do different individuals respond differently to cancer risk factors?

4. Does an increase in the percentage of deaths due to cancer necessarily mean that cancer rates have increased? What else could explain such findings? How could you go about determining which of the possible explanations best fits the data?

5. Recently the genetic defect associated with an inherited form of colon cancer was identified as a defect in a DNA-checking protein. This finding was reported in the lay press as the discovery of 'the colon cancer gene.' Is this name misleading? To what extent can a defective repair mechanism be considered to be the same thing as a cause of a cancer?

6. Tobacco smoke contains chemicals that are tumor initiators and other chemicals that are tumor promoters. How does this combination contribute to the carcinogenicity of tobacco smoke?

7. Tobacco and alcohol act synergistically in increasing cancer risks. Can you explain this in terms of what is happening inside cells?

WE CAN TREAT MANY CANCERS AND LOWER OUR RISKS FOR MANY MORE

An understanding of the mechanisms that produce cancer has greatly increased our understanding of how to prevent and treat it. Cancer therapies have improved significantly over the past few decades and continue to do so, resulting increased survival rates for most cancers. In the period from 1999-2016, overall cancer death rates decreased an average of

1-2% per year for men, women, and children. In this section we examine medicine's current strategies for treatment and prevention.

Traditional cancer therapies: surgery, radiation, and chemotherapy

The traditional treatments for cancer comprise a triad of approaches that have been used since the 1940s: surgery, radiation, and chemotherapy. Surgery aims to remove visible tumors, but it cannot eliminate cancer cells circulating in the bloodstream that have the potential to spawn metastatic tumors or existing metastases that are too small to detect. Therefore, surgery is often combined with radiation or chemotherapy. With either radiation therapy or chemotherapy, the strategy is the same: cancer cells are dividing cells; therefore, agents that interfere with cell division should stop tumor growth or even cause tumors to shrink due to apoptosis. Radiation causes breaks in the DNA of dividing cells that are so large that the cell cannot repair them and usually cannot live with the damage. Chemotherapeutic drugs prevent DNA synthesis at several steps. Some of the drugs inhibit the synthesis of the nucleotides needed to build DNA; some substitute for certain nucleotides in newly synthesized DNA, preventing its further replication; and some inhibit an enzyme needed to unwind and rewind the double helix during its replication. Other chemotherapeutic drugs, some of which are natural plant products, prevent RNA synthesis or damage the mitotic spindle, thus blocking mitosis.

An important drawback to radiation and chemotherapy treatments is that, because they damage DNA, they increase the risk of secondary cancers derived from damaged cells that survive the treatments. Another drawback is that both radiation and chemotherapy are nonspecific: both kill any type of dividing cell. Two examples are hair follicle cells and immune cells. A high proportion of cells in hair follicles are dividing, so

hair loss frequently accompanies these treatments. A large percentage of cells of the immune system are also dividing and so are killed. Not all hair follicle and immune cells are killed because not all were dividing at the time of treatment, so they can repopulate. Hair grows back, and people regain their full immune function. During the time when people's immune systems are compromised, they need to avoid exposure to infectious diseases. Both radiation and chemotherapy may destroy memory B and T cells, which 'remember' which diseases the person has been exposed to or vaccinated against (see Chapter 14). If these memory cells are killed, even a person who has regained the ability to form new immune responses has lost previous immunities and therefore may need to be revaccinated.

A further risk from chemotherapeutic drugs is that they put a selective pressure on the population of transformed cells. As a result, any cells that become resistant to the drug quickly out-compete the drug-susceptible cells. Because cancer cells mutate quickly, they often acquire mutations that confer resistance to specific drugs. Several drugs, each of which works by a different mechanism of action, are often used in combination to minimize the development of drug resistance. However, cancer cells may also develop mutations that prevent them from transporting drug molecules across their membranes or start expressing cell-surface 'pump' proteins that can pump a range of different drugs out of the cell. This phenomenon of 'multi-drug resistance' is a major problem in cancer treatment.

Despite the drawbacks and risks, surgery, radiation, and chemotherapy have been very effective, increasing the survival rates of many types of cancers. Moreover, in the vast majority of patients, melanoma, Hodgkin lymphoma, and breast, prostate, testicular, cervical, and thyroid cancers can now be 'cured' by these treatments, as measured by five-

year survival rates. Leukemia also has very high 'cure' rates in patients under 55, and especially in children. It is impossible to know whether cancer cells have been completely eliminated from a person's body, so many physicians and scientists hesitate to use the word 'cure' when talking about cancer outcomes. More often, they say that a person's cancer is 'in remission,' which means that cancer cells cannot be detected, but there is a chance that it could recur at a later time. Recurrence rates vary widely depending on a number of factors, including the type of cancer, the cancer stage at diagnosis, and the treatment received. In some cases, even patients who have been in remission for more than five years are monitored in the hope than any recurrence can be detected early. For some cancers, especially those diagnosed at later stages, surgery, radiation, and chemotherapy cannot achieve complete remission; they may not fully eliminate solid tumors or circulating tumor cells, but they cause tumors to shrink and the numbers of circulating tumor cells to be significantly reduced. In such cases, cancer is treated as a chronic disease, with patients continually receiving treatment to try to prevent further progression of their cancers without achieving true remission.

Targeted cancer drugs

Research on cancer treatments continues at a rapid pace. New chemotherapeutic agents, both natural and artificial, continue to be sought and developed. Some of these have properties similar to 'traditional' chemotherapies, in that they target features common to all dividing cells. However, there is greater emphasis on therapies that exploit specific features of cancer cells that are not shared by normal cells, which minimizes side-effects due to generalized cell death and maximizes killing of cancer cells. Such agents are often

referred to as ‘designer drugs’ and are the basis of a new trend in cancer therapy called ‘precision medicine.’ In precision medicine, a patient is not just diagnosed with a particular type of cancer, but the specific features of the cancer cells are assessed at the genetic and molecular level to identify possible routes of targeted therapy. For example, DNA may be isolated from cells taken from a tumor that has been removed surgically (or from a small piece of a tumor taken in a ‘biopsy’), and the sequences of certain genes known to be commonly mutated in that cancer (e.g. driver mutations in oncogenes and tumor suppressor genes) will be determined to identify mutations. In most cases, genetic testing of tumors focuses on a panel of specific driver genes, but given the rapid advances in next-generation DNA sequencing (see Chapter 4), the entire genomes of tumors are sometimes sequenced. Other types of tests may detect the expression level of particular proteins known to be overexpressed in certain types of cancers.

One of the first precision drugs approved for use in patients targets a specific chromosomal rearrangement that occurs in more than 90% of cases of chronic myelogenous leukemia (CML). This chromosomal mutation fuses the coding sequences of two signaling proteins, Bcr and Abl, creating a fusion protein that has hyperactive growth-promoting activity, as mentioned earlier in this chapter. Scientists determined the three-dimensional structure of the Bcr-Abl oncoprotein and designed a drug that could bind specifically to this protein and block its function (see Figure 4.17). The result was the drug Gleevec®, which was approved by for use in the United States in 2001. About 90-95% of CML patients survive five years or more after diagnosis when treated with Gleevec. CML accounts for only 15% of leukemia cases, but the Bcr-Abl oncogene is also implicated in a small percentage of cases of another leukemia (acute lymphoblastic leukemia, ALL), and Gleevec is highly effective for those patients as well.

As described earlier in this chapter, the hormone estrogen drives the proliferation of many breast cancers. Therefore, drugs that block the estrogen signaling pathway can be effective in treating so-called estrogen-dependent breast cancers. About 80% of breast cancers express the estrogen receptor. These can be treated with a class of drugs called selective estrogen receptor modulators (SERMs). The SERMs bind to the estrogen receptor and block its ability to promote breast cancer cell division. Estrogen has additional functions in cells apart from promoting cell division; these include maintenance of bone density and cholesterol regulation. Importantly, while SERMs block the proliferative effect of estrogen, they do not block its other, desirable effects. Tamoxifen is one example of a SERM that has proved effective in reducing mortality from breast cancer and in decreasing its onset in women at high risk due to genetic predisposition or age (over 60). Another class of drugs, the aromatase inhibitors, block an enzyme required to make estrogen. These are frequently used to treat estrogen-dependent breast cancer in post-menopausal women, but they do not have the selective action of the SERMs, so patients taking them must be monitored and sometimes treated for bone loss and elevated cholesterol.

Another type of targeted therapy aims to deliver toxins selectively to tumor cells but not normal cells in a ‘seek and destroy’ tactic. One such approach, called photodynamic therapy, is used for esophageal cancer, for a type of lung cancer that affects the large breathing tubes (bronchi), and for pre-cancerous skin lesions. In this treatment, the patient is given an intravenous injection of a photosensitizing dye, which accumulates in tumor cells, while being expelled by normal cells. The patient’s tumor is then illuminated using fiber optics. Light reacts with the dye in the tumor cells to create a toxin that kills the cells. Photodynamic therapy is limited to tumors that are accessible to the

fiber optic light required to activate the toxin. Other toxin-based therapies are delivered in nanoparticles that can be injected into the bloodstream. When it was first developed in the mid-1990s, nanoparticle-mediated cancer therapy relied on the fact that the extra blood vessels that develop to feed tumors have 'leaky walls;' when injected into the bloodstream, nanoparticles bound to chemotherapy drugs can exit the leaky blood vessels into tumors but are too large to exit intact vessels in normal tissues. In more recent years, nanoparticles have been developed that use targeting molecules such as antibodies that bind specifically to proteins found preferentially on cancer cells to increase the concentration of the drug-carrying nanoparticles in tumors. Another approach uses nanoparticles made of metals such as gold or silver; once these have been given time to concentrate inside tumors, a laser pulse or radiation is administered, which causes the metal nanoparticles to heat up and kill tumor cells. Many cancer researchers are working to develop additional nanoparticle-mediated therapies, but one paper published in 2019 cautions that care must be taken to ensure that nanoparticles do not inadvertently create larger holes in blood vessels that will promote migration of tumor cells into the vessels, thereby stimulating metastasis.

Because we now know so much about the changes that occur in cells during transformation and cancer progression, scientists have pursued many different routes to exploit the varied characteristics of cancer cells that we discussed earlier in the chapter. These include the following: drugs that inhibit the enzyme telomerase, which confers 'immortality'on cancer cells; angiogenesis inhibitors that target production of new blood vessels required to provide nutrients and oxygen to tumors; inhibitors of many proteins that function in growth factor signaling pathways or drive cell cycle progression; and inhibitors of the proteasome, which regulates many cellular pathways via protein

degradation. Unfortunately, none of these has proved to be a 'magic bullet;' in other words, there does not seem to be one specific feature of cancer that can be targeted that will result in its eradication. Rather, the best outcomes are typically achieved by combining multiple angles of attack, and different strategies work better for different types of cancers and even different patients with the same basic type of cancer.

One additional behavior of cancer cells that could be a very important clinical target is the process of metastasis. Since the vast majority of cancer deaths are due to metastasis, a treatment that inhibits the formation of second-site tumors could make a very large impact on cancer mortality rates. One study published in 2018 described a compound the authors called 'metarrestin' that suppressed metastasis in mouse models of pancreatic cancer. The researchers hope that clinical trials testing the safety and efficacy of this drug in humans will begin soon.

After extensive laboratory research, new therapies are tested only on patients who have given their informed consent (see Chapter 1) to be part of the studies; for the first stage of clinical trials patients accepted to participate are usually the very sickest who have exhausted all other options, since unexpected, possibly dangerous side effects can occur, and it would not be ethical to subject healthier patients to those potential dangers. Once a new therapy has been deemed safe, other types of patients are accepted into later rounds of clinical trials that are designed to test its efficacy and, particularly, to determine whether it works better than the treatments currently used for a given cancer. The gold standard of clinical trials is a double-blind trial, where neither the patient nor the physician knows which patients are receiving the therapy and which are receiving a mock therapy, or 'placebo.'

Because cancer is greatly feared and not always curable, some people put their hopes in unproven remedies. For a number of years, a compound called laetrile (also known as amygdalin) achieved a large and devoted following as an alternative therapy. Laetrile is a bitter compound found in some fruit pits, raw nuts, and other plant parts; when taken into the body, it is converted into cyanide, which was supposedly effective at killing cancer cells. The supporters of laetrile became so influential that, in the early 1980s, the National Cancer Institute conducted careful clinical trials. The clinical trial results showed no positive effects of laetrile, but, even years later, it is still produced in Mexico (without batch standardization) and is sought by some patients as a last resort.

Immunotherapies

In the past few decades, much progress has been made toward understanding how the human immune system responds to cancer and these discoveries have yielded a number of novel therapies that are achieving significant clinical results (Table 10.7). As discussed earlier, cancer cells represent unique targets of the immune system because they are 'self,' but they may also have features that distinguish them from normal cells. A variety of animal and human studies have shown that, during the course of cancer progression, an unrelenting 'tug of war' plays out between the cancer cells and a person's immune system. Cancer cells may express novel, mutated proteins that function as antigens to attract the attention of immune cells, resulting in elimination of some of the cancer cells; however, additional mutations typically occur in the cancer cells that cause changes in gene expression that allow new sub-populations of cancer cells to escape immunosurveillance. Thus, there is a continual back-and-forth between the cancer cells and the immune system,

with one temporarily gaining ground and then the other fighting back. The goal of cancer immunotherapies is to tip the balance in favor of the patient's immune system.

One type of immunotherapy is 'passive immunization.' In this approach, patients are given immune products such as antibodies or antigen-specific immune cells that stimulate destruction of the cancer cells. One of the first and most successful antibody-based passive immunization treatments is a drug called Herceptin, which consists of an antibody that recognizes a cell-surface receptor called HER2 that is highly expressed on about 25–30% of breast cancers. Herceptin works in several ways: its binding to the HER2 receptor can block signaling pathways that stimulate breast cancer cell proliferation; it can also cause the HER2 protein to be taken into the cell and degraded (thus also blocking signaling), and, further, it can attract natural killer (NK) cells that kill cancer cells by injecting them with cytotoxic granules.

Another type of passive immunization, called adoptive cell transfer, involves giving a patient immune cells, either from a donor or expanded cell populations derived from the patient themselves. One type of adoptive cell transfer has been used for many years in bone marrow transplants to replace blood-cell generating bone marrow in patients with blood cancers whose own bone marrow has been destroyed by radiation treatments. In recent years, it has become evident that the best outcomes are achieved when the surface proteins on the bone marrow donor cells differ very slightly from the patient's so that the donor immune cells recognize remaining cancer cells as foreign and destroy them. (Donor cells that are too different from the patient can cause a lethal immune response, so patients must be very carefully matched with donors.) Another passive immunization therapy that has been approved for clinical use more recently is called chimeric antigen receptor T-cell therapy (CAR-T). In this strategy, a patient's own T-cells are harvested

from their blood and genetically engineered in the laboratory to express a particular receptor protein (a chimeric antigen receptor) that will recognize a molecule found on the surface of the patient's cancer cells. These engineered T cells are then infused into the patient's bloodstream, where they seek out cancer cells expressing their target antigen and kill them. CAR-T therapies have been approved for several blood cancers, and more are being developed. One final example of passive immunization involves administration of tumor infiltrating lymphocytes (TILs, or T- and B-cells harvested from excised tumors) that have been allowed to proliferate in tissue culture, often stimulated by activating immune signal molecules. These expanded TIL populations are introduced back into the patient as 'reinforcement troops' to kill remaining tumor cells. Studies have shown that patients with tumors that naturally have a higher number of TILs have better prognoses. TIL therapy has been especially effective for metastatic melanoma, allowing some patients to achieve complete remission and significantly improving three-year survival rates.

In addition to the passive immunization strategies just described, several active immunization approaches to cancer therapy have been developed in recent years. These aim to stimulate the body's own immune response, rather than delivering immune cells or molecules. One such strategy is called immune checkpoint blockade. The immune system has natural mechanisms that limit the activity of stimulated T-cells so that out-of-control immune responses are not produced that might attack normal cells. In the case of cancer, these so-called immune 'checkpoint' mechanisms can limit the efficacy of anti-tumor immune responses. Therefore, molecules that interfere with the immune checkpoint responses can maximize the cancer killing potential of a patient's own T-cells. Antibodies that bind and block the function of three key immune checkpoint proteins, PD-1, PDL-1, and CTLA-4, are currently in use to treat many different types of cancer and are being

studied for additional applications. Former U.S. President Jimmy Carter is one high-profile success story for immune checkpoint inhibitors. He had melanoma that had spread to his liver and his brain; after three months of checkpoint inhibitor therapy, no remaining cancer could be detected anywhere in his body. Such dramatic results are seen in only about 20% of patients, but they provide hope to many patients with advanced cancers.

Cancer vaccines are another type of active immunization treatment. In contrast to vaccines against viruses or bacteria, which are administered to prevent infections, cancer vaccines are designed to prime the immune system to fight existing tumors. Only one cancer vaccine is currently approved for clinical use, but other are in development. The approved vaccine is called Provenge or sipuleucel-T, and it is used to treat advanced prostate cancer. In this treatment, dendritic cells, which are a type of immune cell that process and 'present' antigens to other immune cells, are isolated from a patient's blood and cultured in vitro in the presence of a protein called 'PAP' that is nearly universallsy expressed by prostate cancer cells. When these dendritic cells are infused into the patient, they stimulate the patient's B-cells to produce antibodies against the prostate-cancer specific antigen and activate T-cells that can kill the cancer cells. Because most prostate cancers express PAP, this vaccine can be used in most prostate cancer patients. One small clinical study reported in 2017 developed personalized vaccines for six melanoma patients that consisted of small segments of up to 20 novel proteins ('neoantigens') expressed by the melanoma cells but not by normal cells; the neoantigen mixture for each patient was generated by sequencing the complete genomes of the patient's cancer cells and their normal cells, identifying cancer-specific mutations likely to be antigenic, and expressing protein fragments corresponding to those mutated genes. Melanoma was chosen for this study because it is caused by exposure to UV light, which is highly mutagenic, so

melanoma cells tend to have higher overall numbers of mutations than other cancers, including many 'passenger' mutations that do not contribute to malignant behaviors but cause the expression of novel proteins not present in normal cells. Of the six patients in the study, four achieved complete remission with the vaccine alone, while the other two went into remission after receiving the vaccine plus an immune checkpoint inhibitor. While these results are very promising, scaling up production of individualized vaccines for larger numbers of patients will present great financial and technical challenges.

The cost of cancer treatment

The process of developing a new cancer drug is estimated to take 15 years of more, including the time spent on laboratory research to identify and optimize a drug and as many as eight years of clinical trials prior to approval by regulatory agencies (in the United States, the Food and Drug Administration). This is very expensive. Drug companies typically cite a figure of 2.7 billion dollars for the whole process; an independent study claimed the real figure may be closer to 650 million dollars, but this is still a high price. Once a drug is marketed, patients and their insurance companies also pay high prices, as drug companies seek to recoup their investments and earn a profit. For many patients, the cost of cancer therapy can lead to bankruptcy or other serious financial problems. In order for a new cancer drug to be approved for treatment of a given cancer, it must not only be safe and effective, but it must produce better outcomes than the current standard of care for that cancer. However, the definition of a 'better outcome' is often measured in months of extended survival rather than years. For example, the prostate cancer vaccine mentioned earlier in this chapter was shown to extend the time for which

50% of trial participants survived by only about 4 months compared to the placebo; the cost of this treatment is approximately $93,000 per patient. Extending a patient's life by a matter of months may enable that person to attend a child's wedding or see the birth of a grandchild; however, it is important to consider whether the benefits of a short extension of life outweigh the financial costs of treatment. This is a difficult ethical question, and its emotional content makes it extremely difficult to enact governmental policies that aim to place any sort of limits on late-stage cancer care, even when there is very little change that a patient will survive. Some cancer therapies are extremely expensive but have a better chance of actually producing lasting remission. For example, one type of CAR-T therapy was shown to produce complete remission two years or more in more than 50% of patients; the cost of CAR-T for a single patient is currently 1.5 million dollars. As cancer treatments become ever more specialized and the numbers of patients affected by cancer remain high, the cost of cancer care will be an issue that cannot be ignored.

Cancer detection and predisposition

Early detection greatly increases the probability of successful cancer treatment. Monthly breast self-examination is very effective at finding tumors while they are treatable. Diagnostic breast X-rays (mammograms) and ultrasound (sonograms) detect tumors that are too small to be felt but are less effective in younger women than in postmenopausal women whose breast tissue is less dense. Microscopic examination of tissue from the cervix taken during a medical examination, called a Pap smear, is effective at early detection of cervical cancer. Testicular cancer, although rare, is the most frequent cancer in men between the ages of 15 and 34; as for breast tumors, monthly self-examinations to detect lumps in the testes are an important method of early detection. As mentioned

earlier, regular colonoscopies in people older than 50 have also contributed to decreased mortality from colon cancer.

An emphasis on early detection has contributed to the increased survival rate for a number of cancers. For other cancers, it is not so clear what is meant by an increase in survival rate and how this relates to a 'cure.' When we try to evaluate the meaning of statistical statements like 'survival rates,' we need to know a lot about how the numbers were gathered and what definitions are being used for certain terms. What is often reported as survival rate refers to the proportion of persons with cancer still living after 5 years compared with the proportion of surviving persons without cancer. For example, it is estimated that it takes 9 years for a breast cancer to develop, spread, and kill a person. If past methods of detection led to discovery of the cancer 7 years after its inception, very few people would have been alive 5 years after its discovery. Now, with better detection methods and better public education for breast self-examination, the 5-year survival rate looks much improved. Does this mean that therapies have improved, or does it simply mean that people are now finding the cancers at 2 years into their development rather than at 7 years? It is not always easy to distinguish advances made through better or earlier diagnosis from advances made in the treatment of cancers once they have reached comparable stages of development.

In addition to the tests mentioned above, two types of laboratory cancer tests currently exist. One type is for the early detection of existing cancers. The other type is genetic testing for cancer predisposition.

An example of the first type is the PSA test for prostate cancer. This test measures the level of PSA (prostate-specific antigen) in a person's blood. This protein is elevated when prostate cancer begins to develop. Thirty percent of people with elevated PSA are

found to have cancer when the test is followed by a biopsy (tissue sample examined by microscope). The PSA test can detect cancer up to 5 years before there are other symptoms. The clinical question then becomes what to do about it. Cancers detected by PSA tests are generally still localized and can therefore be successfully removed surgically. However, prostate cancer is very slow growing, and it has been said that most men die with prostate cancer, not because of it. One-third of men have some form of prostate cancer by the time they are over the age of 50, but only 3% die from it. For many individuals, after a biopsy has confirmed the presence of prostate cancer the recommended treatment is therefore to do nothing. However, a recent study has shown that men with newly diagnosed prostate cancer who have their prostate surgically removed have a 48% reduction in their risk of dying from this disease (4.6% mortality) compared to a similar group of men who underwent "watchful waiting" (8.9% mortality). There are similar uncertainties related to some types of breast cancer. Many breast tumors develop very slowly and have a low probability of spreading to other sites, so, especially in women over age 75, minimal treatment may be the best option.

The second type of laboratory test does not detect cancer, but instead identifies DNA sequences that are statistically correlated with an increased probability of someday acquiring the disease. Tests of this type are available for *BRCA1* and *BRCA2* (breast cancer predisposition mutations), DNA mismatch repair genes (predisposing to some kinds of colon and uterine cancers), APC (predisposing to colon cancer), *p53* (predisposing to a range of tumors) and a few other tumor suppressor genes and oncogenes.

These tests for genetic predispositions for cancer are controversial for many reasons. They are very expensive and do not give much more useful information than is

gained from knowing your family medical history. A negative test does not mean that a person will not get cancer; cancers arise spontaneously in people with no family history of the disease, so the recommended preventive measures discussed below should still be followed. A positive test is also not a guarantee that a person will get cancer. The probability is increased but we cannot always tell by how much. The increase in risk is different for different mutations, but none increases the probability to 100%. The genetic mutation cannot be repaired, so there is little that a person with increased risk can or should do beyond what is recommended for everyone (regular check-ups and the lifestyle choices summarized below). Still, because some of these cancers are difficult to detect early, being aware of a predisposition for them can ensure that physical examinations are done even more thoroughly than usual, and perhaps more often.

Some women with increased breast cancer risk due to mutations in *BRCA1* (or family history) have opted for 'prophylactic mastectomies,' that is, removal of their breasts before there is any evidence of disease. A recent study was widely publicized as showing that women who had their breasts removed reduced their risk of dying by 90%. While this statement is not untrue, it is only part of the story and is an example of reporting 'relative risk' instead of 'absolute risk.' In the study, 639 women had their breasts removed. On the basis of calculations made from the number of deaths among their sisters who faced the same increased susceptibility but did not have their breasts removed, it was estimated that 20 of the 639 women would have died. Only 2 actually did die, so the relative risk was decreased by 90% [$(20 - 2)/20 \times 100 = 90\%$]. When absolute risk is considered, it can also be correctly stated that 97% of the women had their breasts removed unnecessarily [$(639 - 20)/639 \times 100 = 97\%$]. The difficulty for any woman faced

with such a choice is that there is no way to predict whether she will be one of the 18 saved by the procedure or one of the 619 who did not need it.

Cancer management

Some people feel that more research dollars should be spent on cancer management, not just cancer treatment. Cancer management includes the development of drugs or strategies to minimize the side-effects of cancer treatments. Examples include cold capping, a procedure in which a cold pack is applied to the scalp during chemotherapy so as to slow cell division in hair follicle cells, thus decreasing hair loss. Another possibility is the development of anti-nausea drugs. Use of medicinal marijuana to overcome nausea and restore appetite in chemotherapy patients is becoming increasingly common (see Chapter 13). As of 2019, 33 U.S. states and the District of Columbia have legalized medicinal use of marijuana, and 13 others have approved restricted medicinal use; however, a 2019 study reported that, while 73% of oncologists (cancer specialists) believed marijuana could provide benefits to their patients, only 46% felt comfortable recommending it to patients. Because of the federal ban on marijuana, there has been little funding for proper scientific studies of its efficacy; however, this is beginning to change as more states legalize even recreational marijuana and the stigma surrounding this drug is reduced.

Cancer management also includes providing psychological and emotional support through group or individual therapy and complementary therapies such as massage, acupuncture, yoga, meditation, and nutritional counseling. The aim of these approaches is to improve the quality of life—to treat the person, not the disease. Women who were in support groups after recurrent breast cancer lived longer than those who were not. Survival rates have been found in some cases to be influenced by mental attitude (see Chapter 14).

Patients who were optimistic, who were aggressive, or who were 'determined fighters' had statistically longer survival rates and higher cure rates than those who were pessimistic or who resigned themselves early to their fate. Complementary therapies aim to keep the body and mind as strong as possible while an individual endures the often grueling treatments required to fight their cancer.

Cancer prevention

As we learn more about the causes of cancer, it seems that preventing cancer may be far easier and less expensive than curing it.

Smoking remains a major cause of cancer (Fig. 10.29). The Centers for Disease Control and Prevention state that cigarette smoke causes 30 times more lung cancer deaths than all regulated air pollutants combined. Exposure to secondhand smoke (for example, living in a home with someone who smokes) causes death from lung cancer in over 7000 nonsmokers a year in the United States. Tobacco smoke contains both tumor initiators and tumor promoters, and it suppresses the immune system.

Some dietary regimens have been associated with a decreased risk of cancer: lower total calorie and lower fat intake, for example. Food containing antioxidants may also help, as these compounds protect cells from the DNA damaging effects of mutagens; such antioxidants include beta-carotene and vitamins A, C, and E (see Chapter 10), which can be found in a wide range of fruits and vegetables (especially brightly colored ones), nuts, and even dark chocolate. Fresh vegetables of the plant family Cruciferae (including cabbage, radish, turnip, broccoli, cauliflower, kale, kohlrabi, mustard greens, and brussels sprouts) are especially rich in these and other protective compounds, and are recommended by the National Cancer Institute for that reason. Clinical studies have shown

that some vitamin supplements are not as effective in preventing cancer as the same vitamins obtained from foods, probably because other food ingredients are also at work or because the vitamins in the supplements are not absorbed as well as from foods. High-fat diets and obesity are important risk factors in colorectal cancer and in postmenopausal breast cancer. There is also evidence that high-fiber diets lower the risks of colon and rectal cancers, mainly by decreasing calorie intake and helping people maintain a healthy weight.

Given the available evidence, the most important actions you can take to lower your cancer risks are the following.

1. *Don't smoke*! Also, avoid secondhand smoke from poorly ventilated rooms where others smoke. *These are the single greatest steps you can take to reduce your cancer risk, far outweighing all other possible measures*.
2. Limit alcohol consumption; the American Cancer Society recommends no more than two alcoholic drinks per day for men and no more than one per day for women.
3. Follow a diet low in fats, high in fiber, and high in antioxidants, and strive to maintain a healthy weight through both diet and regular exercise.
4. Avoid occupational exposures to potential carcinogens; minimize exposure through the appropriate use of safety equipment.
5. Avoid exposure to radioactive substances and X-rays above necessary minimum levels, and avoid needless exposure to ultraviolet radiation from the sun or tanning booths.
6. As you age, be sure to get checkups at regular intervals, including screening that detects the common cancers in their earliest and most easily treated stages. If you are

a woman, learn to practice breast self-examination and, if you are a man, testicular self-examination.

THOUGHT QUESTIONS

1. Explain how the theory of evolution accounts for the development of cancer cells that are resistant to chemotherapy.
2. What characteristics would you look for in an ideal chemotherapeutic drug for the treatment of cancer?
3. Secondhand smoke is a cancer risk. Does this biological reality change the ethical debate about smoking in restaurants or smoking in the workplace? What rights are in conflict on either side?
4. Do you agree with the following statements? When someone's chances for survival are predicted to be very low, any and all treatments are justified. In other words, any treatment is good as long as it is not harmful. Try to apply this thinking to such unproven remedies as laetrile.
5. Is it ever ethically permissible to give up on treatment? Are there things other than treatments that can be done for a dying person? Do you think people who have terminal cancer should be told of their condition? Try to justify your answer. What ethical assumptions underlie your argument?

CONCLUDING REMARKS

Research directed toward understanding the basic biology of cancer continues. Some people feel that treatment cannot be rationally designed unless the underlying biology is

known, while others feel that such basic understanding is not important. The former group point to the development of new treatments such as tamoxifen and Herceptin. The latter group use arguments such as the following: we still do not know the basic biology underlying the disease polio, but development of a vaccine for its prevention has eliminated our need to know. The real dilemma is a problem in the allocation of resources. How much money should we spend on treating cancer patients, how much on improving methods of treatment, how much on laboratory research to discover more information on the causes of cancer, and how much on cancer prevention activities? How much funding should be aimed at particular types of cancers, such as breast cancer as compared with colon cancer? There are no clear answers here because we cannot accurately predict how well or how soon funds spent on certain activities (especially research) will translate into a reduction of cancer incidence rates or cancer deaths. Cancer prevention is clearly very cost-effective, but the cost-effectiveness of the other alternatives may be very difficult to assess.

On an individual level, we can reduce our exposure to cancer risk factors by many choices that we make in our lives. However, not all types of exposure among those listed above are matters of personal choice. Dumping of carcinogens on the land and water of poor people with little or no political power has become a global environmental issue. Air pollution affects people at great distances from the source. Therefore, as part of any effort to prevent cancer, people need to work together to prevent or remove environmental hazards from work places and communities.

CHAPTER SUMMARY

- All organisms are built of **cells** that maintain an efficient ratio of surface area to volume for the organism.
- Normal cells occasionally enter the **cell cycle** and divide. They always stop dividing when enough cells are present because of such phenomena as contact inhibition and other processes that maintain **homeostasis** of cell number.
- Cells also **differentiate** as they divide, becoming more and more fully **determined**, that is, restricted in their **potentiality** to form different kinds of cells that in most species are organized into **tissues**.
- **Stem cells** retain the ability to differentiate into many different kinds of cells.
- **Cloning** can begin with stem cells, increasing them in number for therapeutic purposes. Cloning for reproductive purposes begins with the insertion of a diploid nucleus into an enucleated egg to asexually reproduce a new individual.
- Both cell division and cell differentiation result from differential **gene expression**, and both are influenced by molecular signals secreted by other cells. These signal molecules are bound by receptors and this information is then transferred to the cell nucleus by **second messengers**. Signals that trigger cell division are called **growth factors**.
- Cancer cells are cells that have undergone **transformation** and differ from normal cells in abnormal responses to growth control signals—they do not stop dividing (they are 'immortal'), and they remain less differentiated.
- A few **cancers** have genetic predispositions, but most are caused by environmental factors. These factors include exposures to certain viruses and infective agents, dietary and other behavioral factors, and exposure to a long list of **carcinogens** including ionizing radiation (radioactivity), ultraviolet radiation, tobacco smoke, and a variety of chemicals.
- **Mutagens** damage DNA, and other chemicals can then increase the risk that these mutated cells will develop into cancer. Mutagens and tumor promoters have synergistic effects, that is, the increased risk of exposure to both is greater than the sum of the risks of the two separately.
- Cancerous **tumors** can be removed surgically, but other forms of cancer therapy target any cells that are dividing, destroying many healthy cells along with the

cancer. New, more specific chemotherapies are being developed, including therapies based on boosting the body's immune system.

- We can best reduce our risks for cancer by avoiding tobacco smoke and other carcinogens (including ultraviolet and ionizing radiations) and by eating a diet low in fats, high in fiber, and rich in beta-carotene and vitamins A and C.

KEY TERMS TO KNOW (boldface in the text).

Adult stem cell: An undifferentiated cell that retains into adulthood the ability to divide and differentiate; bone marrow cells capable of differentiating into blood cells are an example..

Angiogensis: The stimulation of nearby blood vessels to grow into a structure and supply it with blood.

Apoptosis: Programmed cell death (or cellular suicide) that begins with breakage of a cell's DNA, followed by cell shrinkage and detachment from its surroundings.

Basement membrane: A membrane to which cells are attached at their base, especially in epithelial (sheetlike) tissues.

Benign tumor: A tumor that has not broken through its basement membrane.

Biofilms: Layered aggregates of bacteria or other cells, with different molecules expressed in the several layers.

Blastula: An early embryonic stage consisting of a hollow ball of cells.

Cancer: A group of diseases characterized by DNA mutations in growth control genes and in which some cells divide without regard to growth control signals.

Carcinoma: A cancerous growth of epithelial (sheetlike or glandular) tissue.

Carcinogen: A physical, chemical, or viral agent that induces cancer; its action is called carcinogenesis.

Cell: The smallest unit of living organisms that shows the characteristics of life; can either be free-living or part of a multicellular organism.

Cell cycle: The process by which a cell duplicates its DNA and then divides into two cells.

Cell of origin: The initial cell whose transformation begins a cancerous growth.

Cell theory: The theory that cells are the building blocks and functional units of all organisms.

Checkpoints: Molecules that do not allow the cell cycle to proceed until certain repairs are made or processes are completed.

Cloning: Production of a new individual having the complete genome of another individual.

Compartmentalization: The division of a eucaryotic cells into separate compartments that carry out certain functions differently from the rest of the cell.

Contact inhibition: The inability of a normal cell to divide if it is surrounded by other cells.

Cyclin: A protein whose abundance varies cyclically, increasing at a particular time in the cell cycle that it appears to control.

Cyclin-dependent kinase: An enzyme that controls progress through the cell cycle by adding phosphate groups to other proteins in response to the abundance of cyclins.

Cytokinesis: Division of the cytoplasm to form two cells.

Determined: A state of development in which the future identity of a cell's progeny is predictable.

Differentiation: The process of becoming different; a restriction of the set of future possibilities of a cell's progeny.

Embryonic stem cell: An undifferentiated cell in an embryo that is able to divide and differentiate.

Endocrine: A form of secretion in which a product is secreted into the bloodstream to be carried to a target elsewhere.

Epidemiology: The study of the frequency and patterns of disease in populations.

Extracellular matrix: Material produced by cells but located outside any cell. Connective tissues have large amounts of extracellular matrix.

Gastrula: An embryonic stage in all animals except sponges, consisting of an outer layer of cells (ectoderm), an inner layer of cells (endoderm) which line a cavity open to the outside, and in most cases also a third or middle layer of cells (mesoderm) between them.

Growth factor: A messenger molecule that stimulates a cell to divide.

Homeostasis: The ability of a complex system (such as a living organism) to maintain conditions within narrow limits. Also, the resulting state of dynamic equilibrium, in which changes in one direction are counteracted by other changes that bring the system back to its original state

Immortal: A property of transformed cells that relieves them from having a limit on the number of times they can divide.

Malignant: A tumor that has grown through the basement membrane or extracellular matrix.

Maternal effect gene: A gene that is transcribed in an egg prior to fertilization.

Metastasis: The ability of transformed cells to leave the original tumor, travel through the body, and adhere and form new tumors in other locations.

Multipotent: Capable of forming a restricted variety of cellular progeny of several different types.

Mutagen: An agent that causes mutations in DNA.

Necrosis: Death of a damaged or diseased cell by breakage of its outer membrane and release of its contents.

Oncogene: A mutated growth control gene that leads to the transformation of a cell, which may then lead to cancer.

Organizer: An embryonic tissue whose chemical secretions induce the differentiation of other cells.

Paracrine: A form of secretion in which a product is secreted locally, affecting only nearby cells.

Phagocytes: Cells that engulf and digest other cells or the remains of dead cells.

Phosphorylation: Addition of a phosphate group to a protein, usually causing activation.

Pluripotent: Capable of forming a wide variety of cellular progeny, such as all ectodermal cell types.

Potentiality: The range of possible futures for a cell's progeny.

Promoter: A DNA sequence where RNA polymerase binds and where transcription of a gene therefore begins.

Proto-oncogene: A normal gene from which an oncogene is derived; it encodes a product that regulates cell division.

Regulated: Causing a process to occur either more or less in a given time period, based on some sort of informational input.

Sarcoma: A cancerous growth of originating in connective tissue.

Second messengers: Molecules within the cytoplasm of a cell that carry information from membrane receptors to other locations in the cell.

Sporadic: Arising in somatic cells, and therefore not inherited.

Stem cell: An undifferentiated cell that retains the ability to divide and differentiate.

Synergistic effect: A physiological response to two drugs given simultaneously that is greater than the sum of the effects of the same two drugs given separately.

Telomerase: An enzyme that lengthens the telomeres of embryonic cells or cancer cells to counteract their shortening during cell division.

Telomere: A structure at the end of a chromosome whose gradual loss during cell division limits the cell's capacity to go on dividing forever.

Tissue: A group of similar cells and their extracellular products that are built together (structurally integrated) and function together (functionally integrated).

Tissue culture: A growth of cells and tissues in a laboratory, artificially maintained outside any organism.

Totipotent: Capable of forming cellular progeny of all different types, as in embryonic stem cells.

Transformation: The multistage process that a cell undergoes in changing from a normal cell to an unregulated, less-differentiated, immortal cell lacking contact inhibition and anchorage dependence. NOTE: Bacterial transformation, discussed in Chapter 2, is unrelated.

Tumor: A solid mass of transformed cells that may also contain induced normal cells such as blood vessels.

Tumor initiator: An agent that begins the process of transformation by causing permanent changes in the DNA; mutagens and radiation are tumor initiators.

Tumor promoter: An agent that completes the process of cell transformation after the process is started by a tumor initiator; tumor promoters are not mutagenic by themselves but cause partly transformed cells to go into cell division.

Tumor suppressor genes: Genes whose protein products normally inhibit cell division.

CONNECTIONS TO OTHER CHAPTERS

Chapter 2: Cancers are caused by mutated growth-control genes or by DNA damage during chromosome crossovers.
Chapter 3: Cancers can result from changes that bring normal genes to abnormal locations in the genome.
Chapter 7: Several cancers have different incidence rates in different human populations.
Chapter 9: High-fat diets with low fiber content increase the risks for several cancers.
Chapter 14: Good mental outlook and immunological health can improve cancer survival rates.
Chapter 15: Certain otherwise rare cancers occur more frequently in AIDS patients.
Chapter 17: Many cancer-fighting drugs are plant products.
Chapter 18: Rainforest plants have been sources for new cancer-fighting drugs.
Chapter 19: Pollution increases the incidence rates of several forms of cancer.

PRACTICE QUESTIONS

1. What are the four phases of the cell cycle and what happens in the cell during each phase? Is G_0 part of the cell cycle?
2. In which cells is cell division inhibited by contact with other cells, normal cells or cancer cells?
3. Compartmentalizing an organism by dividing it into cells increases which of the following: the volume or the surface area?
4. Which of the following develop by differentiation from the zygote: tracheal cells in the lungs, muscle cells, or cells of the eye? Which develop from the gastrula? Which develop from the endoderm?
5. How long does an average human tongue cell live? How long do human liver cells live on average? Human nerve cells?

6. When the telomere region of the chromosomes becomes too short, which of the following happens?

   a. The cell can no longer divide.
   b. The cell becomes cancerous.

c. The cell can no longer differentiate.

7. Do the protein products of proto-oncogenes induce cells to divide or do they prevent cells from dividing? What about the protein products of tumor suppressor genes?

8. How many cells need to be transformed for cancer to develop? Does every transformed cell result in cancer? Why or why not?

9. Which of the following causes more cases of cancer: heredity, smoking, or viruses?

10. What processes are induced in a cell by binding of a growth factor to its receptor? Does a growth factor induce these processes in every cell?

11. Are the same genes transcribed and translated in every cell of the body?

12. Does cellular differentiation take place in adult animals or only in embryos?

BOX 12.1 The Ames Test

Tens of thousands of known chemical substances have never been tested as possible carcinogens in animals. Animal testing is expensive and slow; it would take many, many decades (and many billions of research dollars) to test all these substances. Clearly, we need a quick screening method that tells us which substances are more likely to be carcinogenic; these substances can be tested first, while the testing of less likely carcinogens can wait.

The Ames test, devised by cell biologist Bruce Ames of Cornell University in the 1970s, is a screening method that detects mutagens capable of causing particular types of mutations in a culture of *Salmonella* bacteria, as shown in the diagram below. The bacteria used are from a strain called *his*$^{-}$, which are unable to synthesize histidine, an amino acid required for the manufacture of bacterial proteins and hence for bacterial growth. Most bacteria are *his*$^{+}$, meaning that they can make their own histidine from other materials. In the Ames test, *his*$^{-}$ bacteria are grown in a medium containing just a small amount of histidine, which allows just enough growth for mutations to have a chance to occur. Soon, however, the histidine is used up, and the bacteria die unless they have mutated from *his*$^{-}$ to *his*$^{+}$ and thus have become able to make their own histidine. The rate of spontaneous mutation is very low. If a chemical is added to the culture medium and many more bacterial colonies grow than in a culture without this addition, the chemical can be assumed to have caused the mutations—i.e., to be a mutagen. Counts of the numbers of colonies also identify stronger and weaker mutagens.

Remember that the Ames test was designed as a *screening method* for carcinogens. A positive result in the Ames test does not guarantee that a chemical will be carcinogenic to animals or humans, but it identifies chemicals that should be tested further, first in mammalian mutagenesis assays (in tissue culture) and then in animal studies if the tissue culture tests are also positive. Reasons why a chemical that is mutagenic in bacteria might not be carcinogenic to animals include differences in the rate of uptake of the chemical by cells, differences in bacterial vs. mammalian cell metabolism, and differences in DNA repair. Bruce Ames, the originator of the Ames test, has also pointed out that nearly *any*

substance is mutagenic in a sufficiently high dose. Therefore, the effective dose to which cells in a whole animal are likely to be exposed must also be considered. Another limitation of the Ames test is that it cannot identify animal carcinogens that are tumor promoters, not tumor initiators, since these exert their cancer-causing effects without causing mutations.

[EDITOR AND ARTIST: This Box contains an illustration that can be copied without change from Box 12.1 (page 449) in BT 3rd ed.]

TABLE 10.1

Average life spans of human differentiated cell types.

CELL TYPE	**LIFE SPAN (DAYS)**
Intestinal lining	1.3
Stomach lining	2.9
Tongue surface	3.5
Uterine cervix	5.7
Stomach mucus	6.4
Cornea	7
Epidermis: abdomen	7
Epidermis: cheek	10
Lung alveolus	21
Lung bronchus	167
Kidney	170
Bladder lining	333
Liver	450
Adrenal cortex	750
Brain nerve	27,375+ (75+ years)

TABLE 10.2

Characteristics of normal cells and transformed (cancer) cells.

CELLULAR BEHAVIOR	NORMAL	TRANSFORMED
Limit to the number of cell divisions	Finite	Immortal
Differentiation	Present	Inhibited
Transport of nutrients across cell membrane	Slower	Faster
Nutrient requirement	Higher	Lower
Contact inhibition	Present	None
Requirement for attachment	Present	None
Adhesiveness	High	Low
Secretion of protein-degrading enzymes	Low	High
Genetic material	Stable	Unstable

TABLE 10. 3 Cancer Cases, Deaths and Five-Year Survival Rates in the United States, arranged in decreasing order of the number of deaths (totals estimated by the National Cancer Institute from incidence rates found in the Surveillance, Epidemiology and End Results [SEER] Program).

TYPE OF CANCER	NEW CASES*	DEATHS*	5-YEAR SURVIVAL RATE (%)‡
Lung	228,150	142,670	19
Colon and rectum	145,600	51,020	65
Pancreas	56,770	45,750	9
Breast	271,270†	42,260†	90
Liver & intrahepatic bile duct	42,030	31,780	18
Prostate	174,650	31,620	98
Leukemia	61,780	22,840	65^
Lymphoma	82,310	20,970	74, 88^
Brain & other nervous system	23,820	17,760	Vary widely by type & age at diagnosis
Urinary bladder	80,470	17,670	77
Kidney & renal pelvis	73,820	14,770	75
Ovary	22,530	13,980	47
Myeloma	32,110	12,960	52
Uterus, endometrium	61,880	12,160	81
Oral cavity and pharynx	53,000	10,860	65
Skin, melanoma	96,480	7,230	92
Soft tissue sarcoma	12,750	5,270	81
Uterus, cervix	13,170	4,250	66

* 2019 American Cancer Society Facts and Figures. Numbers are estimates for 2019 based on data from 2001-2015.

† Includes 2670 cases and 500 deaths in men.

‡ Percentage of people diagnosed with cancer who are still alive after five years, compared with percentage in a comparable, cancer-free population. Data are for all clinical stages at diagnosis; cancers that have already spread regionally at the time of diagnosis have somewhat lower survival rates, and those that have metastasized have much lower survival rates. Unless otherwise indicated, data are from 2019 American Cancer Society Facts and Figures.

^2019 Leukemia and Lymphoma Society. Survival rates for leukemia are much higher in younger people. Five-year survival rate for non-Hodgkins lymphoma, 74%; for Hodgkins lymphoma, 88%

TABLE 10. 4 Examples of genes mutated in familial cancers and the cancers they cause

GENE	PROTEIN FUNCTION	TYPE OF CANCER	ASSOCIATED RISK*
RB	G_0 cell cycle checkpoint	Retinoblastoma	90%†
XPA, XPB, XPC, XPD, XPE, XPF, XPG & XPV	DNA repair (UV damage)	Various skin cancers, including melanoma	~100%[#]
APC	Promotes cell differentiation	Colon	~100%[#]
BRCA1	DNA repair (via recombination)	Breast, ovarian	72% (breast) ‡, 44% (ovarian) ‡
BRCA2	DNA repair (via recombination)	Breast. ovarian	69% (breast) ‡, 17% (ovarian) ‡

***** Data indicate the approximate percentages of people with inherited mutations in the given genes who will develop the associated cancers.
†2019, American Cancer Society
[#]2019, National Institutes of Health, Genetics Home Reference
‡2017, National Cancer Institute; data are for risk of developing cancer by age 80

TABLE 10.5

Age-specific probabilities of developing breast cancer.

At age	Probability of developing breast cancer in the next 10 years	
	%	1 in:
Birth to 49	2	51
50-59	2.3	43
60-69	3.5	29
70 & older	6.7	15
Birth to death	12.4	8

2019 American Cancer Society Facts and Figures. Percentages based on 2013-2015 data.

TABLE 10.6

Carcinogens in the workplace.

CARCINOGEN	Cancer Type	Exposure of General Population	Examples of Workers Frequently Exposed or Exposure Sources
CHEMICAL AGENT			
Arsenic	Lung, skin	Rare	Insecticide and herbicide sprayers; oil refinery workers
Asbestos	Lung, other sites	Uncommon	Brake-lining; shipyard; insulation and demolition workers
Benzene	Bone marrow	Common	Painters; distillers and petrochemical workers; dye users; furniture finishers; rubber workers
Diesel exhaust	Lung	Common	Railroad and bus-garage workers; truck operators; miners
Formaldehyde	Nose, pharynx	Rare	Hospital laboratory workers; manufacture of wood products, paper, textiles, garments and metal products
Heavy metals (cadmium, uranium, nickel)	Prostate	Rare	Metal workers
Man-made mineral fibers	Lung	Uncommon	Wall and pipe insulation; duct wrapping
Hair dyes	Bladder	Uncommon	Hairdressers and barbers (inadequate evidence for customers)
Mineral oils	Skin	Common	Metal machining
Nonarsenical pesticides	Lung	Common	Sprayers; agricultural workers
Painting materials	Lung	Uncommon	Professional painters
Polychlorinated biphenyls (PCBs)	Liver, skin	Uncommon	Heat transfer and hydraulic fluids and lubricants; inks; adhesives; insecticides
Soot	Skin	Uncommon	Chimney sweeps and cleaners; bricklayers; insulators; firefighters; heating-unit service workers
Vinyl chloride	Liver	Uncommon	Plastic workers
PHYSICAL AGENT			
Ionizing radiation	Bone marrow, several others	Common	Sunlight; nuclear materials; medicinal products and procedures
Radon	Lung	Uncommon	Mines; underground structures

Data modified from *Scientific American*, September 1996.

TABLE 10.7

Summary of various causes of cancer worldwide.

CAUSE	RELATIVE PERCENTAGE OF CANCER DEATHS*
Smoking & tobacco use	22
Diet	30
Alcohol	6
Food additives (salt)	1
Sedentary lifestyle	3
Radiation	2†
Pollutants (air, water)	2
Viruses	20
Chemical carcinogens	Variable‡
Genetic susceptibility	<10

* Derived from statistical analysis of epidemiological data. A figure of 30 means that 30% of all cancer deaths worldwide are attributable to that particular cause.
† Over 90% of skin cancers are caused by UV radiation.
‡ The percentage of deaths in the general population attributable to carcinogen exposure is low, but can be locally very high, for example in industries with high or prolonged exposure.

FIGURE CAPTIONS:

Figure 10.1 Subdividing a volume into cells increases the effective surface area. Cube A is one unit on each side, while cube B is three units on each side; cube C is also three units on each side but is subdivided into smaller cubes, each 1 unit per side. What is the total volume of each cube? What is the total surface area? What is the ratio of surface area to volume? [EDITOR & ARTIST: **Same as BT 3ed Fig. 12.1**]

Figure 10.2 Regulation of transcription. A, RNA polymerase binds to genes at promoter sequences to begin transcription. B, When activator proteins bind to DNA, they help RNA polymerase bind to the promoter and increase the rate of transcription. C, When repressor proteins bind to DNA, they block RNA polymerase and prevent transcription. [EDITOR AND ARTIST: New figure; **please see file Fig1002.jpg**]

Figure 10.3 The activity of transcriptional activators and repressors is also regulated. A, A signaling molecule binds to an activator and causes the activator to bind DNA. B, Chemical modification of an activator causes it to bind DNA. C, A signaling molecule binds to a repressor and prevents it from binding DNA. D, Chemical modification of a repressor prevents it from binding DNA. [EDITOR AND ARTIST: New figure; **please see file Fig1003.jpg**] [New figure incorporating panel D of existing Fig. 12.5—can find something similar in Alberts MBoC or Essential Cell Biology]

Figure 10.4 Six steps in the process of gene expression at which regulation can take place. [1, Transcription turned on or off; 2, mRNA is chemically modified to allow exit from the nucleus (show capping, splicing, polyadenylation in figure); 3, Translation initiation regulated to produce more or less protein; 4, Chemical modification and protein folding; 5, Binding of an effector molecule or chemical modification to regulate protein activity; 6, Regulation of protein destruction.] [EDITOR AND ARTIST: **Please see file Fig1004.bmp or Fig1004.jpg**]

Figure 10.5 Signals can travel short or long distances to reach target cells. A, paracrine signals are released by one cell and affect neighboring cells. B, endocrine signals enter the bloodstream and travel throughout the body to reach distant target cells. [New figure; e.g. Fig. 15-4 from Alberts 4th ed.

ARTIST: OK for parts A & B to be either side-by-side or stacked vertically.] [**Please see file Fig1005.jpg**]

Figure 10.6 Signal reception and signal transduction A, Steroid hormones cross the cell membrane to bind internal receptors that regulated gene transcription. B, Other signals bind cell-surface receptors to activate a signal transduction cascade inside the cell. 1) Signal binding changes the shape of the internal portion of the receptor. 2) Internal signaling proteins are activated by the bound receptor. 3) Some internal signaling proteins are enzymes that produce internal signals called second messengers. 4) Second messengers activate proteins that cause the final outcome of a signal, such as changes in gene expression. [EDITOR AND ARTIST: **Please see file Fig1006.bmp or Fig1006.jpg**]

Figure 10.7 The cell cycle. Hours shown are the approximate lengths of time for each phase in a cell with a 24-hour cell cycle, typical of many eucaryotic cells. [EDITOR AND ARTIST: **Please see file Fig1007.bmp or Fig1007.jpg**]

Figure 10.8 Cyclin dependent kinases (CDKs) drive cell cycle progression. A) CDKs are activated by binding to cyclins; phosphorylation of target proteins triggers cell cycle transitions. B) Different cyclins accumulate and are destroyed at different times in the cell cycle, causing activation of the appropriate CDKs at the right times to trigger cell cycle events. [new figure; could modify Alberts 4th ed. 17-15 and 17-16; maybe indicate rising levels of cyclins w/ wedges like in Lodish 13.29]

Figure 10.9 Most normal cells need to be attached in order to divide (anchorage dependent) and stop dividing when they have formed a complete layer (contact inhibition); cancer cells do not obey either of these rules. Contact inhibition will cause normal cells to stop dividing when they form a monolayer, but cancer cells will continue to grow and form a tumor or heap. [EDITOR AND ARTIST: Please combine BT 3ed Fig. 12.4 with BT 3ed Fig. 12.14 and arrange at artist's discretion; no changes needed to either]

Figure 10.10 Telomeres are structures at the ends of eucaryotic chromosomes that shorten with each cell division. A, Telomeres contain many repeated DNA sequences that bind to proteins, forming a ‘cap.’ B, In normal somatic cells, telomere shortening with each round of cell division leads to cell aging and eventually lack of further division. In cancer cells, the enzyme telomerase is active and

restores the telomeres, allowing the cells to continue dividing without limit. [EDITOR AND ARTIST: **Please use file Telomere.bmp or Telomere.png or Telomere.jpg**]

Figure 10.11 Types of cell death: apoptosis vs. necrosis. [new fig.; e.g. Fig. 17-37 from Alberts 4th ed]

Figure 10.12 Virtually all somatic cells contain the complete genome, but different genes are expressed by the cells of different tissues. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.10; no changes needed**]

Figure 10.13 Experimentally induced differentiation. By manipulating cells from the gastrula stage of frog embryos, Hans Spemann and Hilde Mangold showed that an area called the 'dorsal lip' served as an organizer to induce the formation of a nervous system. At the locations of both host and transplanted dorsal lip cells, neural plates developed and went on to form brains and other head structures. Subsequent experiments showed that the transplanted donor cells were not actually part of the induced neural plate. Rather, the dorsal lip secretes 'organizer' signals that cause the overlying host cells to differentiate. Spemann was awarded the Nobel Prize for these experiments in 1935. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.11; no changes needed to the art.**]

Figure 10.14 Differentiation of various cell types during embryonic development. A, Cell layers form as a blastula develops into a gastrula. B, As cells in the ectoderm, mesoderm, and endoderm divide, they differentiate, eventually becoming specialized cells. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.8; no changes needed.**]

Figure 10.15 Gurdon's experiment demonstrating that a differentiated cell contains all the genes needed for the development of a complete organism. The nucleus of a frog egg was destroyed by ultraviolet irradiation and was replaced by the nucleus from the fully differentiated skin cell of another frog. The egg with its transplanted nucleus was allowed to grow and it developed into a normal tadpole. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.9 w/o any changes**]

Figure 10.16 Establishing asymmetry (polarity) in a developing embryo. A) Some eggs, such as those of fruit flies, have asymmetry even before fertilization. B) In other organisms, including frogs, symmetry is established based on the position where the sperm enters upon fertilization. [new figure; modify from Alberts 4th ed 21-30 and ***(need to find this)]

Figure 10.17 When a stem cell divides, one daughter cell remains as an undifferentiated stem cell and the other begins the process of differentiating into a specialized, non-dividing cell, passing through a stage of multiple cell divisions as a transit amplifying cell. [alter Figure 12.13 to become more like Fig. 12.1 in Biology of Cancer]

Figure 10.18 Continuous signaling of cell division in transformed cells by the protein products of five types of oncogenes that lead to the cell's escape from the regulation of cell division. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.15 w/o any changes**]

Figure 10.19 Only one oncogene mutation but two tumor suppressor mutations are required to promote cancer. [new figure showing dominant/recessive behavior of cancer-causing genes]

Figure 10.20 The risks for cancer increase strongly with age. [e.g. figure like https://scienceblog.cancerresearchuk.org/2018/06/20/age-the-biggest-cancer-risk-factor/] [Alternate: https://www.cancer.gov/about-cancer/causes-prevention/risk/age]

Figure 10.21 DNA mismatch mutations and their repair. (A) If a mismatch occurs during replication, it can lead to a permanent mutation if not corrected on the new strand. (B) Instead, a mismatch repair protein (spell-checking protein) checks for mismatched bases on the new strand as synthesis proceeds. If a mismatch is detected by the shape of improperly bonded nucleotides between the strands, part of the new strand is trimmed back before synthesis can restart and allow the correct base to be inserted. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.16 with new caption but no change to the art**]

Figure 10.22 The growth of a tumor in the human breast. [Modify existing Fig. 12.17 by adding "transformed cells broken free from tumor can migrate to new sites through blood vessels, establishing secondary tumors." Also add arrow showing increasing accumulation of mutations] [EDITOR AND ARTIST: **Please use file Fig1022.jpg or Fig1022.png** ; OK to redo any lettering within the figure]

Figure 10.23 Deaths from cancers in the United States. (A) Death rates from all cancers combined (B) Death rates for different cancer sites in males (C) Death rates for different cancer sites in females. (D) Lung cancer death rate and cigarette consumption. (A, from National Center for Health Statistics data as analyzed by NCI; B,C from American Cancer Society, Cancer Facts & Figures, 2020; D, redrawn from several sources) [ARTIST AND EDITOR: **For A,B,C, use the files Fig1023a.jpg**

Fig1023b.png Fig1023c.png For D, use the file Fig1023p.xlsx by combining both graphs on a single set of axes, with vertical axis title and scale for Cigarettes per capita on left (omit grid lines) and vertical axis title and scale for Lung cancer deaths per 100,000 (with horizontal grid lines) on the right.]

Figure 10.24 Familial cancers. (A) Retinoblastoma [e.g. https://commons.wikimedia.org/wiki/File:Rb_whiteeye.PNG] (B) Xeroderma pigmentosum [e.g. https://commons.wikimedia.org/wiki/File:Xeroderma_pigmentosum_02.jpg] (C) Familial adenomatous polyposis (precursor to colon cancer) [e.g. https://visualsonline.cancer.gov/details.cfm?imageid=10067 public domain but see source for credit info] [EDITOR AND ARTIST: **Use files Fig1024a.png Fig1024b.jpg and Fig1024c.jpg** arranged at artist's discretion]

Figure 10.25 Lifetime probabilities of acquiring malignant cancers (U.S., 2014-2016), based on data from National Cancer Institute, 2019. [ARTIST AND EDITOR: Use the file **Fig1025t.xlsx** Figures may be used as they are or rotated left 90 degrees. Please omit decimals from numerical axes.]

Figure 10.26 Tumor viruses carry oncogenes. [figure showing virus picking up normal gene, it getting mutated to form an oncogene, and viral insertion into a host to cause a tumor—I can draw this]

Figure 10.27 UV irradiation induces abnormal bonds between neighboring nucleotides. (figure showing T-T dimers)

Figure 10.28 Synergism between alcohol and cigarettes in producing cancers of the mouth and throat. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.19 w/o any changes**]

Figure 10.29 The effects of smoking on the incidence of cancer. [EDITOR AND ARTIST: **Please use BT 3ed Fig. 12.20 w/o any changes**]

[NEEDED HERE: List of figures for production staff; proofread number changes.]
[10-08: Needed; also 16, 17; 19,20,23, 26, 27 20,23 exist]